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Monitoring immune checkpoint inhibition in advanced solid tumors using genome-wide cfDNA fragmentomes

Jamie E. Medina,[1*] Evanthia T. Roussos Torres,[1,2*] Alessandro Leal,[1] Vilmos Adleff,[1] Keith Lumbard,[3] Laurel Keefer,[3] Jacob Carey,[3] Adam Brufsky,[4] Patricia LoRusso,[5] Joseph Paul Eder,[5] Vincent Chung,[6] Melinda Downs,[1] Ashley O'Connor,[1] Richard Piekarz,[7] Howard Streicher,[7] Elizabeth M. Jaffee,[1] Robert B. Scharpf,[1] Vered Stearns,[1] Roisin M. Connolly,[1,8*] Victor E. Velculescu[1*]

[1]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2]Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [3]Delfi Diagnostics, Inc., Baltimore, MD, USA; [4]University of Pittsburgh Cancer Institute and UPMC Cancer Center, Pittsburgh, PA, USA; [5]Yale Cancer Center, Yale School of Medicine, New Haven, CT, USA; [6]City of Hope, Duarte, CA, USA; [7]Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, USA; [8]Cancer Research at UCC, College of Medicine and Health, University College Cork, Cork, Ireland

BACKGROUND

- Conventional computed tomography (CT) imaging is the gold standard of care to guide clinical decisions for monitoring patients with cancer receiving immunotherapy.
- Despite the advantages of CT imaging, relying on imaging assessments to rapidly identify disease progression in patients receiving immune checkpoint inhibitors (ICIs) is challenging.
- Cell-free DNA (cfDNA), acquired through noninvasive liquid biopsies, may offer a new opportunity to detect and serially monitor responses to immunotherapy in a timely manner.
- We investigated genome-wide cfDNA fragmentation profiles[1,2] to molecularly detect disease progression in patients with advanced solid tumors receiving ICIs.

APPROACH

Study participants

- Participants in the phase 1 study of entinostat and nivolumab ± ipilimumab for advanced solid tumors (ETCTN-9844; NCT02453620) received an entinostat run-in 2 weeks before the addition of anti-PD-1 with or without anti-CTLA-4 ICIs.[3]
- Clinical disease assessments by CT scan were based on RECIST v1.1 criteria and used to identify clinical responders (CR/PR) and clinical non-responders (SD/PD).

Sample and data collection

- Blood samples were collected from 50 participants at baseline (BL), 48 participants after the entinostat run-in at week 2, and 27 participants at 10 weeks after ICI treatment initiation (Figure 1).
- cfDNA was extracted from plasma, constructed into genomic libraries, and sequenced at low coverage across the whole genome (1-2×). All samples from an individual were included in a library batch that also included an interbatch control to limit any batch-specific effect.

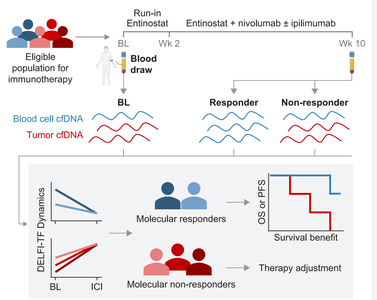
Molecular monitoring

- Blood samples were evaluated using a molecular approach termed DELFI-Tumor Fraction (DELFI-TF) that analyzes genome-wide cfDNA fragmentation patterns (Figure 1).[1,4]
- Genome-wide cfDNA fragmentation features were included in a Bayesian model to approximate the DELFI-TF as compared to best response by RECIST v1.1.

Statistical analyses

- Progression-free survival (PFS) was defined as time to progression, death, or last known alive date.
- Molecular response was defined as reduction (≥30%) in DELFI-TF between the BL and 10-week timepoints.

Figure 1. Schema of the DELFI-TF for monitoring during immunotherapy.



Participants with liquid biopsy draws at BL and after treatment with ICIs are evaluated for cfDNA fragmentation patterns. Responders to ICIs demonstrate decreased tumor-derived cfDNA in the blood, compared with non-responders. The dynamics of DELFI-TF are monitored in each participant and molecularly characterized. This framework may allow for the adjustment of therapy depending on participant response.

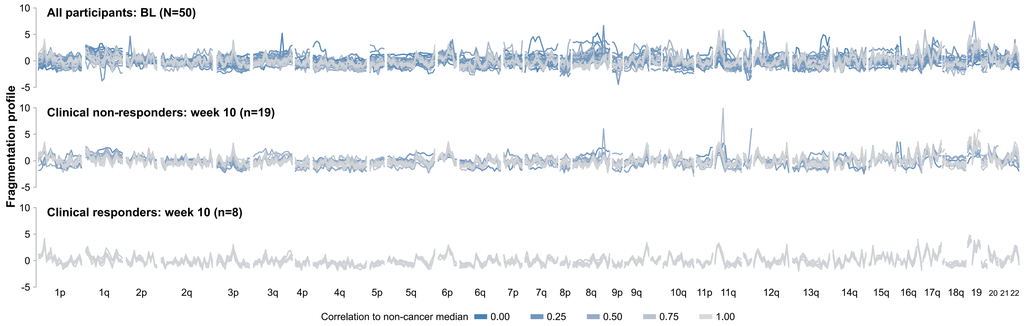
Can a blood-based cfDNA fragmentome approach detect disease progression in patients receiving immunotherapy?

Table 1. Participant characteristics.

	BL N=50	Week 2 n=48	Week 10 n=27
Age, n (%)			
>50 y	35 (70)	33 (69)	18 (67)
≤50 y	15 (30)	15 (31)	9 (33)
Sex, n (%)			
Female	47 (94)	45 (94)	25 (93)
Male	3 (6)	3 (6)	2 (7)
Best overall RECIST, n (%)			
CR	1 (2)	1 (2)	1 (4)
PR	7 (14)	6 (13)	7 (26)
SD	18 (34)	18 (34)	12 (44)
PD	15 (30)	13 (27)	7 (26)
Discontinued	9 (18)	8 (17)	—
Histology, n (%)			
Adenoid cystic	4 (8)	4 (8)	2 (7)
Apocrine	1 (2)	1 (2)	1 (4)
Bile duct	3 (6)	3 (6)	2 (7)
Bowel	1 (2)	1 (2)	1 (4)
Breast	28 (56)	26 (54)	16 (59)
Cervix	1 (2)	1 (2)	—
Colorectal	1 (2)	1 (2)	—
Endometrial	1 (2)	1 (2)	1 (4)
Gastric	1 (2)	1 (2)	—
Liposarcoma	1 (2)	1 (2)	1 (4)
Lung	2 (4)	2 (4)	2 (7)
Ovarian	1 (2)	1 (2)	—
Parotid	2 (4)	2 (4)	—
PEComa	1 (2)	1 (2)	—
Sarcoma	1 (2)	1 (2)	1 (4)
Uterine	1 (2)	1 (2)	—

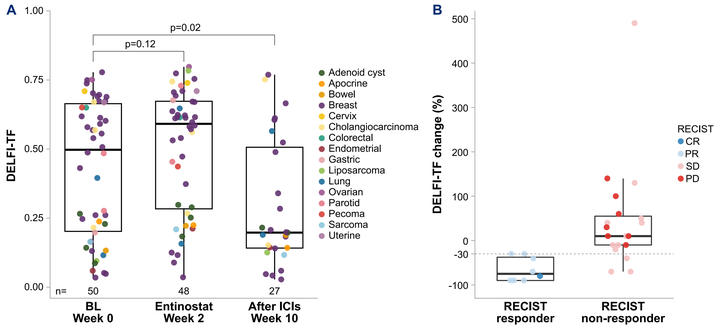
CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Figure 2. Genome-wide fragmentation profiles reflect participant responses during immunotherapy.



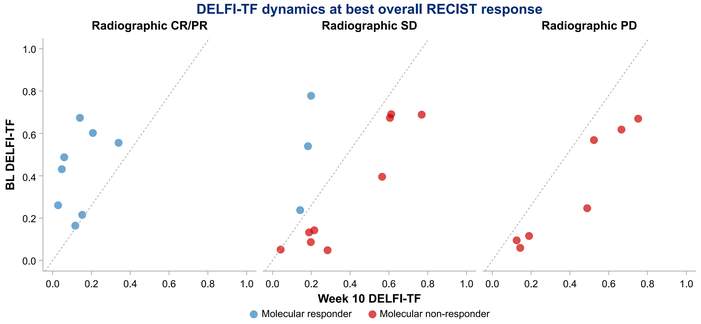
- Fragmentation profiles at BL (N=50; multiple cancer types) revealed genome-wide fragmentation alterations (blue) evident by low correlation to profiles of non-cancer individuals.
- Compared with participants categorized as non-responders (n=19) by RECIST v1.1 clinical evaluation post-immunotherapy week 10, those categorized as responders (n=8) showed fragmentation profiles with higher correlation to profiles of non-cancer individuals (gray).

Figure 3. DELFI-TF identifies molecular responders and non-responders.



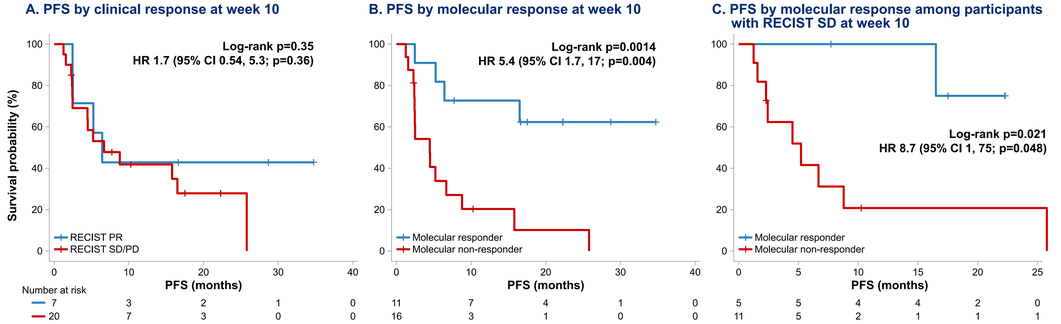
- [A] Distribution of DELFI-TF estimates across serial blood collection timepoints and different cancer types showed no difference between BL and entinostat/week 2 but a significant difference between BL and after ICIs/week 10 (Welch two sample t-test).
- [B] A 30% reduction in DELFI-TF from BL to week 10 captures RECIST responders.

Figure 4. DELFI-TF molecular response categorization adds to radiographic assessment.



- Within radiographic categories, molecular responses as determined by the change in DELFI-TF from BL to week 10 were assessed.
- Gray dashed line represents the threshold separating responders from non-responders.
- Importantly, three participants with radiographic SD showed ≥30% reduction in DELFI-TF at week 10.

Figure 5. DELFI-TF response categorization is associated with PFS.



- [A] Median PFS was 6.4 months (PR) *vs* 6.7 months (SD/PD)
- [B] Median PFS was not reached (responder) *vs* 4.5 months (non-responder)
- [C] Median PFS was not reached (SD/molecular responder) *vs* 5.2 months (SD/molecular non-responder)

Our analyses provide a proof-of-concept framework that a cfDNA fragmentome-based approach may have broad applicability in the setting of monitoring disease in patients receiving immunotherapy.

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JEM declares no competing interests. RBS is a founder and consultant of Delfi Diagnostics and owns Delfi Diagnostics stock, subject to certain restrictions under university policy. VEV is a founder of Delfi Diagnostics, serves on its Board of Directors and as a consultant, and owns Delfi Diagnostics stock, subject to certain restrictions under university policy. Johns Hopkins University owns equity in Delfi Diagnostics. VEV divested his equity in Personal Genome Diagnostics to LabCorp in February 2022. VEV is an inventor on patent applications submitted by Johns Hopkins University related to cancer genomic analyses and cfDNA for cancer detection that have been licensed to one or more entities, including Delfi Diagnostics, LabCorp, Qiagen, Sysmex, Agios, Genzyme, Esoterix, Ventana, and ManaT Bio. Under the terms of these license agreements, the University and inventors are entitled to fees and royalty distributions. VEV is an advisor to Danaher, Takeda Pharmaceuticals, and Viron Therapeutics. These arrangements have been reviewed and approved by Johns Hopkins University in accordance with its conflict-of-interest policies.

References: 1. Cristiano S, et al. *Nature*. 2019;570:385-9. **2.** Mathios D, et al. *Nat Commun*. 2021;12:5060. **3.** Roussos Torres ET, et al. *Clin Cancer Res*. 2021;27:5828-37. **4.** Lumbard K, et al. *Cancer Res*. 2022;82 (12_Supplement):Abstract 2224.