Kinetics of Plasma cfDNA Predicts Clinical Response in Non-Small Cell Lung Cancer Patients

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INTRODUCTION

Lung cancer is the most common cancer worldwide with a high morbidity (11.6% of the total cases) and mortality (18.4% of the total cancer deaths)[1]. In 2018 there was an estimated 2.1 million new cases and 1.8 million deaths, representing 1 in 5 cancer deaths[1]. The main histological categories of lung cancer are non–small cell lung cancer (NSCLC, 85% of patients) and small cell lung cancer (SCLC,15%)[2]. NSCLC consists of several subtypes, predominantly lung adenocarcinoma (LUAD, 40%), lung squamous-cell carcinoma (LUSC, 25-30%), and large-cell carcinoma (LULC, 5 10%)[3]. Genotyping tumor tissue with next generation sequencing (NGS) represents an effective way to capture actionable genetic alterations as potential biomarkers in clinical oncology[10]. However, tissue biopsy may be limited due to insufficiency of sampling or inaccessibility for biopsy and only 25-50% of lung cancer patients have sufficient tissues for genotyping[11]. However, the clinical value of cfDNA application in NSCLC has not been well-established due to inconsistent reports[33-36]. Our recent study confirmed that plasma cfDNA concentration was significantly increased in patients with advanced gastric cancer and can serve as a potential biomarker for chemotherapy monitoring[37]. Here we sought to investigate the predictive value of cfDNA in efficacy of treatment and prognosis for NSCLC patients with chemotherapy, targeted therapy, immunotherapy or combined treatment.

METHOD

Assessment of Peripheral cfDNA

All patients were subjected to peripheral blood samples collection before (baseline) and after (post-therapy) the first target cycle of chemotherapy. The cfDNA concentration was determined by QuantiDNA™ Direct cfDNA Test Kit (DiaCarta. Inc., CA, USA) according to the manual and our previous publication[37]. The method is based on a patent technology with convenience and cost-effective. In brief, 2-3 ml peripheral blood was drawn and subjected to 10 minutes centrifugation in 1900 xg for plasma isolation. The plasma sample were centrifuged 10 min at 13000 x g in 4C. Plasma samples were first diluted at 10-fold by adding 10 µL of plasma into 90 µL of 1xPBS (pH7.4). Diluted plasma samples were heated at 95°C for 5 minutes for DNA denaturation and then immediately chilled on ice. Next, 20 µL of prepared plasma samples were loaded to a 96-well microplate (Greiner Bio-One, USA) together with 80 µL of Working Probe Solution containing Lysis buffer, DNA probe set, Blocking reagent, and Proteinase K. The microplate was incubated at 55°C overnight (15-18 hours) with shaking at 600rpm followed by sequential hybridization with Pre-amplifier probe (55°C 40 minutes), Amplifier probe (55°C 40 minutes), Label probe (50°C 40 minutes), and SAPE (Streptavidin, R-Phycoerythrin Conjugate) (37°C 30 minutes). All of the probes were manufactured by DiaCarta, Inc (CA, USA). Lastly, plate reading and data acquisition were performed on Luminex MAGPIX instrument with xPONENT software (Luminex, USA).

Statistical Analysis

We stratified the treatment evaluation by dug combination regiment which consisted of four groups: 1) chemotherapy only; 2) chemotherapy plus VEGF/VEGF receptor inhibitors (VEGFIs); 3) chemotherapy plus tyrosine kinase inhibitors (TKIs); and 4) chemotherapy plus immune checkpoint inhibitors (ICIs). The primary outcome was 1) progression-free survival (PFS) /disease-free survival (DFS); and secondary outcomes was 2) objective response ratio (ORR), defined as the proportion of CR and PR in all subjects. An initial model without interactions was used to identify the prognostic impact of baseline cfDNA, post-therapy cfDNA, and the cfDNA ratio respectively. Other demographic or clinical factors which may be associated with PFS/DFS were also evaluated via univariate Cox model separately and multivariate Cox model together. Survival curves were plotted by the Kaplan-Meier method with R package 'survival' and 'survminer'.

RESULTS

Pathological and Demographic Characteristics

The pathological and demographic characteristics of the 154 patients were summarized (Table 1).

Table 1. Baseline Characteristics of Patients

Characteristics	Number (N=154)	Proportion
Sex & Age		
Male	107	69.5%
Female	47	30.5%
Median age (yr)	62（34-79）	
Histology		
LUAD	128	83.1%
LUSC	26	16.9%
Clinical Stage		
I	2	1.3%
II	4	2.6%
III	19	12.3%
IV	126	81.8%
ECOG (Eastern Cooperative Oncology Group, baseline)		
0	6	3.9%
1	72	46.8%
2	1	0.7%
NA	75	48.7%

Peripheral cfDNA Baseline Correlates with Tumor Burden

Overall, a weakly positive correlation between TB and cfDNA was observed at baseline (N=80, Pearson's coefficient = 0.24; 95% confidence interval (CI): 0.017-0.433; P = 0.03, Fig 1A), while no significant correlation was found for post-chemotherapy (N=47, Pearson's coefficient = 0.124; 95% CI: -0.169-0.397; P = 0.4, Fig 1B).

Fig 1

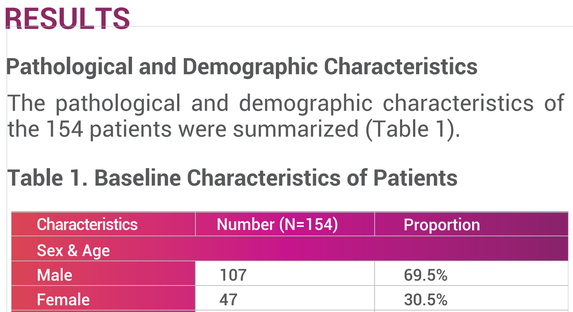

Fig 1. **Scatter plot showing a weakly positive correlation of baseline cfDNA with baseline tumor burden.** Tumor burden was evaluated by Response Evaluation Criteria in Solid Tumors, version 1.1. cfDNA was quantified by QuantiDNA Direct cfDNA Test Kit (Diacarta. Inc., CA, USA) according to the manual both (A) at baseline and (B) post-chemotherapy. We selected those whose interval between cfDNA test and TB evaluation was within 7 days, so 80 cases were qualified (A) at baseline, and 47 cases were qualified (B) post-chemotherapy. A weakly positive correlation between TB and cfDNA was observed at baseline (N=80, Pearson's coefficient = 0.24; 95% CI: 0.017-0.433; P = 0.03), while no significant correlation was found for post-chemotherapy (N=47, Pearson's coefficient = 0.124; 95% CI: -0.169-0.397; P = 0.4).

Plasma cfDNA Relates to Objective Response Rate (ORR) and Progression-Free Survival (PFS)/ Disease-Free Survival (DFS)

Overall, the responsive group trended toward higher baseline cfDNA (median 17.68 ng/mL) than the non-responsive (median 13.70 ng/mL) (P=0.058, Wilcoxon rank-sum test, Fig 2A). However, we found no significant difference in post-chemotherapeutic cfDNA between the two (P=0.6, Wilcoxon rank-sum test, Fig 2B), although the median post-chemotherapeutic cfDNA in the responsive (17.18 ng/mL) was modestly lower than that of the non-responsive (19.15 ng/mL). Notably we found a significantly lower ratio in the responsive group (median 0.87) than that of the non-responsive (median 1.21) (P=0.012, Wilcoxon rank-sum test, Fig 2C). These data suggested that cfDNA can be used to discriminate responsive patients from the non-responsive well, especially with cfDNA ratio which reflected the dynamic change of plasma cfDNA.

Significantly improved PFS benefit was observed for Ratio_low (HR: 0.54 (95% CI: 0.29-1.01); Log-rank test, P=0.05, Fig 3A) compared with Ratio_high, while no significant difference was found between Baseline_low and Baseline_high group (Log-rank test, P=0.86, Fig 3B) and between Post-chemotherapy_low and Post-chemotherapy_high group (Log-rank test, P=0.57, Fig 3C). After a median follow-up of 6.4 months, the median PFS of Ratio_low group was 6.1 months which was 2 months longer than that of Ratio_high group (4.1 months).

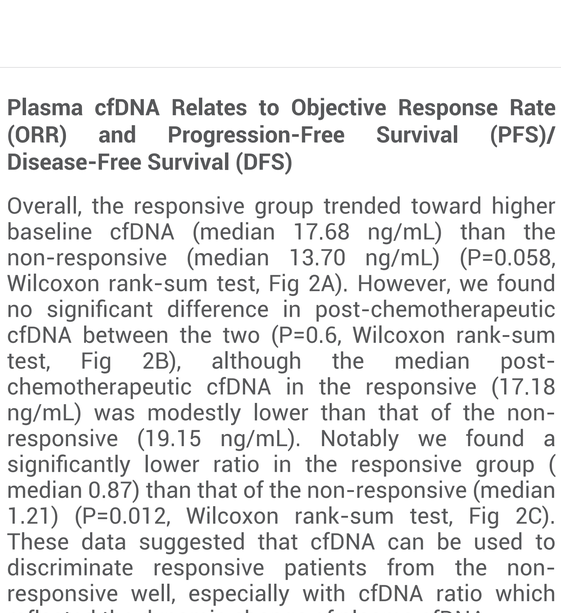

Fig 2. **Comparison of cfDNA levels and cfDNA ratio between the responsive group and non-responsive group**. Boxplots from top to bottom showed the baseline value (A), post-therapy value (B), and ratio value (C) of cfDNA respectively in both the responsvethe two was estimated by Wilcoxon test.

Fig 3

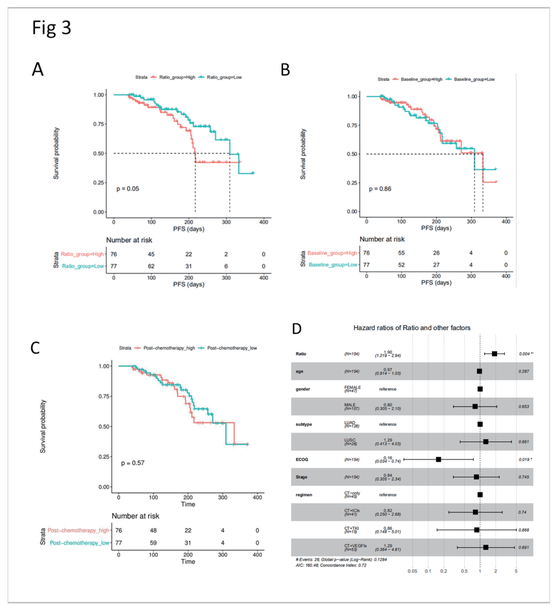

Fig 3. **Progression-free Survival (PFS)/disease free survival (DFS) in the overall cohort (N=154)**. Kaplan-Meier curves for comparisons of progression-free survival between (A) high cfDNA Ratio and low cfDNA Ratio groups, (B) high cfDNA baseline and low cfDNA baseline groups, (C) high post-therapy cfDNA and low post-therapy cfDNA groups (cut-values were set as median value), respectively. (D) The hazard ratios of cfDNA ratio and other important clinical factors by multivariate Cox model. Cut-values were set as the median value of the overall cohort, respectively. PFS/DFS was assessed according to Response Evaluation Criteria in Solid Tumors, version 1.1 through investigators' review, and tick marks represent data censored at the last time the patient was known to be alive and without disease progression.

We compared the demographic (age and gender), pathological (subtype, stage, and ECOG scores), and therapeutic (therapy regimens) characteristics between Ratio_low and Ratio_high group, and found no significant difference (chi-square test) in all these factors (Table 2).

Table 2. Comparisons between Ratio_high group and Ratio low group

Ratio	High	Low	P
n	77	77	
age (mean (SD))	60.30(8.68)	61.65(9.16)	0.349
gender = MALE (%)	53(68.8)	54(70.1)	1
subtype = LUSC (%)	9(11.7)	17(22.1)	0.132
ECOG (mean (SD))	0.95(0.32)	0.93(0.27)	0.721
Stage (mean (SD))	3.75(0.59)	3.81(0.51)	0.483
response (%)			0.157
PD	17(22.4)	11(14.3)	
PR	22(28.9)	33(42.9)	
SD	139.59(76.16)	33(42.9)	
PFS/days (mean (SD))	139.59(76.16)	179.08(83.75)	0.003
regimen (%)			0.227
CT-only	25(32.5)	20(26.0)	
CT+ICIs	17(22.1)	24(31.2)	
CT+TKIs	5(6.5)	10(13.0)	
CT+VEGFIs	30(39.0)	23(29.9)	

Stratification Analysis by Subtype

For the LUAD group (N=128), we found a significantly improved PFS benefit for the Ratio_low group (HR: 0.42 (95% CI: 0.20-0.86); P=0.015, Fig 4A) compared with Ratio_high group. The median PFS of Ratio_low group was 6.3 months which was 2.1 months longer than that of Ratio_high group (4.2 months). Additionally, ORR of Ratio_low group (43.3%) was also higher than that of the Ratio_high group (29.4%). no significant difference of PFS was found between Baseline_low and Baseline_high group (Fig 4B) or between Post-chemotherapy_low and Post-chemotherapy_ high group (Fig 4C) when stratified by LUAD and LUSC, respectively.

Fig 4

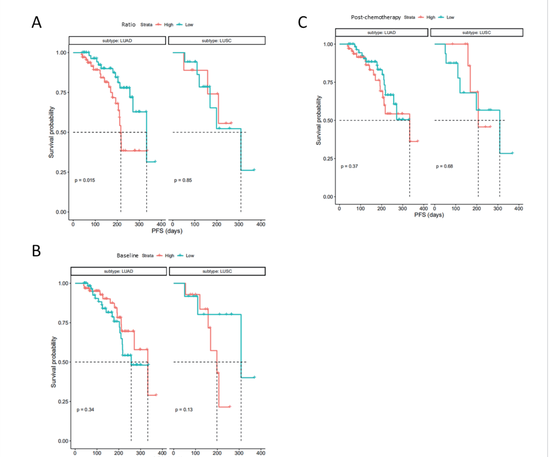

Fig 4. **Progression-free Survival (PFS)/disease free survival (DFS) analysis by pathological subtype.** Stratification analysis of PFS/DFS by pathological subtype (LUAD, N=128 and LUSC, N=26) (A) high cfDNA Ratio and low cfDNA Ratio groups, (B) high cfDNA baseline and low cfDNA baseline groups, (C) high post-therapy cfDNA and low post-therapy cfDNA groups (cut-values were set as median value), respectively. PFS/DFS was assessed according to Response Evaluation Criteria in Solid Tumors, version 1.1 through investigators' review, and tick marks represent data censored at the last time the patient was known to be alive and without disease progression.

Stratification Analysis by Treatment

Only the Ratio_low group of patients received chemotherapy plus VEGFIs treatment showed significantly prolonged PFS compared to those in Ratio_high group (HR: 0.23, 95% CI: 0.06-0.88; P=0.02, Fig 5A).

Fig 5

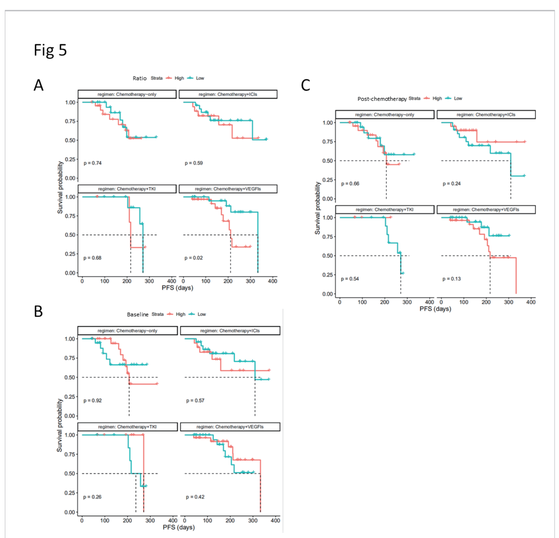

Fig 5. **Progression-free Survival (PFS)/disease free survival (DFS) in subgroups by therapy regimen.** Stratified analysis of Kaplan-Meier curves by therapy regimen (chemotherapy alone (N=45), chemotherapy plus TKIs (N=15), chemotherapy plus VEGFIs (N=53), and chemotherapy plus ICIs (N=41)) for comparisons of PFS/DFS between (A) high cfDNA Ratio and low cfDNA Ratio groups, (B) high cfDNA baseline and low cfDNA baseline groups, (C) high post-therapy cfDNA and low post-therapy cfDNA groups (cut-values were set as median value), respectively. PFS/DFS was assessed according to Response Evaluation Criteria in Solid Tumors, version 1.1 through investigators' review, and tick marks represent data censored at the last time the patient was known to be alive and without disease progression.

CONCLUSION

- We confirmed that the kinetics of plasma cfDNA (Ratio, post-/pre-) is well correlated with clinical response (ORR) and progression free survival (PFS) at least in chemotherapy with VEGF inhibito targeted therapy.
- A positive correlation between tumor burden and cfDNA baseline in NSCLC.
- Ratio_low group has a significantly improved PFS with 2 months longer than that of Ratio_high group (4.1 months).
- The Ratio-low group enjoyed an ORR more than 1.5 times higher than that of Ratio-high group (42.8% vs 28.5%) regardless of treatment regimen.

ABSTRACT

Tyrosine kinase inhibitors (TKIs), VEGF/VEGF receptor inhibitors (VEGFIs) and immune checkpoint inhibitors (ICIs) have revolutionized the treatment of advanced cancers including non-small-cell lung cancer (NSCLC). This study aims to evaluate the utility of plasma cell-free DNA (cfDNA) as a prognostic biomarker and efficacy predictor of chemotherapy (CT) with or without these precision therapies in NSCLC patients. Peripheral cfDNA levels in 154 NSCLC patients were quantified before and after the first target cycle of chemotherapy. The correlations of cfDNA with tumor burden, clinical characteristics, progression-free survival (PFS)/disease-free survival (DFS), objective response ratio (ORR), and therapy regimens were analyzed respectively. Baseline cfDNA, but not post-chemotherapeutic cfDNA, positively correlates with tumor burden. Notably, cfDNA kinetics (cfDNA Ratio, the ratio of post-chemotherapeutic cfDNA to baseline cfDNA) well distinguished responsive individuals (CR/PR) from the non-responsive (PD/SD). Additionally, cfDNA Ratio was found negatively correlated with PFS in lung adenocarcinoma (LUAD), but not lung squamous-cell carcinoma (LUSC) which may be due to a limited number of LUSC patients in this cohort. LUAD patients with low cfDNA Ratio have prolonged PFS and improved ORR, compared to those with high cfDNA Ratio. When stratified by therapy regimen, the predictive value of cfDNA Ratio is significant in patients with chemotherapy plus VEGFIs, while more patients need be included to validate the value of cfDNA Ratio in other regimens. Thus, the kinetics of plasma cfDNA during chemotherapy may function as a prognostic biomarker and efficacy predictor for NSCLC patients.

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