Original Article

Longitudinal multi-gene panel assessment of circulating tumor DNA revealed tumor burden and molecular characteristics along treatment course of non-small cell lung cancer

Gloria Y. F. Ho[1], Tao Wang[2], Hoi-Hin Kwok[3], Rehana Rasul[1], Rita Peila[2], Maria Guzman[1], Mary S. M. Ip[3], David C. L. Lam[3]

[1]Department of Occupational Medicine, Epidemiology and Prevention, Feinstein Institute for Medical Research, Northwell Health, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Manhasset, NY, USA; [2]Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, NY, USA; [3]Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Contributions: (I) Conception and design: GYF Ho, DCL Lam; (II) Administrative support: DCL Lam, MSM Ip (III) Provision of study materials or patients: DCL Lam; (IV) Collection and assembly of data: GYF Ho, T Wang, HH Kwok, R Rasul, R Peila, M Guzman, DCL Lam; (V) Data analysis and interpretation: GYF Ho, DCL Lam, MSM Ip; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: David C. L. Lam, MD, PhD, FRCP. Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China. Email: dcllam@hku.hk.

Background: Most studies associating circulating tumor DNA (ctDNA) with outcome in lung cancer treatment were either cross-sectional or, if longitudinal, only analyzed a limited number of genes. This study evaluated the potential of utilizing ctDNA profiled by a panel of common cancer genes to monitor tumor burden and to reveal molecular characteristics of tumor along treatment course.

Methods: Twenty Chinese non-small cell lung cancer (NSCLC) patients with serial plasma samples collected (I) before starting on either first- or second-line treatment, (II) at stable disease on treatment, and (III) upon disease progression, were analyzed for mutations in ctDNA using the PGDx 64-gene panel. Paired statistics compared mutation profiles between any two of the three time points.

Results: Proportions with detectable ctDNA decreased from 65% at baseline to 35% at stable disease and rose to 80% at progression (P=0.012, between stable disease and progression); median ctDNA levels (mutated fragments per mL) were 7.8, 0, and 24.7 at the three time points, respectively (P=0.013 between baseline and progression; P=0.007 between stable disease and progression). Although plasma epidermal growth factor receptor (*EGFR*) mutations were commonly detected, 15% of patients had mutations other than *EGFR* detected during progression, such as various types of *TP53* mutations.

Conclusions: ctDNA profiling in serial blood samples reflected tumor burden over time, and a multi-gene panel was more sensitive in indicating lung cancer progression on treatment than a single gene approach. The detection of additional oncogenic mutations or their disappearance suggested evolution of tumor heterogeneity along treatment course.

Keywords: Lung cancer; circulating tumor DNA (ctDNA); epidermal growth factor receptor (*EGFR*); longitudinal assessment; tumor burden

Submitted May 16, 2020. Accepted for publication Aug 03, 2020.

doi: 10.21037/tlcr-20-675

View this article at: http://dx.doi.org/10.21037/tlcr-20-675

© Translational Lung Cancer Research. All rights reserved.

Introduction

Lung cancer is the leading cause of cancer death worldwide (1). For patients with advanced stage lung cancer, treatment options include conventional systemic chemotherapy or molecular-targeted therapy, and the latter is indicated with identification of therapeutic tumor targets (2). Actionable tumor targets, such as epidermal growth factor receptor (*EGFR*) mutations and anaplastic lymphoma kinase (*ALK*) translocation, are present more commonly in lung adenocarcinomas in Asians, and the presence of these actionable targets indicate that the tumour would be sensitive to specific tyrosine kinase inhibitors (TKIs) (2). Therefore, the identification of actionable mutations in tumors can guide treatment choice. Nevertheless, it is often impractical to obtain tumor tissue biopsy for molecular profiling, let alone to repeat tissue samplings over time to evaluate acquired resistance due to further genetic aberrations. The liquid biopsy approach, which offers the option of detecting molecular characteristics of circulating tumor cells or circulating tumor DNA (ctDNA) in blood (3), has the potential to revolutionize clinical care in cancer patients (2). Longitudinal follow up with liquid biopsy may be a new way to inform clinicians of subtle changes in underlying tumor characteristics or early knowledge of disease progression. This may allow for discovery of acquisition of oncogenic mutations that could be biomarkers for the next line of anti-cancer treatment or for prognostication (4,5).

ctDNA is cell-free DNA released from tumor cells into the circulation (6). Our group had previously shown the feasibility of detecting concordant *EGFR* mutations in plasma and tumor tissue of advanced stage lung cancer patients, and the presence or increasing plasma levels of ctDNA was associated with a worse prognosis (7). Similarly, it has been reported in other studies that somatic mutations in ctDNA of patients with non-small cell lung cancer (NSCLC) reflect molecular characteristics in tumor tissue, and ctDNA levels correlated with tumor stage and tumor burden (8-10). These observations have prompted subsequent studies to examine if ctDNA testing is useful for monitoring tumor status and tumor response to therapy over time. However, most of these previous studies tested for either a single gene (e.g., *EGFR*) or a few genes in ctDNA (11-13) or when a larger panel of genes was used, ctDNA was only examined at one single time point (14,15). In this proof-of-concept study, we employed a longitudinal design and a panel of 64 genes to examine whether (I) serial testing of ctDNA over time in the same patient was feasible for monitoring tumor burden, and (II) sequencing a panel of genes, as compared to the single gene approach, provided additional advantage in revealing tumor status and acquisition of new ctDNA mutations along treatment course. We present the following article in accordance with the AME publishing reporting checklist.

The authors present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-675).

Methods

Study population

Consecutive patients with advanced stage lung cancer attending the clinics of the Department of Medicine, Queen Mary Hospital, were recruited. The inclusion criteria were: (I) patients had diagnosis of advanced stage NSCLC and would undergo anti-cancer treatment, with either first- or second-line EGFR-TKI for patients with *EGFR* mutations, or first line chemotherapy for patients with *EGFR* wildtype tumors; and (II) patients gave informed written consents and agreed to have study follow up with plasma samples taken at three time points along their course of treatment: enrollment/baseline, at stable disease, and upon clinical progression of disease according to the RECIST 1.1 criteria. Blood samples of at least 30 mL were collected at each clinical visit time-point along the course of treatment. Plasma and serum samples were processed within 1 hour of collection and stored at –80 ℃. Clinical information of recruited subjects was recorded. At the time of enrollment, each participant also completed a questionnaire pertaining to their demographic information, lifestyle factors, and medical history. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the Ethics Committee of the University of Hong Kong and Hong Kong Hospital Authority Hong Kong West Cluster Institutional Review Board (IRB Reference Number UW 16-104). Informed consent was obtained from all the participants.

The first patient was recruited in April, 2016 and the last patient who completed all the follow-up with disease progression had the last blood sample collected in October 2018. Stable disease was confirmed with review of all the records for the 20 recruited subjects, with neither recent sufficient shrinkage to qualify for partial or complete response nor sufficient increase to qualify for progressive

© Translational Lung Cancer Research. All rights reserved.

disease.

ctDNA measurements

For each patient, a plasma sample of at least 1 (median: 2, range: 1 to 4) mL, processed from EDTA tubes from each time point was shipped frozen overnight to Personal Genome Diagnostics (PGDx, Baltimore, MD, USA) for ctDNA analysis (16,17). Samples were labeled with a unique sample number, and serial samples of the same participant could not be identified by laboratory personnel. Circulating cell-free DNA was extracted and analyzed for a panel of 64 well-characterized cancer genes (PlasmaSELECT™ 64) by next generation sequencing with about 30,000× coverage (16,17). PlasmaSELECT™ 64 allows the identification of single nucleotide variants (SNVs), indels, amplifications, translocations, and microsatellite instability with sensitivity of 99.4% for SNVs and indels and a per base specificity of >99.9% (16,17). Somatic mutations in ctDNA (point mutations, insertion, deletion and substitution) were identified by the proprietary VariantDx bioinformatics pipeline that incorporates information from public databases, such as dbSNP, the 1000 Genome Project, and COSMIC, and excludes potential germline as well as hematopoietic variants (18-21). The total level of ctDNA in a sample was assessed as the total number of mutant fragments per mL of plasma. The ctDNA level of a particular gene (e.g., *EGFR*) was reported as the total number of mutant fragments detected in that gene per mL of plasma.

Statistical analysis

Our goal was to determine if mutation profiles in ctDNA reflected tumor burden over time and hence varied with tumor status at enrollment/baseline, stable disease, and progression. Statistical analysis focused on within-individual pairwise comparison of ctDNA profiles at any two of the three time points. Wilcoxon signed-rank test and exact binomial McNemar's test were used for comparison of continuous variable (ctDNA quantitative level) and categorical variable (ctDNA detection), respectively. Reported P values are two-sided.

Results

Twenty patients who fulfilled inclusion criteria were recruited and their data analyzed. Their demographics, disease staging, driver mutations, treatment, tumor measurements by the longest diameter, sites of metastasis and concentration of ctDNA at baseline, stable disease and progressive disease were all listed in *Table 1*. A summary of these patients' baseline characteristics are shown in *Table 2*. All patients were of Chinese descent and had stage IV NSCLC. The majority of patients were females, never-smokers, diagnosed with adenocarcinoma of lung, and 16 of them (16/20, 80%) were tested positive for *EGFR* mutations in tumor tissue. The median time interval between ctDNA samples were 84 days [inter-quartile range (IQR): 53–193] between baseline and stable disease, 145 days (IQR: 80–252) between stable disease and progression, and 259 days (IQR: 171–376) between baseline and progression. Median cell-free DNA yield per mL of plasma sample was 20.5 ng (IQR: 12.4–26.2), and the median total yield was 37.3 ng (IQR: 28.7–51).

The detection frequencies of ctDNA in these 20 patients at the three time points are summarized in *Table 3*. Even in this cohort of patients with a high prevalence of *EGFR* driver mutations, more than 30% of patients tested positive for ctDNA have alterations in non-*EGFR* genes (38.5%, 42.9%, and 31.3% at baseline, stable disease, and progression, respectively). None of the patients had microsatellite instability, amplification, or rearrangement. In general, the proportion of patients with detectable ctDNA decreased from baseline (65%) to stable disease (35%), and rose to the highest at the time of progression (80%) (P=0.012, pairwise comparison of ctDNA detection between stable disease and progression). Similar patterns were observed for the detection of somatic *EGFR* and *TP53* alterations over the three time points. ctDNA quantities in terms of the number of mutant fragments per mL of plasma are shown in *Table 3*. Median levels of ctDNA were 7.8 at baseline, zero at time of stable disease, and 24.7 at disease progression (pairwise difference: P=0.013 between baseline and progression; P=0.007 between stable disease and progression). ctDNA quantitative levels in individual patients over three time points are plotted in *Figure 1*.

The largest difference in ctDNA detection frequency was observed between stable disease and progression, within-subject results are detailed in *Table 4*. Using a 64-gene panel, we found that in 50% of the patients, ctDNA detection changed from negative at the time of stable disease to positive during disease progression. Contrarily, change in *EGFR* detection from absence to presence occurred in 35% of the patients when disease progressed (mostly in *EGFR* T790M and/or E746_A750del). Therefore, if a single

© Translational Lung Cancer Research. All rights reserved.

Table 1 A summary of the baseline demographics of recruited patients and their tumor characteristics and treatment received

Patient characteristics	N (%)
Average age (± SD), years	61.3±7.9
Gender	
Male	7 (35)
Female	13 (65)
Smoking status	
Never-smoker	14 (70)
Former smoker	5 (25)
Missing information	1 (5)
Histology	
Adenocarcinoma	18 (90)
NSCLC-NOS	2 (10)
EGFR mutation in tumor tissue at baseline	
Wildtype	4 (20)
Mutant	16 (80)
Treatment	
First line EGFR-TKI	14 (70)
Second line EGFR-TKI	2 (10)
Chemotherapy	4 (20)
Average number of days between blood draws (± SD)	
Baseline and stable disease	114.8±78.2
Stable disease and progression	197.6±169.8

SD, standard deviation; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; *EGFR*, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

gene of *EGFR* were assessed instead of using a multi-gene panel, the change in ctDNA pattern indicative of disease progression would have been missed in 15% of the patients who had ctDNA mutations in non-*EGFR* genes.

The detection of additional ctDNA mutations or their disappearance during treatment course in the 20 patients is detailed in *Table 5*. Based on their tumors being *EGFR* mutants or wildtype, and the serial changes in ctDNA mutation patterns, recruited patients could be broadly divided into four groups:

- ❖ Group 1 (patients 1–5): patients had an *EGFR* mutation, but not *EGFR* T790M, in tumor tissue at diagnosis, were treated with first line EGFR-TKI (gefitinib, erlotinib or afatinib) at baseline and had mainly *EGFR* T790M mutation detected at disease progression;
- ❖ Group 2 (patients 6–14): these patients were similar to Group 1 in terms of having had a drug-sensitive *EGFR* mutation in tumor tissue and were treated with EGFR-TKI, but they never had *EGFR* T790M mutation detected along their treatment course;
- ❖ Group 3 (patients 15 and 16): patients had sensitizing *EGFR* mutations and disease progression after first line EGFR-TKI, switched to second line EGFR-TKI (osimertinib) with baseline blood taken when they started osimertinib, and followed with stable disease then further disease progression on osimertinib;
- ❖ Group 4 (patients 17–20): *EGFR* wildtype subjects on first line platinum-based chemotherapy (pemetrexed-platinum) at baseline.

Among the 14 *EGFR* mutant subjects on first line EGFR-TKI treatment, 5 (5/14, 35.7%) (Group 1) had detection of *EGFR* T790M mutation at disease progression (*Table 5*). In these five subjects, other oncogenic mutations were also found at the time of disease progression, such as *MYC* and *MET* mutations. Nine of the 14 patients (64.3%) progressed without *EGFR* T790M mutation detected, but new ctDNA mutations were also found in various genes such as *BRCA1*, *CD274* and *TP53*.

Two *EGFR* mutant patients (Group 3) showed persistent detection of *EGFR* T790M at osimertinib baseline through stable disease on treatment. At further disease progression on osimertinib, one of them (patient 15) lost the *EGFR* T790M in ctDNA while the other one (patient 16) showed persistent *EGFR* T790M mutation detection at further disease progression. Neither patient had new mutations detected at time of progression.

In some patients with either *EGFR* mutations or wildtype *EGFR* in tumor tissue, different mutations in the *TP53* gene were detectable in ctDNA at different time-points. Nevertheless, *TP53* mutations appeared to be more prevalent and were more likely to accumulate diverse types of point mutation in the wildtype *EGFR* tumors when the respective patients were treated with chemotherapy (Group 4).

Discussion

In this proof-of-concept longitudinal study using a multi-gene panel, we showed that ctDNA detection and quantity reflected tumor status over time—after initiation

© Translational Lung Cancer Research. All rights reserved.

Table 2 A list of recruited patients with demographics, disease staging, targets, treatment received, primary tumor site and measurements (the longest diameter), metastatic sites and ctDNA concentration at baseline, stable disease and at disease progression

Patients	Gender	Age	Smoking	Stage	Target	Baseline				Stable disease			Disease progression		
						Primary tumor site & size	Metastatic sites	ctDNA conc (ng/mL)	Treatment	Primary tumor site & size	Metastatic sites	ctDNA conc (ng/mL)	Primary tumor size	Metastatic sites	ctDNA conc (ng/mL)
1	M	57	Ex	IV	Del 19	RLL 2.1 cm	L SCF LN, R pleura, R pleural effusion	10.78	Gefitinib	RLL 2.0 cm	All metastatic sites improved	9.37	RLL 24 cm	Worsening of all metastatic sites	10.2
2	F	57	NS	IV	Del 19	LLL 8.5 cm	R lung, L pleura, L effusion, LN, liver, bone	10.45	Afatinib	LLL 3.9 cm	Resolution of L pleural effusion. Same for other site metastasis	15.83	LLL 13.0 cm	Worsening of all metastatic sites	21.07
3	F	64	NS	IV	Del 19	RUL 2.6 cm	R pleura, LN, liver	47.6	Afatinib	RUL 0.8 cm	All metastatic sites improved	11.63	RUL 1.2 cm	New intrapulmonary nodules and increased R pleural metastasis, same LN and liver metastasis	33.63
4	F	65	NS	IV	L858R	RLL 7.7 cm	RML, LN, bone	18.95	Afatinib	RLL tumor 1.2 cm scarring	Similar to baseline	21.9	RLL 3.8 cm	Worsening of all metastatic sites	74.45
5	F	50	NS	IV	L858R	Numerous bilateral lung nodules 1–1.5 cm	R cervical LN, bone	68.4	Gefitinib	LUL 1.3 cm, all other bilateral lung nodules reduced to <1 cm	Disappearance of R cervical LN. Same bone metastasis	14.6	LUL 2 cm	Worsening of all metastatic sites	44.3
6	F	66	NS	IV	Del 19	RUL 6 cm	R pleura, R pleural effusion, LN, bilateral adrenal, pancreatic tail, bone	25.5	Gefitinib	RUL 3.5 cm	All metastatic sites improved	13.1	RUL 5 cm	Worsening of all metastatic sites	64.5
7	F	59	NS	IV	Del 19	RLL 7.3 cm	R SCF LN, liver, bone	34.2	Afatinib	RLL 2.6 cm	All metastatic sites improved	27.3	RLL 2.7 cm	Worsening of all metastatic sites	44.2
8	F	62	NS	IV	Del 19	RLL 5.2 cm	Intrapulmonary, LN	23.3	Gefitinib	RLL 3.6 cm	Same as baseline	23.6	RLL 4.5 cm	Worsening of all metastatic sites	14.35
9	F	60	NS	IV	L858R	LUL 5.4 cm	Bilateral lung, LN, L pleura, L pleural effusion, pericardial effusion, L adrenal	6.27	Gefitinib	LUL 3.5 cm	All metastatic sites improved	26.17	LUL 3.5 cm	No lung met, increased L pleural effusion and pericardial effusion, same L adrenal met	12.33
10	M	55	NS	IV	Del 19	Multiple lung nodules both lungs	LN, bone	21.4	Gefitinib	All lung nodules decreased in sizes	All metastatic sites improved	13.2	All lung nodules increased in size	All lung and bone metastasis increased in size. New right adrenal met	38.3
11	F	64	NS	IV	Del 19	R hilar mass 1.7 cm	L pleura, L effusion	28.8	Gefitinib	Not measurable	All metastatic sites improved	30.8	Not measurable	New LLL lesions, L pleural metastasis	19.45
12	M	77	Ex	IV	Del 19	RUL 4.1 cm	LN, bone	25.1	Afatinib	RUL 3 cm	All metastatic sites improved	11.05	RUL 3.3 cm	Worsening of all metastatic sites	21.8
13	M	55	NS	IV	Del 19	LUL 2.3 cm	Multiple LN, pleural deposits, massive L pleural effusion	29.93	Erlotinib	LUL 2.1 cm	All metastatic sites improved	12.45	LUL 3 cm	Worsening of all metastatic sites	12.37
14	F	65	NS	IV	L858R	RUL 2.9 cm	L lung nodules, R pleural effusion, rib	16.8	Gefitinib	RUL 2.9 cm	Same as baseline	16.55	RUL 4 cm	Worsening of all metastatic sites	12.7
15	F	45	EX	IV	L858R	LLL 2.7 cm	L pleural deposits, LN, large L pleural effusion	15.75	Gefitinib	LLL 1.4 cm	All metastatic sites improved	20.4	LLL 2.6 cm	Worsening of all metastatic sites	10.3
16	M	66	Ex	IV	L858R	LLL 1.7 cm	LN, bone	21.45	Erlotinib	LLL 1.7 cm	Same as baseline	25.7	LLL 3.7 cm	New L pleural metastasis, same LN and bone metastasis	39.6
17	M	66	Ex	IV	WT	RUL 12.3 cm	R intrapulmonary, bone	10.63	G/Cis	RUL 9.5 cm	Same as baseline	21.43	RUL 12.2 cm	Worsening of all metastatic sites	23.3
18	F	67	NS	IV	WT	RLL 5.3 cm	Intrapulmonary, liver, bone	12.43	P/Carb	RLL 4.4 cm	Same as baseline	16.33	RLL 6.1 cm	Worsening of all metastatic sites	83.23
19	M	51	NS	IV	WT	RUL 1.8 cm	LN, bone	25.75	P/Cis	RUL 1.4 cm	Same as baseline	20.5	RUL 3.2 cm	New LUL mass, increased LN and bone metastasis, new bilateral pleural effusion	8.97
20	F	70	NS	IV	WT	RUL 4 cm	R pleural effusion, bone	22.45	P/Carb	RUL 3 cm	All metastatic sites improved	17.35	RUL 2.5 cm	Worsening of all metastatic sites	4.83

ctDNA, circulating tumor DNA; NS, non-smoker; Ex, ex-smoker; Conc, concentration; WT, wildtype; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; LN, lymph node; G/Cis, gemcitabine/cisplatin; P/Carb, pemetrexed/carboplatin; P/Cis, pemetrexed/cisplatin.

© Translational Lung Cancer Research. All rights reserved.

Table 3 A summary of ctDNA profiles with mutation detection in recruited lung cancer patients at three time points at baseline, stable disease and disease progression

ctDNA mutation levels	Baseline	Stable disease	Disease progression
Any detectable mutations in ctDNA, n (%)*			
Yes	13 (65)	7 (35)	16 (80)†
No	7 (35)	13 (65)	4 (20)
Any *EGFR* mutations in ctDNA, n (%)*			
Yes	8 (40)	4 (20)	11 (55)†
No	12 (60)	16 (80)	9 (45)
Any *TP53* mutations in ctDNA, n (%)*			
Yes	8 (40)	4 (20)	10 (50)
No	12 (60)	16 (80)	10 (50)
ctDNA levels, median [IQR]‡			
Number of mutant molecules/mL	7.8 [0, 47.6]	0 [0, 11.4]	24.7 [1.5, 449.2]†§
Number of *EGFR* mutant molecules/mL	0 [0, 21.9]	0 [0, 0]	1.7 [0, 254.8]†§
Number of *TP53* mutant molecules/mL	0 [0, 19.3]	0 [0, 5.6]	7.0 [0, 263.8]†§

*, Paired comparison of ctDNA detection frequencies between two time points by exact binomial McNemar's test; †, P<0.05 for comparing ctDNA at stable disease *vs.* progression; ‡, Paired comparison of ctDNA quantities between two time points by Wilcoxon signed rank test; §, P<0.05 for comparing ctDNA at baseline *vs.* progression. ctDNA, circulating tumor DNA; *EGFR*, epidermal growth factor receptor; IQR, inter-quartile range.

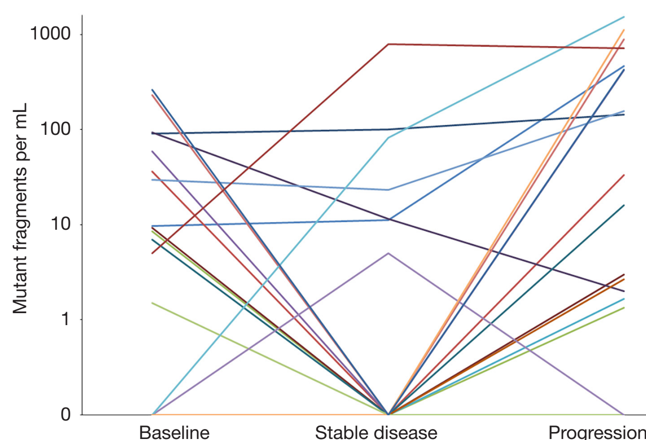

Figure 1 Quantification of mutant fragments expressed as amount of ctDNA per mL of plasma in the 20 lung cancer patients included at three time points showing the changes in the quantity of ctDNA changes in the treatment time course. ctDNA, circulating tumor DNA.

of treatment, levels decreased during stable disease and then increased again when disease progressed. Multi-gene ctDNA assessment also revealed mutations acquired and accumulated during anti-cancer treatment and reflected the underlying heterogeneous tumor biological characteristics.

Previous cross-sectional studies had found ctDNA levels to be positively associated with tumor stage, tumor burden, or tumor volume of lung cancer (8,10,22). In a study using CAPP-Seq for 139 genes, ctDNA detection increased from 50% in stage I NSCLC tumors to 100% among stage II–IV patients, and ctDNA levels correlated with tumor volume as measured by imaging (R^2=0.89) (8). Because of the cross-sectional nature of these studies, they were not able to evaluate whether ctDNA changed with the evolution of tumor characteristics over time or with course of treatment. On the other hand, longitudinal studies on the utility of ctDNA in monitoring tumor status were mostly done with testing of *EGFR* gene only. In general, these studies showed that increased levels of plasma-mutant *EGFR* and detection of specific *EGFR* mutations in ctDNA at baseline were associated with poorer response to EGFR-TKI and poor progression-free and overall survivals (9,12,23). In serial samples, the levels of ctDNA containing *EGFR* mutations usually declined after the start of treatment (11,24). Only a few longitudinal studies had used a multi-gene panel for ctDNA analysis (25,26). In a study that utilized a similar gene

© Translational Lung Cancer Research. All rights reserved.

Table 4 A table showing the within-subject detection of ctDNA and comparison of mutation pattern changes at the times of stable disease and disease progression, with highlights of changes in mutations detected in *EGFR* and *TP53* genes at disease progression

Pattern of ctDNA mutation changed between stable disease and progression			Number of individuals (%) with the corresponding ctDNA pattern change at disease progression		
ctDNA pattern	Stable disease	Progression	Any mutations in 64 genes	Mutations in *EGFR*	Mutations in *TP53*
A	No	No	3 (15%)	9 (45%)	9 (45%)
B	No	Yes	10 (50%)	7 (35%)	7 (35%)
C	Yes	No	1 (5%)	0 (0%)	1 (5%)
D	Yes	Yes	6 (30%)	4 (20%)	3 (15%)

ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor.

panel and sequencing method as our study, it was reported that ctDNA reflected tumor load over time. Among 12 patients who had radiographic response to target therapy, their mutant allele concentrations in ctDNA decreased from an average of 10.8% at baseline to 0.2% at a median time of 19 days after treatment initiation. Contrarily, among five patients who did not respond to treatment and had radiographic progressive disease, they showed a relatively high average level of mutant allele fraction at baseline (14.2%) and a modest variation after initiation of therapy (11.8%) were found (26). Our longitudinal study, nevertheless, was more advantageous to demonstrate the utility of ctDNA in monitoring tumor status than previous studies—all our 20 subjects had completed follow-up from baseline, through stable disease, to disease progression, and hence our data showed within-subject variations in ctDNA pattern over the entire course of treatment.

To date, available data support the potential utility of ctDNA as a tumor biomarker for monitoring treatment response and tumor burden over time, particularly when repeat biopsy is not feasible. One question of interest is whether testing for mutations in multiple genes is advantageous over a single gene approach. Our data showed that even in NSCLC patients with a high prevalence of *EGFR* mutations, testing for multiple genes in ctDNA could help to identify additional patients who had disease progression. Not all mutations detected in ctDNA are actionable or have clinical significance, but changes in ctDNA pattern and quantitative levels informed by a large gene panel are more sensitive in reflecting tumor load than a single gene analysis. Additional benefits of a multi-gene panel include the potential of identifying actionable targets when more drugs are available and revealing therapy resistance mechanisms (9). *EGFR* mutant patients (Group 3) showed persistent detection of *EGFR* T790M at Osimertinib baseline through stable disease on treatment. Neither patient had new mutations detected at the time of progression. This observation may imply that tumors bearing acquired *EGFR* T790M could continue to evolve and may change acquired resistance pathways, through other mechanisms like epithelial-mesenchymal transition (EMT) rather than tumor mutational changes (27).

Different types of *TP53* mutations were detectable at different time-points in all the 20 subjects (overall 10/20, 50%), although *TP53* mutations appeared to be more prevalent in *EGFR* wildtype subjects on chemotherapy than in patients treated with EGFR-TKI. *TP53* point mutations of various types have been reported to result in loss of tumor suppressor function and promote tumor growth, hence disease progression while on treatment (28,29). The emergence of serial changes in the various different types of *TP53* mutations as well as other non-*EGFR* mutations such as *BRCA1*, *BRCA2*, *MYC* or *MET* mutations along treatment course probably reflected the evolution of tumor heterogeneity. The contributions of these non-*EGFR* mutations towards development of acquired drug resistance, regardless of whether the tumor initially carried sensitizing *EGFR* mutations or not, deserves further investigation (30).

Our study is one of the few in lung cancer literature that assessed serial measurements of ctDNA for a panel of genes. The results provided support for application of serial monitoring of ctDNA mutations in lung cancer patients, especially those on *EGFR* targeted therapies, and such serial monitoring may allow for discovery of new acquired mutations that could be of therapeutic or prognostication importance. However, this study comprised of a small sample size and lacked a time-to-event outcome, as the level of ctDNA might have started to rise well before clinical progression based on RECIST criteria. As such, we were not able to estimate how much sooner ctDNA could

© Translational Lung Cancer Research. All rights reserved.

Table 5 A table listing the serial changes of ctDNA mutations detected along treatment courses in all the 20 recruited subjects

Patients	Tissue *EGFR* mutation at baseline	Tissue *EGFR* T790M detection at re-biopsy (disease progression)	ctDNA mutation detected		
			At baseline (%)	At stable disease (%)	Upon disease progression (%)
Group 1: disease progression with *EGFR* T790M (on first line *EGFR*-TKI: either gefitinib/erlotinib/afatinib)					
1	*EGFR* exon 19 E746_A750del	Not done	*EGFR* exon 19 E746_A750del (2.98)	No mutation detected	*EGFR* exon 19 E746_A750del (3.68)
			–		*EGFR* T790M (2.26)
			TP53 G245V (4.25)		*TP53* G245V (3.45)
2	*EGFR* exon 19 E746_A750del	Not done	–	*EGFR* exon 19 E746_A750del (22.51)	*EGFR* exon 19 E746_A750del (29.07)
				EGFR T790M (12.23)	*EGFR* T790M (14.61)
			RNF43 E380D (0.34)	–	–
			ERBB2 D1048Y (0.42)	–	–
			–	*BRCA1* K1290I (0.23)	*BRCA2* C1573 (0.49)
				MTOR R1987Q (0.11)	–
				TP53 R249S (0.07)	–
				–	*MET* Y234F (0.21)
					MET Y291F (0.25)
					MYC Y192N (0.13)
3	*EGFR* exon 19 E746_A750del	*EGFR* exon 19 E746_A750del	*EGFR* exon 19 E746_A750del (1.7)	No mutation detected	*EGFR* exon 19 E746_A750del (15.83)
		EGFR T790M	–		*EGFR* T790M (0.08)
		–	*TP53* R248W (1.84)		*TP53* R248W (11.93)
			–		*MET* L674F (0.11)
			–		–
4	*EGFR* L858R	Not done	*EGFR* L858R (11.19)	No mutation detected	*EGFR* L858R (16.05)
			–		*EGFR* T790M (4.58)
			TP53 M246del (10.84)		*TP53* M246del (18.69)
			RET E732K (0.24)		–
			–		*ALK* W614 (0.12)
			–		*AR* E323K (0.15)

Table 5 (*ocntinued*)

© Translational Lung Cancer Research. All rights reserved.

Table 5 (*continued*)

Patients	Tissue *EGFR* mutation at baseline	Tissue *EGFR* T790M detection at re-biopsy (disease progression)	ctDNA mutation detected		
			At baseline (%)	At stable disease (%)	Upon disease progression (%)
5	*EGFR* L858R	Not done	No mutation detected	*EGFR* L858R (9.2)	*EGFR* L858R (18.95)
				–	*EGFR* T790M (0.14)
Group 2: disease progression appeared independent of *EGFR* T790M (on first line *EGFR*-TKI: either gefitinib/erlotinib/afatinib)					
6	*EGFR* exon 19 E746_A750del	Not done		No mutation detected	*EGFR* exon 19 E746_A750del (0.14)
			KRAS D33H (0.38)		*KRAS* D33H (0.29)
			PTCH1 E1242K (0.22)		*PTCH1* E1242K (0.15)
7	*EGFR* exon 19 E746_A750del	Not done	*EGFR* exon 19 E746_A750del (13.2)	No mutation detected	No mutation detected
			TP53 V272L (9.38)		
8	*EGFR* exon 19 E746_A750del	Not done	No mutation detected	RNF43 Q543* (0.43)	No mutation detected
9	*EGFR* exon 19 E746_A750del	Not done	No mutation detected	No mutation detected	*CD274* M267V (0.49)
10, 11	*EGFR* exon 19 E746_A750del	Not done	No mutation detected	No mutation detected	No mutation detected
12	*EGFR* exon 19 E746Vfs*16	Not done	No mutation detected	No mutation detected	*EGFR* exon 19 deletion E746Vfs*16 (31.04)
	EGFR exon 19 insertion L747Ffs*20				*EGFR* exon 19 insertion L747Ffs*20 (33.28)
	–				*BRCA1* D1065N (0.71)
	–				*TP53* T155L (15.42)
	–				*TP53* F109L (13.3)
13	*EGFR* exon 19 L747_E749del	Not detected	*EGFR* exon 19 L747_E749del (0.32)	No mutation detected	–
			EGFR exon 19 E749Q (0.38)		–
			EGFR exon 19 A750P (0.38)		–
			RB1 R251* (0.62)		–
			–		*TP53* M237Cfs10 (0.58)
14	*EGFR* L858R	Not done	*EGFR* L858R (1.43)	No mutation detected	*EGFR* L858R (0.21)

Table 5 (*continued*)

© Translational Lung Cancer Research. All rights reserved.

Table 5 (*continued*)

Patients	Tissue *EGFR* mutation at baseline	Tissue *EGFR* T790M detection at re-biopsy (disease progression)	ctDNA mutation detected		
			At baseline (%)	At stable disease (%)	Upon disease progression (%)
Group 3: *EGFR* T790M on osimertinib [on second line EGFR-TKI (osimertinib) after first line EGFR-TKI failed]					
15	*EGFR* L858R	*EGFR* L858R; *EGFR* T790M (re-biopsy after first line *EGFR*-TKI failure)	*EGFR* L858R (8.06)	*EGFR* L858R (0.89)	*EGFR* L858R (0.31)
			EGFR T790M (1.92)	*EGFR* T790M (0.17)	–
			TP53 L35* (2.23)	–	–
16	*EGFR* L858R	*EGFR* L858R; *EGFR* T790M (re-biopsy after first line *EGFR*-TKI failure)	*EGFR* L858R (6.38)	*EGFR* L858R (5.78)	*EGFR* L858R (4.79)
			EGFR T790M (0.66)	*EGFR* T790M (0.67)	*EGFR* T790M (0.80)
Group 4: *EGFR* wildtype [on first line platinum-based chemotherapy (pemetrexed-platinum)]					
17	*EGFR* WT	Not done	*CDKN2A* L63P (2.71)	–	*CDKN2A* L63P (5.27)
			TP53 E294* (3.18)	*TP53* E294* (1.37)	*TP53* E294* (5.07)
			–	*TP53* Y205D (0.22)	*TP53* Y205D (1.04)
			–	*TP53* H193R (0.25)	*TP53* H193R (0.17)
			–	–	*TP53* C238Y (0.12)
18	*EGFR* WT	Not done	*TP53* R267G (1.58)	*TP53* R267G (1.72)	*TP53* R267G (22.96)
19	*EGFR* WT	Not done	No mutation detected	No mutation detected	*TP53* R158H (0.24)
					TP53 R175H (0.15)
20	*EGFR* WT	Not done	*TP53* R248W (0.12)	No mutation detected	No mutation found

The 20 subjects were divided into Group 1: subjects who received first or second generation EGFR-TKI but treatment failed with disease progression and development of EGFR T790M mutations; Group 2: subjects who received first or second generation EGFR-TKI but treatment failed with disease progression but no development of *EGFR* T790M mutations; Group 3: subjects recruited after they have failed primary or secondary *EGFR*-TKI and have their ctDNA followed during the course of osimertinib; and Group 4: *EGFR* wildtype subjects who received systemic chemotherapy. *, stop codon. ctDNA, circulating tumor DNA; *EGFR*, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; WT, wildtype.

© Translational Lung Cancer Research. All rights reserved.

detect disease progression than standard-of-care radiologic imaging, as suggested by some small-scale studies (12,25,26). Future studies need to determine the frequency of ctDNA testing, define the extent of increase in ctDNA, in terms of total quantitative levels or allele fractions of specific genes, to define disease progression, and identify ctDNA biomarkers for early detection of disease progression.

Conclusions

In summary, our results suggest that ctDNA levels in serial blood samples reflect tumor burden over time, and a multi-gene panel would be a more sensitive way of detecting lung cancer disease progression or biomarkers for drug resistance than a single gene approach. The detection of additional oncogenic mutations, some of which are cumulative, or their disappearance in plasma during treatment course, reflects underlying evolution of tumor heterogeneity.

Acknowledgments

The authors thank Ms. Lucinda Wong for data processing and Ms. Peony Chong for data collection and management.
Funding: The research project in this manuscript was supported by the Lee and the Ho Families Respiratory Research Fund.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at http://dx.doi.org/10.21037/tlcr-20-675

Data Sharing Statement: Available at http://dx.doi.org/10.21037/tlcr-20-675

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr-20-675). 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 protocol was approved by the Ethics Committee of the University of Hong Kong and Hong Kong Hospital Authority Hong Kong West Cluster Institutional Review Board (IRB Reference Number UW 16-104). Informed consent was obtained from all the participants.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Arbour KC, Riely GJ. Systemic therapy for locally advanced and metastatic non-small cell lung cancer: a review. JAMA 2019;322:764-74.
3. Zhou J, Kulasinghe A, Bogseth A, et al. Isolation of circulating tumor cells in non-small-cell-lung-cancer patients using a multi-flow microfluidic channel. Microsyst Nanoeng 2019;5:8.
4. Kapeleris J, Kulasinghe A, Warkiani ME, et al. The prognostic role of circulating tumor cells (CTCs) in lung cancer. Front Oncol 2018;8:311.
5. Kulasinghe A, Kapeleris J, Kimberley R, et al. The prognostic significance of circulating tumor cells in head and neck and non-small-cell lung cancer. Cancer Med 2018;7:5910-9.
6. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014;32:579-86.
7. Lam DC, Tam TC, Lau KM, et al. Plasma EGFR mutation detection associated with survival outcomes in advanced-stage lung cancer. Clin Lung Cancer 2015;16:507-13.
8. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014;20:548-54.
9. Esposito Abate R, Pasquale R, Fenizia F, et al. The role of circulating free DNA in the management of NSCLC. Expert Rev Anticancer Ther 2019;19:19-28.
10. Winther-Larsen A, Demuth C, Fledelius J, et al.

Correlation between circulating mutant DNA and metabolic tumour burden in advanced non-small cell lung cancer patients. Br J Cancer 2017;117:704-9.
11. Imamura F, Uchida J, Kukita Y, et al. Early responses of EGFR circulating tumor DNA to EGFR tyrosine kinase inhibitors in lung cancer treatment. Oncotarget 2016;7:71782-9.
12. Taus Á, Camacho L, Rocha P, et al. Dynamics of EGFR mutation load in plasma for prediction of treatment response and disease progression in patients with EGFR-mutant lung adenocarcinoma. Clin Lung Cancer 2018;19:387-94.e2.
13. Yanagita M, Redig AJ, Paweletz CP, et al. A prospective evaluation of circulating tumor cells and cell-free DNA in EGFR-mutant non-small cell lung cancer patients treated with erlotinib on a phase II trial. Clin Cancer Res 2016;22:6010-20.
14. Mayo-de-Las-Casas C, Garzón Ibáñez M, Jordana-Ariza N, et al. An update on liquid biopsy analysis for diagnostic and monitoring applications in non-small cell lung cancer. Expert Rev Mol Diagn 2018;18:35-45.
15. Thompson JC, Yee SS, Troxel AB, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. Clin Cancer Res 2016;22:5772-82.
16. 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.
17. Phallen J, Sausen M, Adleff V, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med 2017;9:eaan2415.
18. Jones S, Anagnostou V, Lytle K, et al. Personalized genomic analyses for cancer mutation discovery and interpretation. Sci Transl Med 2015;7:283ra53.
19. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477-87.
20. McKerrell T, Park N, Moreno T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. Cell Rep 2015;10:1239-45.
21. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 2014;20:1472-8.
22. Zhu YJ, Zhang HB, Liu YH, et al. Quantitative cell-free circulating EGFR mutation concentration is correlated with tumor burden in advanced NSCLC patients. Lung Cancer 2017;109:124-7.
23. Karachaliou N, Mayo-de las Casas C, Queralt C, et al. Association of EGFR L858R mutation in circulating free DNA with survival in the EURTAC trial. JAMA Oncol 2015;1:149-57.
24. Sorensen BS, Wu L, Wei W, et al. Monitoring of epidermal growth factor receptor tyrosine kinase inhibitor-sensitizing and resistance mutations in the plasma DNA of patients with advanced non-small cell lung cancer during treatment with erlotinib. Cancer 2014;120:3896-901.
25. Chaudhuri AA, Chabon JJ, Lovejoy AF, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov 2017;7:1394-403.
26. Phallen J, Leal A, Woodward BD, et al. Early noninvasive detection of response to targeted therapy in non-small cell lung cancer. Cancer Res 2019;79:1204-13.
27. Zhang Z, Yang S, Wang Q. Impact of MET alterations on targeted therapy with EGFR-tyrosine kinase inhibitors for EGFR-mutant lung cancer. Biomark Res 2019;7:27.
28. Blandino G, Di Agostino S. New therapeutic strategies to treat human cancers expressing mutant p53 proteins. J Exp Clin Cancer Res 2018;37:30.
29. Matsumura Y, Owada-Ozaki Y, Suzuki H. Significance of testing for TP53 gene mutations in lung adenocarcinoma using targeted gene sequencing. J Thorac Dis 2018;10:S4147-50.
30. Lim ZF, Ma PC. Emerging insights of tumor heterogeneity and drug resistance mechanisms in lung cancer targeted therapy. J Hematol Oncol 2019;12:134.

Cite this article as: Ho GYF, Wang T, Kwok HH, Rasul R, Peila R, Guzman M, Ip MSM, Lam DCL. Longitudinal multi-gene panel assessment of circulating tumor DNA revealed tumor burden and molecular characteristics along treatment course of non-small cell lung cancer. Transl Lung Cancer Res 2020;9(5):1873-1884. doi: 10.21037/tlcr-20-675

© Translational Lung Cancer Research. All rights reserved.