DELFI as a Real-time Treatment Response Assessment for Patients With Cancer

Keith Lumbard,[1] Laurel A Keefer,[1] Iris van 't Erve,[2] Jacob Carey,[1] Bryan Chesnick,[1] Denise Butler,[1] Michael Rongione,[1] Cornelis JA Punt,[3] Nicholas C Dracopoli,[1] Remond JA Fijneman,[2] Gerrit A Meijer,[2] Robert B Scharpf,[4] Victor E Velculescu,[4] Alessandro Leal.[1]

[1]Delfi Diagnostics, Inc., Baltimore, MD, USA; [2]The Netherlands Cancer Institute, Amsterdam, NL; [3]Utrecht University Medical Center, Utrecht, NL; [4]Johns Hopkins University School of Medicine, Baltimore, MD, USA.

BACKGROUND

- Measurement of tumor-derived DNA molecules in the plasma (ctDNA) has become a useful tool to determine the overall tumor burden in patients with cancer. However, this approach typically relies on prior tumor tissue analyses or knowledge of specific mutations.
- There is a clinical need to develop rapid and accurate noninvasive plasma-only approaches to estimate disease burden dynamics.
- The blood-based DELFI (DNA evaluation of fragments for early interception) method has demonstrated the ability to distinguish cancer from no cancer with high sensitivity and specificity.[1,2]
- We demonstrate that we can use a similar approach to accurately predict tumor burden over time given fragmentation information contained in longitudinal blood draws.

1. Cristiano S, et al. *Nature*. 2019;570(7761):385-9.
2. Mathios D, et al. *Nat Commun*. 2021;12(1):5060.

STUDY DESIGN

- Blood samples were collected longitudinally from patients enrolled in a phase 3 clinical trial (NCT02162563) in The Netherlands (N=78 patients, 312 longitudinal blood draws).
- Patients were initially treatment-naïve, had histological proof of colorectal cancer (CRC), and unresectable metastases confined to the liver according to CT scan.
- Patients were all found to have a *RAS* or *BRAF* mutation through tissue analyses. Cell-free DNA (cfDNA) tumor burden was quantified as the mutant allele frequency (MAF) of the *RAS/BRAF* variant measured by droplet digital PCR (ddPCR).
- After enrollment, patients started first-line treatment with bevacizumab plus either FOLFOX or FOLFIRI.

SAMPLE PREPARATION

- cfDNA was isolated from approximately 4 mL of plasma from samples collected in 10-mL Streck tubes.
- Next-generation sequencing genomic libraries were prepared in batches from the cfDNA of each patient and sequenced at a targeted 8× coverage per genome.
- cfDNA from patients without cancer was independently obtained and used both as a negative control during the model-training process and to define a positivity threshold.

DELFI METHOD

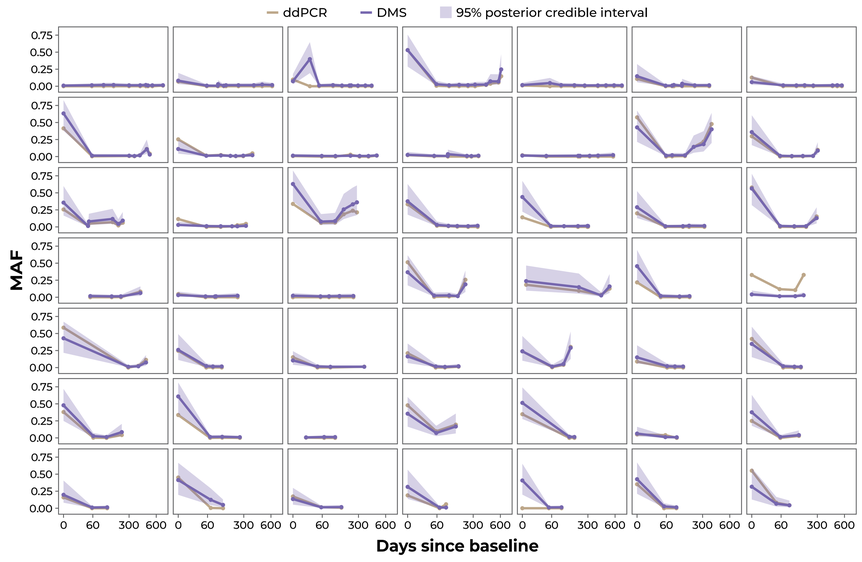
- Fragmentation profiles, which quantify and summarize features of the cfDNA detected in plasma across the genome, were generated for each collected sample.
- A Bayesian regression model was used to predict MAF for a given genomic sample given its fragmentation profile. We define the DELFI Monitoring Score (DMS) as this predicted MAF.
- All scores were derived from leave-one-patient-out cross-validation; consequently, the model that produced DMS for each patient had no access to that patient's data in the model-training process.

STATISTICAL ANALYSES

- We examined the association between DMS and ddPCR MAF by visual inspection and by the Pearson correlation between the two measures.
- We dichotomized samples as above or below a threshold based on the maximum DMS observed in a set of 30 individuals without cancer and examine the Kaplan-Meier curves of the groups that are created as a result of this grouping.

Can the DELFI approach to analyzing cfDNA predict tumor burden in patients with metastatic colorectal cancer?

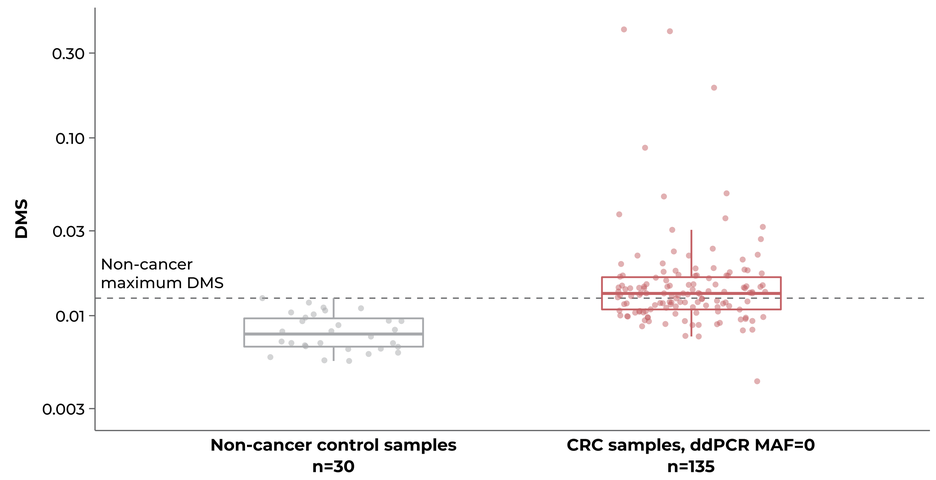
Figure 1. ddPCR MAF measured during therapy, with cross-validated DELFI Monitoring Scores*



*For 49 patients with ddPCR MAF measured at ≥3 timepoints; each panel displays the ddPCR- and fragmentation-based estimated MAF for a single patient.

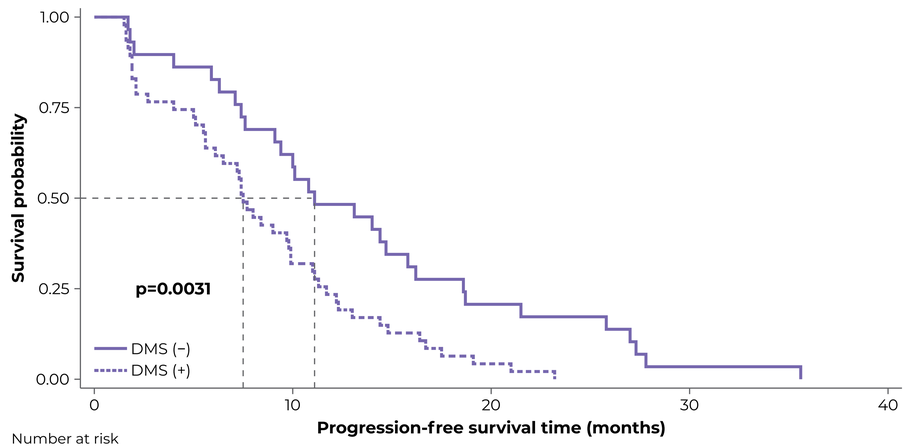
- The fragmentation-based model captures the dynamics of MAF over time with high accuracy: Across all time points and all individuals, DMS and observed ddPCR MAF have a Pearson correlation of 0.85.

Figure 2. DELFI Monitoring Scores are elevated in CRC patient samples with ddPCR MAF=0



- Non-cancer control samples were used to establish a distribution of DMS values that represent background variation.
- Overall, we observed higher DMS among patients with CRC who did not have mutations identified by ddPCR.
- The maximum DMS among non-cancer controls was used to establish a cutoff for ctDNA detection. Patients with DMS scores higher than this cutoff at the first assessment post-treatment were considered positive (DMS +).

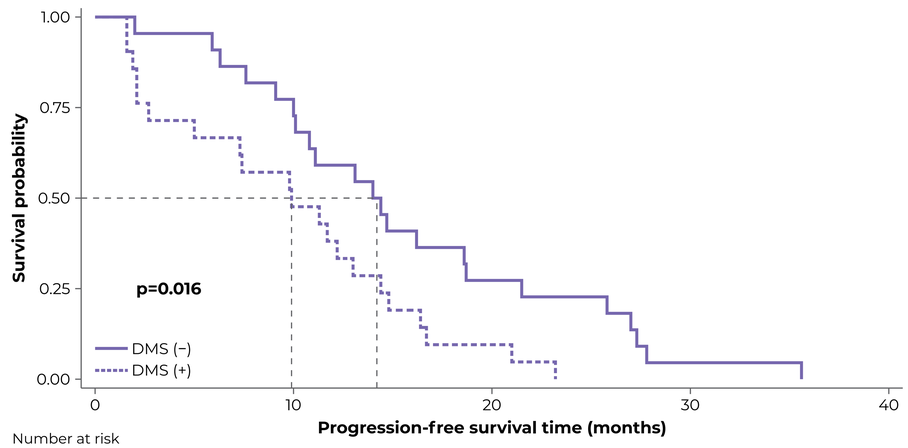
Figure 3a. DELFI Monitoring Score* is associated with PFS at first post-treatment blood draw



Number at risk					
DMS (−)	29	18	6	1	0
DMS (+)	47	15	2	0	0

*Samples were DMS (−) or DMS (+) if the DMS was below or above the maximum DMS observed in non-cancer controls, respectively.

Figure 3b. DELFI Monitoring Score* is associated with PFS at first post-treatment blood draw for samples with ddPCR MAF=0



Number at risk					
DMS (−)	22	17	6	1	0
DMS (+)	21	10	2	0	0

*Samples were DMS (−) or DMS (+) if the DMS was below or above the maximum DMS observed in non-cancer controls, respectively.

- We observed a difference in progression-free survival among these patients using a fragmentation-based measure of tumor burden.

The DELFI Monitoring Score tracks the level of tumor burden over time and shows promise as a prognostic marker for progression-free survival.

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AL: Delfi Diagnostics (founder, employment, stock option, other). IVE, CJAP: nothing to disclose. Johns Hopkins University owns equity in Delfi Diagnostics.

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