Methylated ctDNA dynamics correspond with clinical tumor load in metastatic lung cancer patients on therapy #5588

Patrick Ye[1], Brian Woodward[2], Robb Viens[1], Sydne Langpap[1], Katherine Shelburne[1], Wen Zhou[1], Joyce Zhu[1], Jan Wignall[1], Gary Palmer[1], David Tsao[1], Oguzhan Atay[1], Hatim Husain[2]

[1]BillionToOne, Inc., Menlo Park, California [2]University of California San Diego Moores Cancer Center, La Jolla, CA. Contact: patrick@billiontoone.com

INTRODUCTION

Therapy response monitoring assays are needed to accurately and rapidly assess the efficacy of cancer treatments. While imaging remains the gold standard for monitoring the efficacy of cancer treatment, the use of more sensitive tools, such as liquid biopsy, could be beneficial for the patient's ultimate treatment outcome. Several liquid biopsy-based assays that measure circulating tumor DNA (ctDNA) have been developed to meet this need. However, approaches that rely on quantifying the variant allele fraction (VAF) of somatic variants may be inaccurate or inconsistent due to a scarcity of detected somatic variants in the ctDNA or may be logistically infeasible if they require a tumor biopsy *a priori*.

Methylated ctDNA has shown promise as a biomarker for therapy response monitoring without requiring a tumor biopsy, but current efforts are limited in their ability to precisely quantify the amount of methylation present in the ctDNA. We hypothesize that more precise quantification of methylated ctDNA could enable more accurate correspondence with clinical tumor load and cancer treatment outcomes.

Here, we present a retrospective study characterizing how amounts of methylated ctDNA dynamically change through cancer therapy.

METHODS

Study design

- A retrospective study including N = 75 metastatic lung cancer treatment events (N = 63 patients). A treatment event is defined as a new therapy start. This study was approved by the IRB at UCSD and patients provided consent for specimen analysis.
- Up to 6 mL of plasma and 200 µL of matched buffy coat were collected at one pre-treatment time point and two post-treatment time points
- RECIST measurements were made relative to imaging taken before the pre-treatment time point
- Time to Treatment Failure (TTF) was determined from the medical record (data cutoff was March 9, 2023), censored based on progression, and capped at 1 year

Figure 1: Diagram of patient sample collection

Assay

- Plasma and buffy coat samples were processed with Northstar Response[1], a tumor-naïve, methylation-based therapy response monitoring assay
- The number of methylated molecules is quantified using QCT technology at more than 500 loci known to be hypermethylated in cancer compared to normal tissue

Figure 2: Workflow overview for Northstar Response. cfDNA (cell-free DNA) molecules are hypermethylated (blue rectangles) in ctDNA (circulating tumor DNA) compared to normal tissue at multiple genomic locations (red, green, and blue wavy lines). These cfDNA molecules at targeted hypermethylated loci are co-amplified with Quantitative Counting Templates (QCTs) and sequenced using next-generation sequencing (NGS). This data is analyzed to calculate the number of methylated molecules at these targeted loci and aggregated across all loci to calculate the Tumor Methylation Score.

RESULTS

Patient cohort

Table 1. Baseline Characteristics of Patient Cohort

Characteristic	N	(%)
Patients	63	
Age at first collection (years)		
Median	68	
Range	25-89	
Gender		
Male	30	(48)
Female	33	(52)
Histology Type		
Adenocarcinoma	49	(78)
Squamous Cell Carcinoma	5	(8)
Lung Cancer NOS	9	(14)
Cohort Treatment Designation		
Immunotherapy	12	(16)
Dual immunotherapy	2	(3)
Immunotherapy + chemotherapy	30	(40)
Dual immunotherapy + chemotherapy	2	(3)
Total immunotherapy	*46*	*(61)*
TKI	18	(24)
Dual TKI	1	(1)
Chemotherapy	3	(4)
Chemotherapy + TKI	5	(7)
Antibody drug conjugate	2	(3)
Total non-immunotherapy	*29*	*(39)*

Assay validation

Figure 3: Tumor Methylation Score accurately and precisely quantifies methylated ctDNA in contrived ctDNA samples made from two unique tumors.

Figure 4: Correlation of Tumor Methylation Score with maximum VAF in clinical samples. VAF was measured using a treatment selection assay. Variants present in the buffy coat were removed from analysis

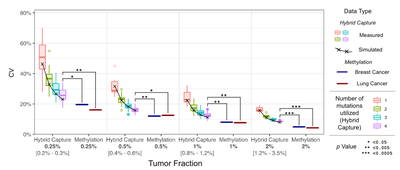

Figure 5: Coefficients of variance for VAF-based and methylation-based therapy response monitoring. VAF-based CVs were calculated through empirical measurements (boxplots) and simulation (black X's). CV of Tumor Methylation Scores were calculated at matching contrived tumor fractions (dark blue and red bars). *p* values for each tumor fraction were calculated comparing the Methylation Score CV for each cancer type to the distribution of average VAF CVs for 4 mutations utilized using a 1-sided T test (black asterisks).

Therapy response monitoring for immunotherapy patients

Initial change in Tumor Methylation Score is predictive of Time to Treatment Failure

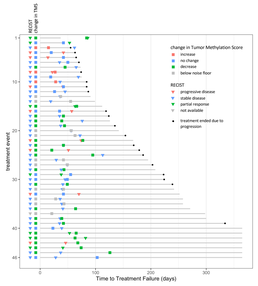

Figure 6: Swimmer plot for patients receiving immunotherapy or combination immunotherapy. Squares indicate the change in Tumor Methylation Score (TMS) measured at post-treatment 1. Triangles indicate the RECIST imaging result that is temporally closest to the TMS measurement. Changes in TMS appears more consistently arranged with Time to Treatment Failure than RECIST.

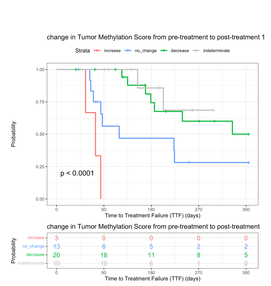

Figure 7: Kaplan-Meier plot for patients receiving immunotherapy, stratifying Time to Treatment Failure by change in TMS from pre-treatment to post-treatment 1

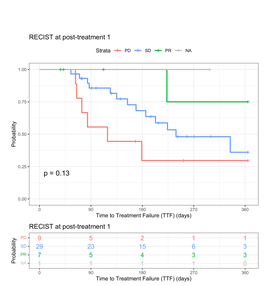

Figure 8: Kaplan-Meier plot for patients receiving immunotherapy, stratifying Time to Treatment Failure by RECIST classification at post-treatment 1

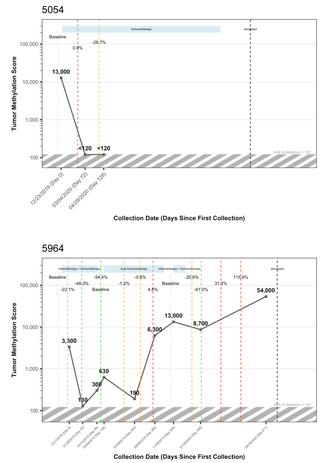

Figure 9: Clinical data and Tumor Methylation Scores for patient 5054. An initial decrease in TMS was observed, and a decrease in tumor size was seen on subsequent imaging. While a new liver lesion was observed in the first RECIST time point, indicating progressive disease, the patient appears to have responded well to treatment. The patient remained on treatment for more than a year and deceased due to a non-cancer cause.

Figure 10: Clinical data and Tumor Methylation Scores for patient 5964. A consistent pattern is seen across all three treatment sets in this patient, where TMS initially decreases but subsequently increases. Imaging is largely concordant with TMS measurements. The overall increase trajectory of TMS is concordant with the patient ultimately passing away due to progression.

Therapy response monitoring for TKI-only therapy patients

Achieving ctDNA clearance as measured by Tumor Methylation Score may be predictive of Time to Treatment Failure

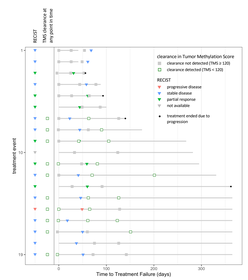

Figure 11: Swimmer plot for patients receiving only tyrosine kinase inhibitor (TKI) therapy. Squares indicate Tumor Methylation Score (TMS) clearance measurements, defined as measuring TMS below the noise floor. Triangles indicate the RECIST imaging result from post-treatment 1. Clearance detected at any point occurs more often in patients with longer Time to Treatment Failure. TMS measurements more than 7 days prior to treatment start are not plotted for clarity.

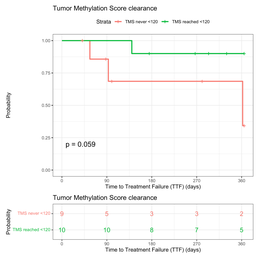

Figure 12: Kaplan-Meier plot for patients receiving TKI-only therapy, stratifying Time to Treatment Failure by whether a patient has experienced clearance in Tumor Methylation Score at any point in time.

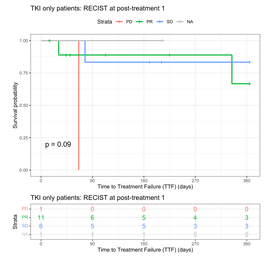

Figure 13: Kaplan-Meier plot for patients receiving TKI-only therapy, stratifying Time to Treatment Failure by RECIST classification at post-treatment 2

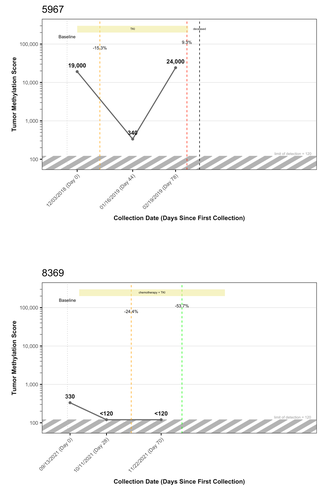

Figure 14: Clinical data and Tumor Methylation Scores for patient 5967. An initial decrease in TMS was observed post-treatment, which was concordant with the initial post-treatment imaging scan. Subsequently, the TMS increased, which was concordant with both the progressive disease measured on imaging and the clinical outcome. In this case, a subsequent imaging scan showed progression.

Figure 15: Clinical data and Tumor Methylation Scores for patient 8369. An initial decrease in TMS was observed post-treatment, which was concordant with the initial post-treatment imaging scan. In this case, a definitive decrease in TMS was detected before the imaging called a partial response.

CONCLUSIONS

- In patients receiving immunotherapy, changes in Tumor Methylation Score at post-treatment 1 are predictive of Time to Treatment Failure
- In patients receiving TKI-only therapy, detecting clearance as measured by Tumor Methylation Score at any point may be associated with longer Time to Treatment Failure

Future directions

- The optimal timing of when to run the assay relative to treatment start needs to be further studied
- Clearance of Tumor Methylation Score may correlate with improved survival for patients with lung cancer

Acknowledgements

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References

1. Ye PP et al. Molecular counting enables accurate and precise quantification of methylated ctDNA for tumor-naive cancer therapy response monitoring. *in preparation*