Methylation-Based ctDNA Serial Monitoring Correlates with Immunotherapy Response in NSCLC

Angela Hsiao[1], Brian Woodward[1], Patrick Ye[2], Matthew G Varga[2], Ghaith Altaie[1], Kevin Lu[1], Naomi Searle[2], Robb Viens[2], Sydne Langpap[2], Zeqian Li[2], Gary Palmer[2], Hatim Husain[1]*

1. University of California San Diego, La Jolla CA
2. BillionToOne, Inc., Menlo Park, CA

UC San Diego Moores Cancer Center

INTRODUCTION

Serial therapy response monitoring assays require improved sensitivity

BACKGROUND

Circulating tumor DNA (ctDNA) from plasma has emerged as an important oncology biomarker used to aid clinical decision-making from therapy selection through on-treatment response monitoring to post-therapy surveillance.

Current ctDNA-based therapy response monitoring strategies employ tracking the variant allele fraction (VAF) of a select few somatic alterations. However, tracking a limited number of somatic mutations has limitations:

- The selected variants may not accurately represent the tumor's composition, especially in late-stage cases where extensive evolution and clonal heterogeneity can be influenced by systemic therapies[1–3].
- Not all tumors have enough somatic variants available for reliable tracking
- For tissue-informed assays, not all patients can be feasibly biopsied to inform liquid biopsy monitoring

To address these limitations, quantification of methylated loci[4–6] from ctDNA has emerged as a viable alternative due to a greater abundance of tumor-derived methylated molecules compared to somatic variants, thereby enhancing assay sensitivity through:

- Reducing sample variability via interrogating more loci
- Limiting reliance on specific or bespoke oncogenic variants
- Enabling the detection of serial changes over time

OBJECTIVE

We utilized a methylation based assay tailored to track tumor-specific ctDNA signals to evaluate whether a change in Tumor Methylation Score (TMS) may be associated with real world progression free survival (rwPFS) for patients on immunotherapy regimens.

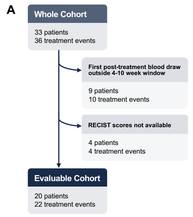
REFERENCES

1. Hasenleithner SO, Speicher MR. A clinician's handbook for using ctDNA throughout the patient journey. Mol Cancer. 2022;21(1):81. doi:10.1186/s12943-022-01551-7
2. De Mattos-Arruda L, Weigelt B, Cortes J, et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: a proof-of-principle. Annals of Oncology. 2014;25(9):1729-1735. doi:10.1093/annonc/mdu239
3. Bettegowda C, Sausen M, Leary RJ, et al. Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. Sci Transl Med. 2014;6(224). doi:10.1126/scitranslmed.3007094
4. Xu RH, Wei W, Krawczyk M, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nat Mater. 2017;16(11):1155-1161. doi:10.1038/nmat4997
5. Taryma-Leśniak O, Sokolowska KE, Wojdacz TK. Current status of development of methylation biomarkers for in vitro diagnostic IVD applications. Clin Epigenetics. 2020;12(1):100. doi:10.1186/s13148-020-00886-6
6. Vrba L, Oshiro MM, Kim SS, et al. DNA methylation biomarkers discovered in silico detect cancer in liquid biopsies from non-small cell lung cancer patients. Epigenetics. 2020;15(4):419-430. doi:10.1080/15592294.2019.1695333
7. Ye, P. P., Viens, R. A., Shelburne, K. E., Langpap, S. S., Bower, X. S., Zhou, W., Wignall, J. C., Zhu, J. J., Woodward, B. D., Husain, H., Tsao, D. S., & Atay, O. (2023). Molecular counting enables accurate and precise quantification of methylated ctDNA for tumor-naive cancer therapy response monitoring. MedRxiv, 2023.05.31.23290555. https://doi.org/10.1101/2023.05.31.23290555

METHODS

Methylated ctDNA therapy response monitoring assay using real-world dataset of NSCLC patients with serial plasma collections

We evaluated a cohort of 20 patients with NSCLC treated with anti-PD1 based immunotherapy that had both baseline and follow-up blood draws as well as outcome data available.



B

Patient Summary	Whole Cohort	Evaluable Cohort RECIST+TMS available
Age		
Median	67	68
Range	26-90	56-85
Sex		
Female	15	8
Male	18	12
Histology		
Adenocarcinoma	27	16
Squamous	4	2
Other/NOS	2	2
Stage		
IB	1	1
III	2	1
IV	30	18
PD-L1 Tumor Proportion Score Immunohistochemistry		
≥ 50%	8	5
1-49%	10	6
0%	9	5
Not reported	6	4
Treatments*		
IO Monotherapy	11	6
IO+ Chemotherapy	23	14
Dual IO + Chemotherapy	2	2

*Treatment events total 36 from 33 patients or 22 from 20 patients. **NOS** = Not Otherwise Specified, **IO** = Immunotherapy

Figure 1. (A) CONSORT diagram of patients in the NSCLC cohort. **(B)** Table of patient demographics and clinical characteristics for the whole cohort (n=33 patients, 36 events) and the evaluable cohort for whom both RECIST scores and TMS in 4-10 week window were available (n=20 patients, 22 events).

Tumor Methylation Score was measured using the Northstar Response assay. The association between TMS and real-world progression-free survival (rwPFS) on therapy was conducted using Cox proportional hazards model and plotted using the Kaplan-Meier method.

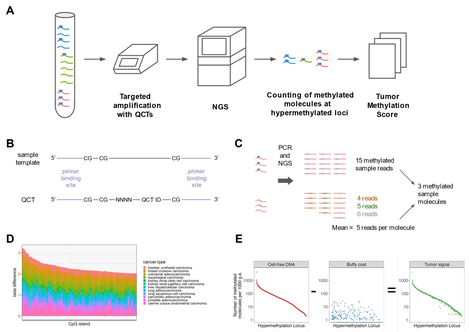


Figure 2. Design and analysis for Northstar Response[7]. (A) Workflow overview for Northstar Response. **(B)** QCTs are designed for each targeted genomic location such that they have identical primer binding site sequences but have an embedded molecular identifier (EMI). **(C)** The number of reads per EMI is averaged across all EMIs for that genomic location. The number of reads per EMI is the number of reads per molecule at that genomic location. The number of methylated sample reads can then be divided by the number of reads per molecule to calculate the number of methylated sample molecules at the start of PCR. **(D)** The top 100 CpG islands ranked by total hypermethylation across 12 cancer types according to TCGA data. **(E)** The numbers of methylated molecules measured in paired cfDNA and buffy coat are first normalized to the estimated input genomic equivalents (g.e.), then background methylation detected in buffy coat is subtracted from the methylation measured in plasma on a per-locus basis to extract tumor-associated signal.

DATA

On-treatment changes in Tumor Methylation Score is a predictive marker of response to Immunotherapy

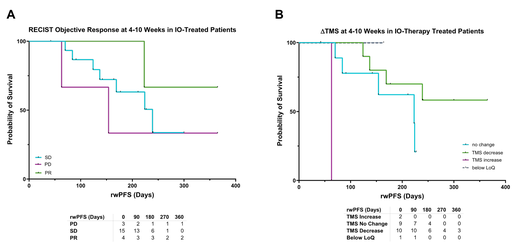


Figure 3. Tumor Methylation Score is predictive of treatment response. (A) Kaplan-Meier plot of the association between RECIST score and rwPFS for IO-treated patients (n=22 events) p=0.55. **(B)** Kaplan-Meier plot of the association between delta TMS and rwPFS for IO-treated patients in the 4-10 week window (n=22 events), p<0.0001.

CLINICAL CASE STUDIES

Methylated ctDNA serial monitoring continues to reflect patient outcomes beyond 4-10 week post-therapy initiation window

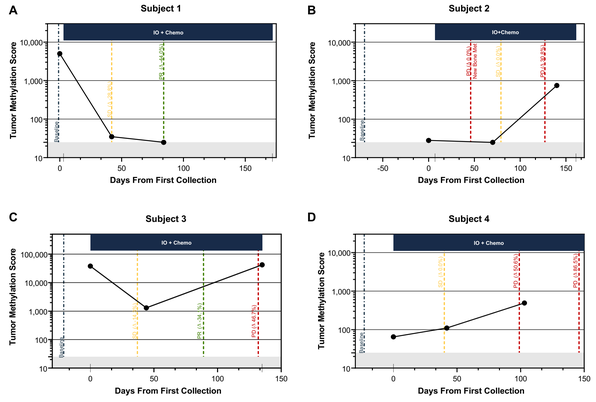


Figure 4. Clinical Validation Case Studies. TMS correlate with disease outcomes across therapy types. **(A,B)** Clinical case studies of TMS corresponding with imaging assessments **(C,D)** Representative clinical case studies in which the trend in TMS precedes the imaging outcome. Dashed lines represent RECIST evaluation objective responses.

RESULTS

Changes in methylation profiles can reflect emergence of new somatic alterations

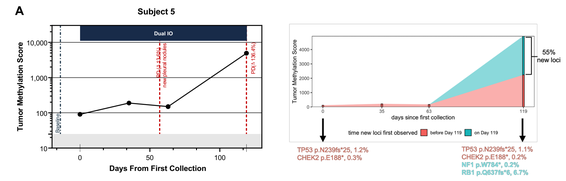


Figure 5. Changes in tumor methylation profile can correlate with appearance of new somatic mutations. (A) TMS increased from 90 to 4900 over 4 months (left). At day 119, 113 newly methylated loci were detected contributing about 55% of total methylation indicating a significant change in tumor methylation profile (right). Somatic alterations were assessed with a CGP assay at Days 0 and 178. **(B)** TMS values increased yet only 1% of methylation at Day 324 came from loci that were never methylated before Day 266. Concurrently, there was no change in the diversity of somatic alterations measured at Day 0 and 324 (red).

CONCLUSION

Serial monitoring of methylated ctDNA can aid clinical decision-making

- In this real-world dataset of NSCLC patients treated with anti-PD1 immunotherapy regimens, the TMS score measured within a 4-10 week window after treatment initiation is predictive of response to therapy.
- Beyond this window, the TMS score can be associated with rwPFS and tumor dynamics.
- Early evidence suggests that changes in the specific methylation profile may be informative for monitoring occurrence of new somatic mutations.