Novel methylation-based, tissue-free ctDNA assay accurately quantifies longitudinal tumor burden changes for precision treatment monitoring

Patrick Ye[1], Robb Viens[1], Xavier Bower[1], Shan Riku[1], David Tsao[1], Oguzhan Atay[1]

[1]BillionToOne, Inc., Menlo Park, California
Contact: patrick@billiontoone.com

#526

INTRODUCTION

As novel cancer treatments become available, the need to identify whether these treatments are effective earlier remains unaddressed. Obtaining earlier feedback on the efficacy of a cancer therapy could prevent a poor treatment outcome by switching to a more effective therapy sooner. Levels of circulating tumor DNA (ctDNA) have been found to be predictive of tumor progression, suggesting that a non-invasive liquid biopsy assay could provide longitudinal ctDNA measurements that accurately track tumor progression. However, while there is interest in using existing minimal residual disease (MRD) detection and treatment selection liquid biopsy assays for treatment monitoring applications, they both suffer from limitations in their ability to precisely and sensitively quantify trends in tumor progression over the course of treatment. In addition, tumor-informed MRD detection assays are often infeasible for treatment monitoring due to unavailability of the initial tissue sample.

We have developed and validated a novel methylation-based liquid biopsy assay for treatment monitoring without the need to obtain a sample from the tumor itself.

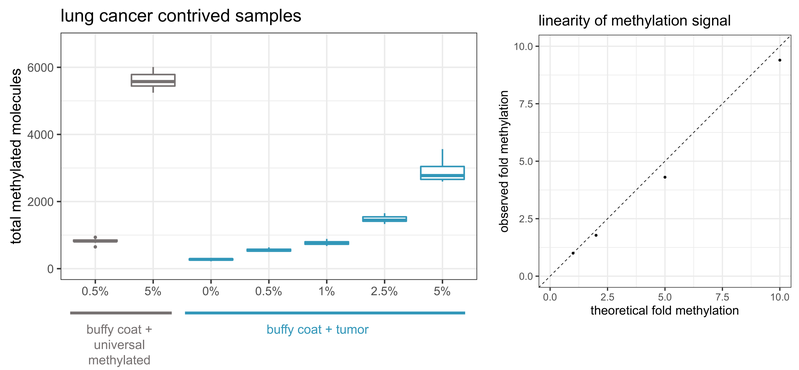
method	CV	multiplex ability	absolute quantification
qPCR	high	poor	no
ddPCR	low	poor	yes
NGS	high	good	no
NGS + QCTs (BillionToOne)	low	good	yes

Table 1: Comparison of methods to quantify methylation in cfDNA

METHODS

- We designed a multiplex PCR targeting 113 locations chosen to maximize hypermethylation in lung adenocarcinoma and lung squamous cell carcinoma tumors compared to normal tissue using publicly available data from the TCGA
- Quantitative Counting Templates[1] were designed and added to the PCR for absolute molecule quantification at each locus
- Samples were bisulfite converted (Diagenode Premium Bisulfite Kit), amplified, indexed, and finally sequenced on an Illumina NextSeq 2000 using P3 100 cycle reagent kits
- Reads were aligned, classified as methylated or unmethylated, and summed. QCT sequences were analyzed to calculate the total number of methylated molecules at each locus

ANALYTICAL RESULTS



tumor fraction	0.5%	5%	0%	0.5%	1%	2.5%	5%
mean	824	5633	275	553	769	1470	2884
standard deviation	64	255	29	41	61	91	298

- Contrived samples mimicking cell-free DNA (cfDNA) were created by mixing sheared tumor genomic DNA (gDNA) with buffy coat gDNA from the same cancer subject at various tumor fractions (5000 genomic equivalents per sample). Contrived samples using sheared universally methylated gDNA were included as a control.
- The low technical noise of the assay means we can distinguish between 0.5% and 0.55% tumor fraction, which is remarkable for a treatment monitoring application.
- Furthermore, our assay can separate 0% and 0.5% tumor fraction samples. This limit of detection is relevant for minimal residual disease applications.
- The amount of background signal can be removed by subtracting the methylation in the 0% tumor fraction condition. The residual relative methylation signal is linearly correlated with the contrived tumor fraction, indicating that our assay can accurately measure the amount of tumor.

CONCLUSIONS

- We have developed a methylation assay that can quantify the absolute number of methylated molecules in a sample without needing tumor tissue.
- Our assay can detect very small levels of tumor fraction (<0.5%) as well as very small differences in tumor fraction in contrived cfDNA samples.
- Longitudinal methylation measurements from an initial cohort of lung cancer subjects appear to correlate with clinical outcomes. Testing additional clinical samples and comparing with clinical outcomes is needed to further validate this assay.
- The promising results from this study suggest that we can achieve similar performance levels for treatment monitoring and potentially minimal residual disease (MRD) applications in other cancer types.

CLINICAL RESULTS

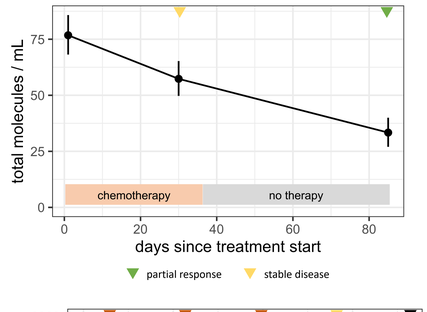
- For this clinical study, we partnered with a clinical research organization to recruit subjects with lung cancer to participate in this longitudinal study. All subjects consented to participating in this study. Clinical outcomes were transcribed from physician notes at the time of sample collection. Sum of longest diameters (SLD) was calculated from radiologist notes of scan images.
- 3 Streck tubes were collected at each time point: one pre-treatment time point and two post-treatment time points. Blood tubes were shipped overnight to BillionToOne, and plasma and buffy coat were isolated upon receipt
- cfDNA and buffy coat samples from an initial cohort of N = 4 subjects were tested. One subject was excluded due to assay failure in cfDNA samples at two collection time points.
- Target loci with high methylation in buffy coat were masked from cfDNA methylation analysis. Plotted error bars indicate 95% confidence interval assuming molecular sampling noise

Subject 107034

71 yo F
Squamous cell carcinoma of bronchus in right lower lobe

Staging at first collection:
Stage IIIB (cT4, cN2, cM0)

Chemotherapy:
paclitaxel 72mg
carboplatin 175mg
1 time a week



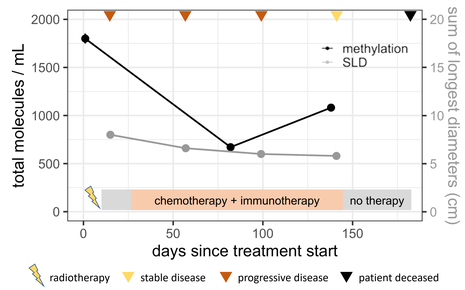
- We measured decreasing methylated molecules post-treatment, suggesting that the chemotherapy was effective at reducing tumor burden.
- Methylation measurements correlated with reported stable disease and partial response clinical outcomes.

Subject 107355

74 yo M
NSCLC

Staging at first collection:
Stage IVB (cT1, cN3, pM1c)

Chemo + immunotherapy:
carboplatin 375mg
pemetrexed 875mg
pembrolizumab 200mg
1-2 times per month



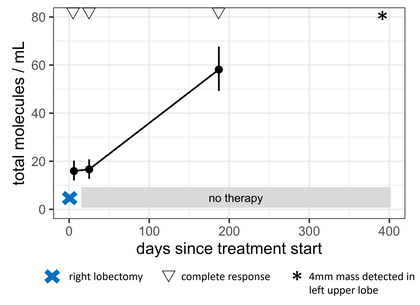
- Our methylation assay measured an initial decrease in methylated molecules which correlated with the decrease in sum of longest diameters (SLD) of target lesions.
- Radiotherapy was most likely effective at initially decreasing tumor burden despite the appearance of new lesions indicating progressive disease.
- Methylation subsequently increased before the subject passed away after 189 days.

Subject 106996

58 yo M
NSCLC

Staging at first collection:
Stage IA3 (cT1c, cN0, cM0)

Surgery:
right lobectomy and lymph node excision



- We did not measure any significant changes in methylation immediately post-surgery.
- Interestingly, we measured an increase in methylation at the third time point, several months ahead of identification of a small mass on imaging.

Acknowledgements

We would like to thank Christian Klosowski, Rafael Velasquez Valle, Randi DeArmitt, and Dorothy Breckner for compiling clinical outcome data, and Ochsner Medical Center and Accio Biobank Online for obtaining the clinical research samples.

References

1. Tsao DS et al. A novel high-throughput molecular counting method with single base-pair resolution enables accurate single-gene NIPT. Sci Rep. 2019 Oct 7;9(1):14382.