Monitoring immunotherapy response using cell-free DNA fragmentomes

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BACKGROUND

- Accurate and timely detection of disease progression in patients receiving immune checkpoint inhibitors (ICIs) is challenging given the lack of reliable biomarkers of clinical response.
- Current targeted next-generation sequencing cell free DNA (cfDNA) assays are costly and rely on prior knowledge of tumor-specific mutations.
- Here, we demonstrate the utility of DELFI Tumor Fraction (DELFI-TF), a tumor- and mutation-independent cfDNA fragmentome approach to monitor immunotherapy response in patients with metastatic non-small cell lung cancer (mNSCLC).

METHODS

- A cohort of 324 longitudinal blood samples were collected from 109 mNSCLC patients treated with ICI-containing regimens (ICI cohort); (Table 1).
- An independent cohort consisting of 47 longitudinal samples obtained from 15 mNSCLC patients undergoing treatment with both immunotherapy and chemotherapy was utilized to show the concordance with MAF and RECIST (Multi-Treatment cohort).
- Plasma-derived cfDNA was processed with whole genome sequencing (WGS) at low coverage (~4x).
- Circulating tumor burden was quantified as the maximum MAF (maxMAF) of tumor-derived variants detected using a 500+ gene panel.
- Matched white blood cells were used to filter out germline and clonal hematopoietic variants.
- DELFI-TF, a random forest regression model trained on MAF data from longitudinal blood samples of stage IV colorectal cancer patients (van 't Erve et. al. 2024), was applied to predict the ctDNA fraction amongst samples.
- The accuracy of DELFI-TF in predicting ICI response was assessed in the ICI and Multi-Treatment cohorts.

RESULTS

- In both the ICI and Multi-Treatment cohorts, DELFI-TF scores were strongly correlated with maxMAF (n=324, r=0.94, p<0.001, Pearson and n=47, r=0.94, p<0.001, Pearson respectively).
- Changes in DELFI-TF and maxMAF at all consecutive timepoints in the ICI cohort were highly correlated (n=215; r=0.9; Pearson).
- DELFI-TF dynamics were consistent with treatment response assessment using imaging.
- Baseline ctDNA levels predicted clinical outcome.
- Landmark molecular response assessment, defined as ctDNA detection above the limit of blank (LOB) at 3 to 9 weeks of ICI initiation, strongly predicted progression-free (PFS) and overall survival (OS).
- LOB estimated as the maximum of 95th percentile of DELFI-TF in 3 non-cancer cohorts.
- Clinical assessment of landmark PFS at 6 months revealed a significant association between ctDNA molecular response and durable clinical benefit (DCB; p < 0.001, Fisher's exact test).
- Patients with molecular response (mR) attained longer PFS and OS (log-rank p<0.001) compared to those in the molecular disease progression (mPD) group.

Characteristic	Number (%)
Participants (samples)	109 (324)
Median Age	68 (36-91)
Sex, n (%)	
Male	59 (54%)
Female	50 (46%)
Stage, n (%)	
Stage III	9 (8%)
Stage IV	100 (92%)
Histology, n (%)	
Adenocarcinoma	84 (77%)
Squamous cell	23 (21%)
Large cell carcinoma	1 (1%)
Unknown histology	1 (1%)
Prior treatment, n (%)	
Yes	72 (66%)
No	37 (34%)
Treatment, n (%)	
Immunotherapy only	80 (73%)
Immunotherapy + chemotherapy	29 (27%)
Clinical Status	
Censored	29 (27%)
Progressive disease	80 (73%)

Table 1. Patient characteristics.

DELFI-TF scores strongly correlate with maxMAF

- DELFI-TF was applied to all longitudinal samples of the ICI and Multi-Treatment cohorts.
- Figure 2 and Figure 3 illustrate the correlation between DELFI-TF and maxMAF in the ICI cohort (n=324, r = 0.94, p < 0.001, Pearson correlation) and the Multi-Treatment cohort (n=47, r = 0.94, p < 0.001, Pearson correlation). Axis are scaled using a square root transformation.

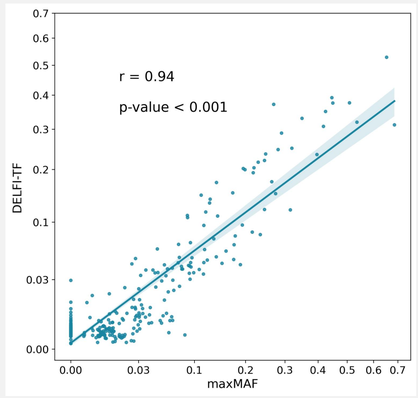

Figure 2. Correlation between DELFI-TF and max MAF in the ICI cohort.

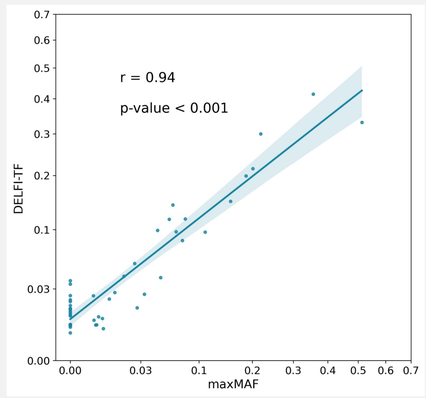

Figure 3. Correlation between DELFI-TF and maxMAF in the Multi-Treatment cohort .

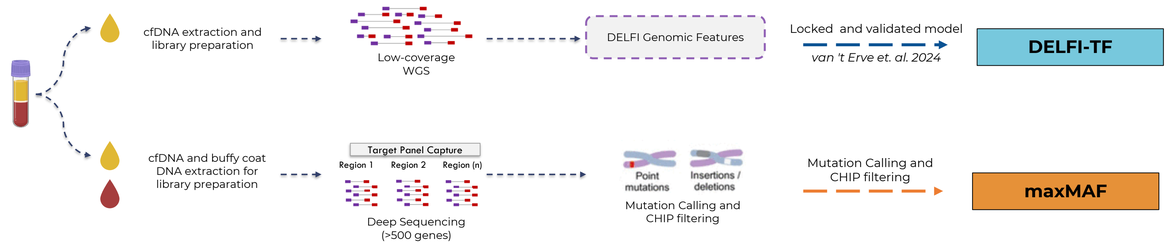

Figure 1. Liquid biopsy analyses performed at baseline and during treatment with ICI evaluated for cell-free DNA fragmentation patterns and mutant allele fraction (MAF).

Conclusions

- DELFI-TF is a cfDNA fragmentome–based tumor- and mutation- independent liquid biopsy approach that reliably and rapidly captures circulating tumor burden.
- Patients with higher fragmentome-TF levels are at higher risk for disease progression and identification of such patients prior to ICI may be informative for risk classification and subsequent intervention.
- DELFI-TF dynamics closely recapitulate radiographic responses by RECIST, which represent the current golden standard for therapy response assessment in patients with solid tumors.
- Landmark molecular response early after ICI initiation identifies patients with differential clinical outcomes, which opens a window of opportunity for therapeutic intervention.

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References

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- Anagnostou et al. "ctDNA response after pembrolizumab in non-small cell lung cancer: phase 2 adaptive trial results" Nature Medicine, 2023.
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DELFI-TF dynamics have strong correlation with maxMAF dynamics and are consistent with the clinical outcomes

- DELFI-TF change between serial timepoints were computed in the ICI cohort. Figure 4 illustrates a strong correlation between DELFI-TF and maxMAF changes.
- DELFI-TF changes captured clinical outcomes. Figure 5 displays DELFI-TF in longitudinal samples of representative patients with radiographic progressive disease (PD), stable disease (SD) and partial/complete response (PR/CR).

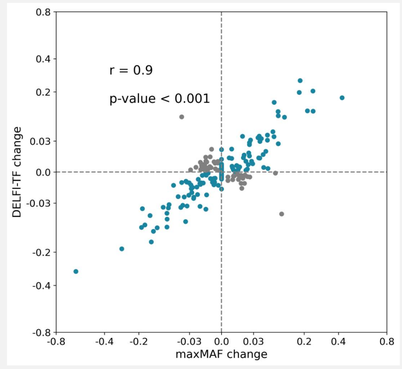

Figure 4. Correlation between DELFI-TF and maxMAF changes (n=215, r = 0.9, p < 0.001).

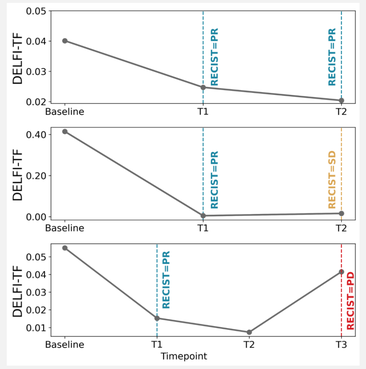

Figure 5. DELFI-TF dynamics for patients with different radiographic responses.

DELFI-TF baseline values and molecular response predict clinical outcomes with ICI

- Baseline DELFI-TF scores were dichotomized by the median value in the ICI cohort; low baseline DELFI-TF scores were associated with longer PFS (4.6 vs 14.7 months for DELFI-TF low and DELFI-TF high subgroups respectively, log rank p=0.0046)
- Absence of detectable DELFI-TF (below LOB) at any sampled timepoint within 3 to 9 weeks of ICI treatment initiation was defined as molecular response.
- Clinical assessment of landmark PFS at 6 months revealed a significant association between DELFI-TF molecular response and durable clinical benefit (DCB; p < 0.001, Fisher's exact test).
- Patients with mR attained longer progression free survival (16.04 vs 2.70 months, log-rank p<0.001) compared to those in the mPD group.
- Patients with mR attained longer overall survival (40.64 vs 6.28 months, log-rank p<0.001) compared to those in the mPD group.

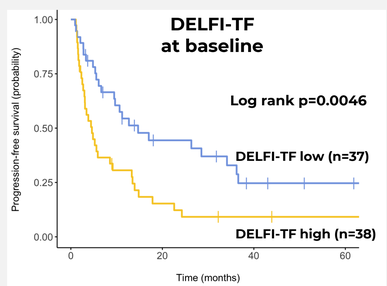

Figure 6. Kaplan Meier curves for progression-free survival by baseline DELFI-TF absolute scores.

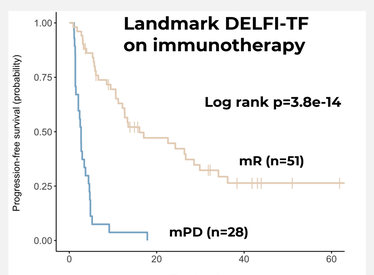

Figure 7. Kaplan Meier curves for progression-free survival by DELFI-TF landmark molecular response at 3-9 weeks on ICI therapy.