Cell-free DNA fragmentomes capture response to immuno-radiotherapy in metastatic non-small cell lung cancer

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Introduction

Analysis of plasma cfDNA is increasingly applied to address key challenges across the continuum of cancer caner. In the context of metastatic disease, early assessment of therapy response is critical and can inform treatment optimization. Genome-wide analysis of cfDNA fragments combined with machine learning can determine presence of ctDNA at small concentrations using low input cfDNA and in a cost-efficient manner. Here, we analyze baseline and on-therapy plasma samples from patients with metastatic NSCLC treated with pembrolizumab ± radiotherapy (RT; Huang, Theelen, Belcaid, et al., *Nat Cancer*, 2025, NCT02492568).

Approach

Patient and sample disposition

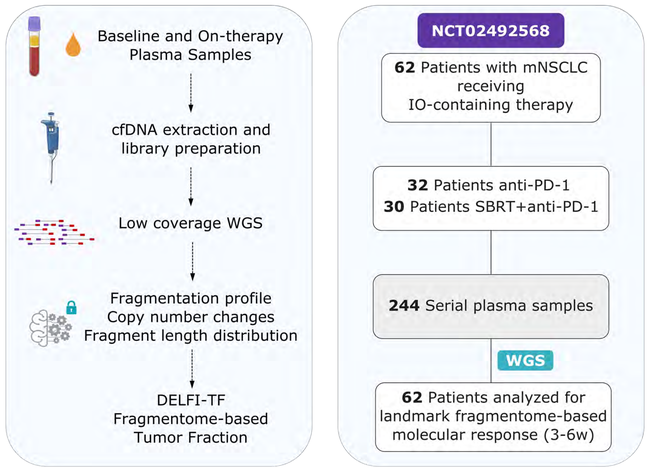

Fig. 1. Approach overview. Plasma samples (n=244) were collected at baseline and on-therapy for 62 patients with metastatic NSCLC treated with pembrolizumab (± RT). cfDNA genomic libraries were analyzed by low pass whole genome sequencing (WGS). The DELFI-TF locked machine learning model, which integrates fragmentation profiles and distribution with plasma aneuploidy was used to estimate cfDNA fragmentome-based tumor fraction (TF).

Results

cfDNA fragmentome DELFI-TF at baseline reflects systemic tumor burden and cancer cell turnover

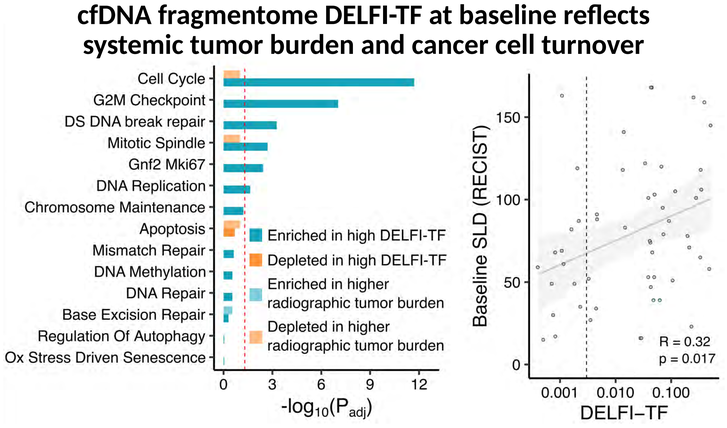

Fig. 2. Association of fragmentome TF with cellular pathways and radiographic tumor burden. Higher DELFI-TF was correlated with increased expression of genes associated with cell cycle progression, while such correlation was absent for higher radiographic tumor burden. Baseline tumor size as characterized by the sum of longest diameters (RECIST1.1) was moderately correlated with DELFI-TF (R=0.32).

Results

Landmark fragmentome molecular response is defined as undetectable DELFI-TF at 3-6 weeks from treatment initiation

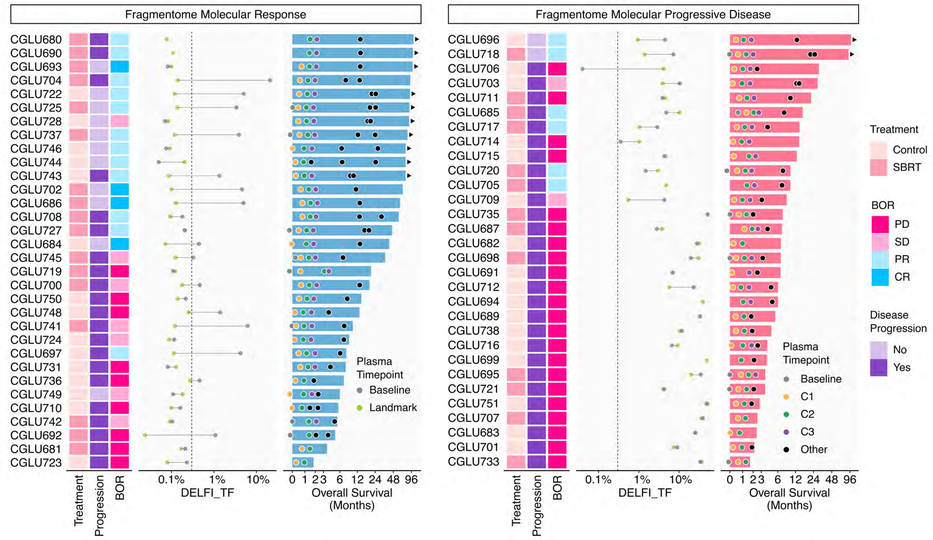

Fig. 3. Cohort characteristics. 62 patients received pembrolizumab alone (51.1%) or following RT (48.4%). The swimmers plot indicates the overall survival as well as the plasma collection schedule; triangles mark censored survival values. Middle panels depict DELFI-TF values for baseline and landmark plasma timepoints.

Differential cfDNA fragmentation patterns at baseline and stratified by fragmentome molecular response (fMR) and molecular progression (fMPD)

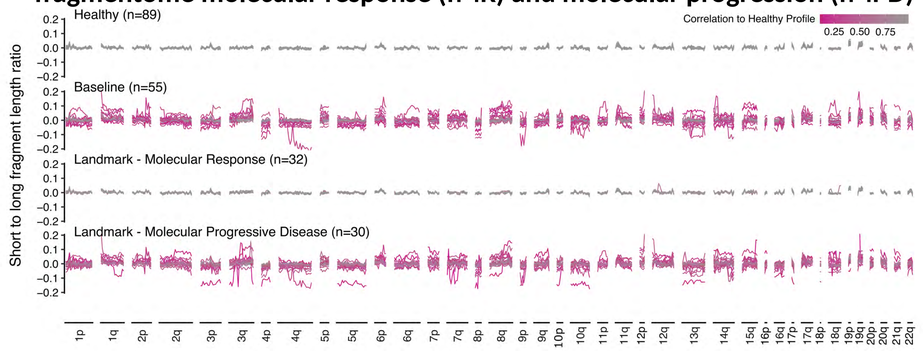

Fig. 4. Genome-wide cfDNA fragmentation profiles. Baseline and on-therapy cfDNA was analyzed to assess short to long fragment ratios in 504 non-overlapping bins across the genome. In patients with fMR, fragmentation patterns resembled non-cancer profiles, while in patients with fMPD, aberrant fragmentation profiles were observed.

Early on-therapy landmark fMR predicts radiographic response

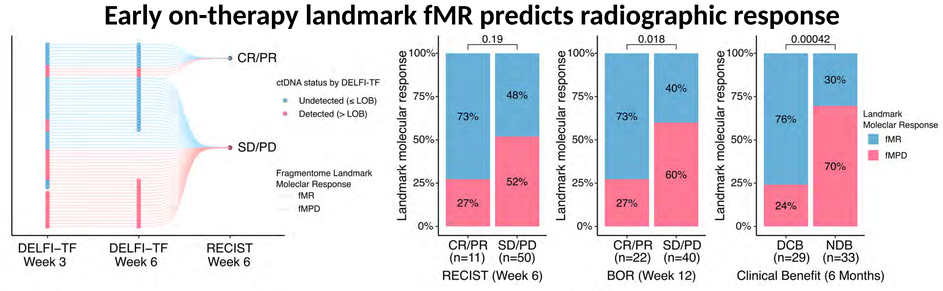

Fig. 5. Correlation of landmark fMR with clinical outcomes. At week 6, 18% patients had radiographic response (CR/PR), while 82% had SD or PD. The CR/PR group was enriched for fMR, while the SD/PD group was highly heterogeneous. fMR was associated with BOR (p=0.018) and clinical benefit (p<0.001).

Fragmentome molecular response predicts progression-free and overall survival

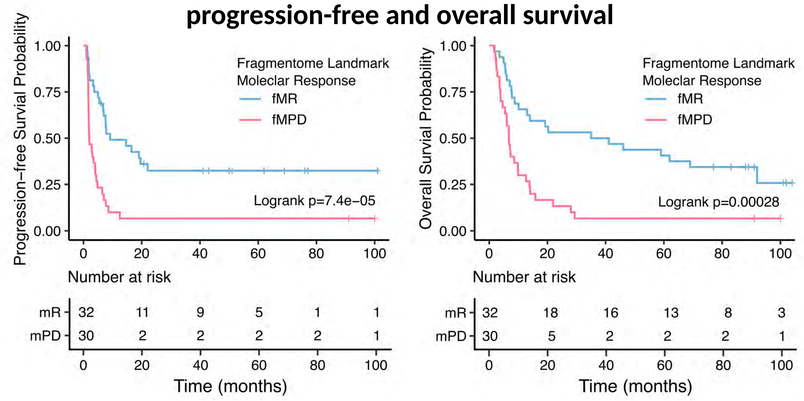

Fig. 6. Prognostication of clinical outcomes by fMR. Landmark fMR was associated with longer PFS (9.2 vs 2 mo, HR=0.32, log-rank P=7.4e-5) and OS (38 vs 6.8 mo, HR=0.35, log-rank P=0.00028).

Fragmentome molecular response is an independent predictor of survival after adjusting for clinical covariates

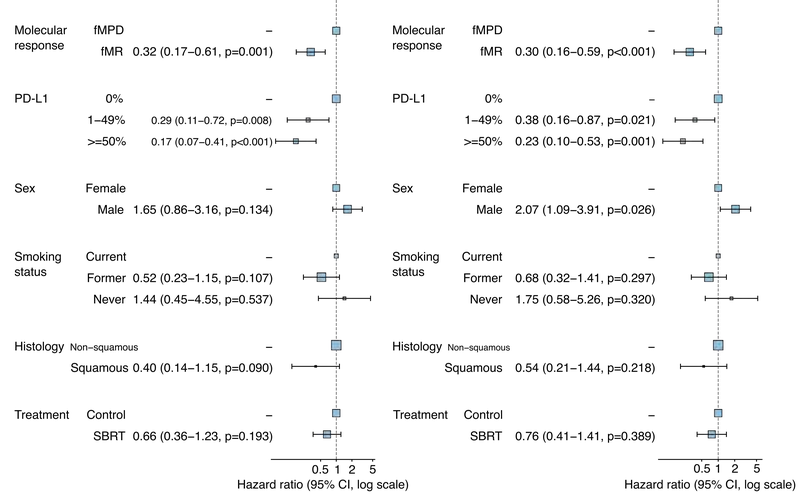

Fig. 7. Independent association of fMR with favorable progression-free and overall survival in multivariate analysis. Fragmentome-based molecular response and higher PD-L1 at baseline were associated with longer progression-free (fMR HR= 0.32, P=0.00055; PD-L1≥50% HR=0.17, P=9.1e-5) and overall survival (fMR HR=0.30, P= 0.00042; PD-L1≥50% HR=0.23, P=0.00056).

Conclusions

Higher levels of DELFI-TF correlate with increased cell turnover and higher overall tumor burden, determined by radiographic assessments. Landmark DELFI-TF based molecular response correlates with radiographic response, as well as progression-free and overall survival. The association of landmark cfDNA fragmentome-based molecular response with survival remains significant after accounting for PD-L1 status and other clinical covariates. Given the cost-effectiveness and logistical benefits of cfDNA fragmentome analyses, fMR shows potential as a clinically viable and readily implementable molecular readout of immunotherapy response in the metastatic setting.

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