Monitoring response to immunotherapy using cell-free DNA fragmentomes

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BACKGROUND

- The rapid 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 cfDNA assays are costly and require a biomarker or prior knowledge about the mutations the tumor harbors.
- Here, we demonstrate the utility of DELFI Tumor Fraction (DELFI-TF), a tumor- and mutation-independent cfDNA fragmentome approach to monitor treatment 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 immunotherapy (ICI cohort); (Table 1).
- In addition, a 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. under review), was applied to predict the ctDNA fraction amongst samples.
- The accuracy of DELFI-TF was assessed using holdout validation in the ICI and Multi-Treatment cohorts.

RESULTS

- In both ICI and Multi-Treatment cohorts, DELFI-TF scores were strongly correlated with maxMAF (n=324, r=0.94, p<0.001, Pearson), (n=47, r=0.94, p<0.001, Pearson) respectively; Figure 2, Figure 3.
- Changes in DELFI-TF and maxMAF at all consecutive timepoints in the ICI cohort were highly correlated (n=215; r=0.9; Pearson); Figure 4.
- DELFI-TF dynamics are consistent with treatment response assessment using imaging; Figure 5.
- At baseline, DELFI-TF and maxMAF both differentiate progressive from non-progressive tumors (p = 0.027 and p = 0.003 for DELFI-TF and maxMAF respectively; Wilcoxon); Figure 6.
- Patients with high DELFI-TF or maxMAF had significantly shorter progression-free survival (PFS) compared to low DELFI-TF or maxMAF (147 vs 518 days, p < 0.001; 147 vs 801 days, p < 0.001; Log-Rank, respectively). Similarly, the overall survival (OS) of patients with high scores was significantly shorter compared to patients with low scores (DELFI-TF high 346 vs low 1236 days, p<0.001; maxMAF high 314 vs low 1038 days, p < 0.001, Log-Rank) using the median to define groups; Figure 7, Figure 8.

TABLE 1. Participant and disease characteristics

Characteristic	ICI
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%)

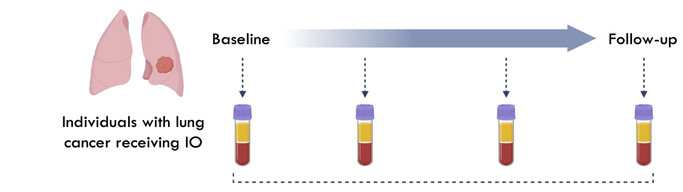

Figure 1A. Patients with liquid biopsy draws at baseline and after treatment with ICI are evaluated for cell-free DNA fragmentation patterns and mutant allele fraction (MAF).

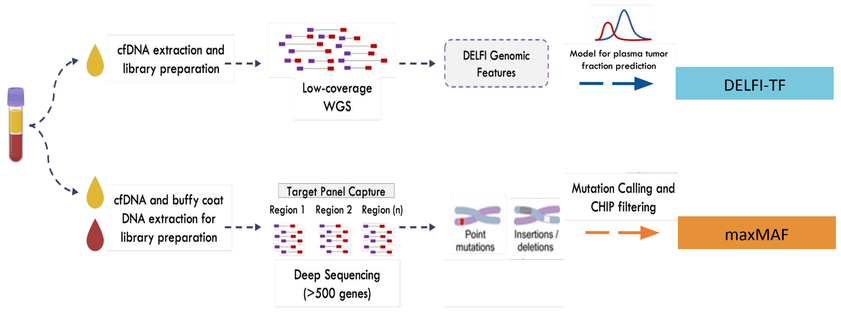

Figure 1B. Plasma cfDNA underwent (~4x) WGS to evaluate cell-free DNA fragmentation. ctDNA was quantified via maxMAF using a 500+ gene panel. Matched white blood cells were employed to filter out germline and clonal hematopoietic variants. The DELFI-TF model is applied on WGS samples and the scores are compared to maxMAF.

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 transform.

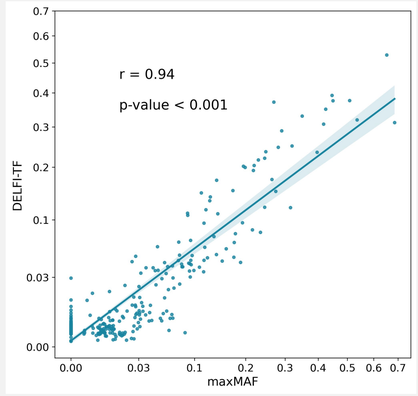

Figure 2. Correlation between DELFI-TF and maxMAF in the ICI cohort.

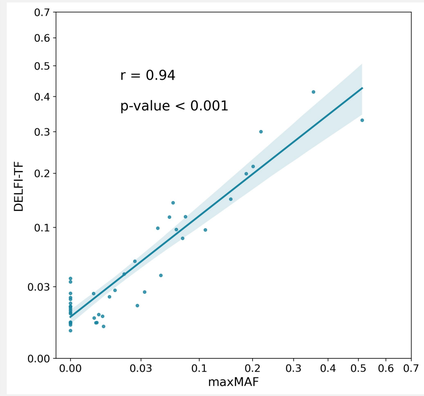

Figure 3. Correlation between DELFI-TF and maxMAF in the Multi-Treatment cohort .

DELFI-TF dynamics have strong correlation with maxMAF dynamics and are consistent with the clinical outcomes

- DELFI-TF change between all consecutive timepoints were computed in the ICI cohort. Figure 4 illustrates a strong correlation between DELFI-TF and maxMAF changes. Axis are scaled using a square root transform.
- DELFI-TF changes are consistent with clinical outcomes. Figure 5 displays DELFI-TF in longitudinal samples of 3 patients experiencing Progressive Disease (PD), Stable Disease (SD) and Partial/Complete Response (PR/CR) during the treatment.

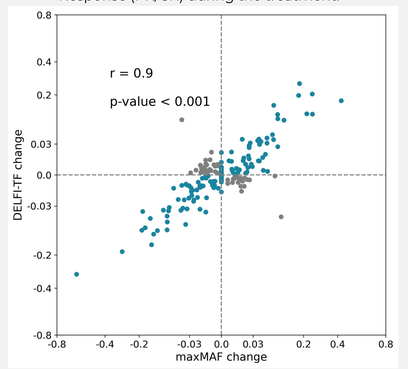

Figure 4. Correlation between DELFI-TF changes and maxMAF changes across all consecutive timepoints in the ICI cohort. (n=215, r = 0.9, p < 0.001, Pearson correlation).

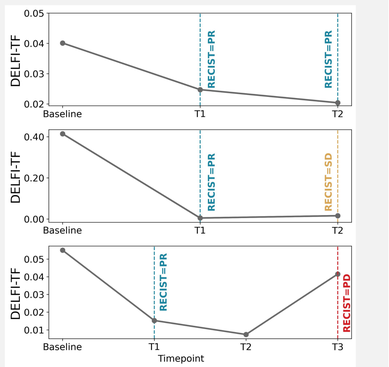

Figure 5. Examples of DELFI-TF dynamics for patients with different clinical outcomes according to RECIST 1.1 in Multi-Treatment cohort.

DELFI-TF scores at baseline are prognostic

- Patients who experienced disease progression, more often exhibited increased DELFI-TF values at baseline timepoints than patients who never presented with disease progression; (p = 0.027 and p = 0.003 for DELFI-TF and maxMAF respectively; Wilcoxon); Figure 6.
- Patients with DELFI-TF below the median at the baseline timepoint experienced significantly longer PFS/OS than patients with high DELFI-TF (Figure 7, Figure 8 respectively).

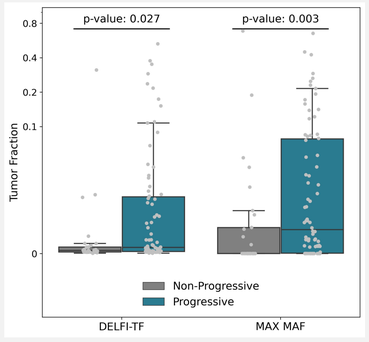

Figure 6. Patients with progressive disease have higher DELFI-TF as well as maxMAF values at baseline compared to patients with non-progressive disease in the ICI cohort.

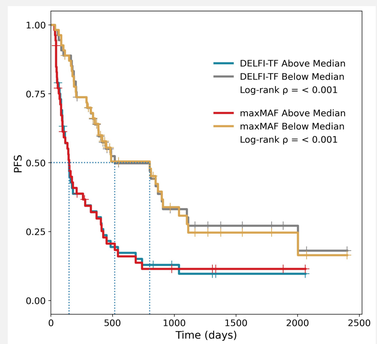

Figure 7. Progression-free survival (PFS) at baseline; DELFI-TF and maxMAF stratified by the median in the ICI cohort. (n=109, p < 0.001 for DELFI-TF and maxMAF, Log-Rank).

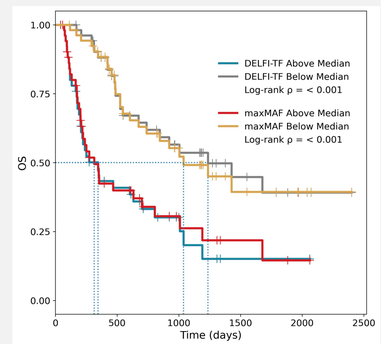

Figure 8. Overall survival (OS) at baseline samples; DELFI-TF and maxMAF stratified by the median in the ICI cohort. (n=109, p < 0.001 for DELFI-TF and maxMAF, Log-Rank).

Conclusions

- DELFI-TF is a tumor- and mutation- independent monitoring approach with high performance comparable to current ctDNA assays.
- DELFI-TF monitors treatment response and detects changes consistent with RECIST in cancer patients.