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A method for Quantifying circulating tumor DNA level and Molecular Response using Methylome Sequencing

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Introduction

Circulating tumor DNA (ctDNA) level and change in ctDNA level on-treatment are promising tools for predicting patient prognosis and response to therapy.

Existing methods commonly use variant allele frequency (VAF) of somatic mutations to quantify circulating tumor fraction (cTF). Their performance can be limited by the number of detectable somatic mutations and the associated limit of detection (LoD), as well as interference from copy number variation and non-tumor alterations, such as clonal hematopoiesis (CH). Moreover, previous studies also show that 30-50% patients with stage I-III cancer, and 15-20% patients with stage IV cancer, lack detectable somatic mutations[1].

GuardantINFINITY, our next-generation oncology liquid biopsy platform, provides a unique combined genomic and epigenomic molecular profile revealing unseen insights distinctive to each sample from a single blood draw. Here, we evaluated the limit of quantitation (LoQ) of a novel cTF method on the GuardantINFINITY epigenomic panel which allows for near genome-wide methylation detection, and compared the performance to a genomic-based method.

Methods

The epigenomics cTF (or methyl cTF) of a single sample is estimated from methylation signals across targeted regions of the GuardantINFINITY methylation panel, calibrated using our internal training data that has clinical blood draw samples of over 5,000 individuals, including cancer-free donors and patients with mixed cancer types. Somatic mutations were also detected through the GuardantINFINITY genomic panel. The genomic cTF (or somatic cTF) is defined as the highest VAF of detected somatic mutations.

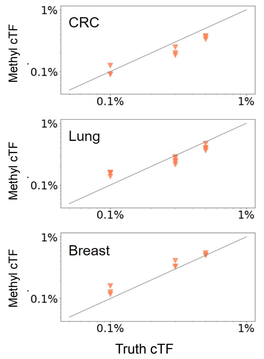
LoD was determined as the minimum cTF level at which >=95% of replicates exhibited methylation signals derived from tumors. LoQ was defined as the minimum cTF level at which the coefficient of variation (CV) across replicates was less than 30%. The accuracy of methylation-based cTF was compared to cTFs derived from the maximum MAF of somatic mutations on 5,045 clinical samples of cancer patients.

Reference

1. Cescon, David W., et al. "Circulating tumor DNA and liquid biopsy in oncology." Nature Cancer 1.3 (2020): 276-290

Results

The performance of methyl cTF on clinical titrations



Truth cTF	%CV of Methyl cTF		
	CRC	Lung	Breast
0.5%	4.7%	9.2%	2.3%
0.3%	6.3%	8.2%	4.9%
0.1%	9.0%	9.6%	5.5%
LoQ	<0.1%	<0.1%	<0.1%

Figure 1 & Table 1. Accuracy, Limit of Quantification (LoQ) and Coefficient of Variation (CV) of methyl cTF in replicates of clinical titrations.

Clinical titrations = clinical cancer samples experimentally titrated into a cancer-free donor sample at known fractions

One colorectal cancer sample, one breast cancer sample, one lung cancer sample, and one cell line sample were titrated into cancer-free backgrounds at target levels ranging from 0.1% to 0.5% MAF. The methylation LoD, which was defined as the lowest concentration of tumor-derived DNA detectable with >95% accuracy, was estimated to be approximately 0.05% at the input level of 5-30ng.(See poster #6601 “Analytical validation of a robust integrated genomic and epigenomic liquid biopsy for biomarker discovery, therapy selection and response monitoring”).

The methyl cTF of clinical samples exhibit a high degree of consistency with underlying titration levels and maintain a strong linearity between different titration levels, as indicated by a Pearson-r of greater than 0.9 and a linearity error less than 5% (Figure 1).

The quantitative precision of the methyl cTF is capable of reaching an LoQ of less than 0.1% in CRC, lung and breast clinical samples (Table 1). The genomic cTF is robust for replicates within the same cTF levels, particularly at cTF levels of 1% or higher (Figure 2, left panel). However, at lower titration levels, the methyl cTF is more stable. The epigenomic cTF can maintain a 100% evaluation rate and has a LoQ down to 0.1% cTF (Figure 2, right panel).

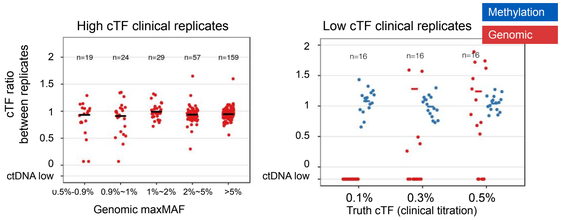
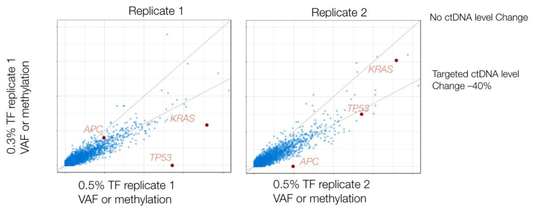


Figure 2. Estimated cTF ratio between replicates.

In somatic-mutation based methods, 15-20% stage iv patients have no detectable signals (“ctDNA low”). With methylation, there are still >1,000 regions with detectable signals at cTF as low as 0.1%.



The robustness of methyl cTF is attributed to the high number of "evaluable" regions in the panel. Specifically, in two technical replicates of a clinical colorectal cancer (CRC) sample with titration levels at 0.5% and 0.3% cTF, the methyl cTF was estimated based on thousands of regions, whereas the genomic cTF can only be estimated from three detectable somatic mutations (Figure 3 and Table 2).

Method	Targeted ctDNA Level Change	#Alterations Tracked	# Tumor Molecules in calculation	ctDNA Level Change
Genomic-only	~40%	3 somatic variants	~100	Rep 1: ~84%, Rep 2:~30%
Methylation	~40%	>>1000 methylated regions	~100,000	Rep 1: ~39%, Rep 2: ~39%

Figure 3 & Table 2. Methylation signals and somatic mutations in two pairs of replicates of clinical titrations (0.5% vs 0.3% cTF).

Methyl cTF on clinical patient samples

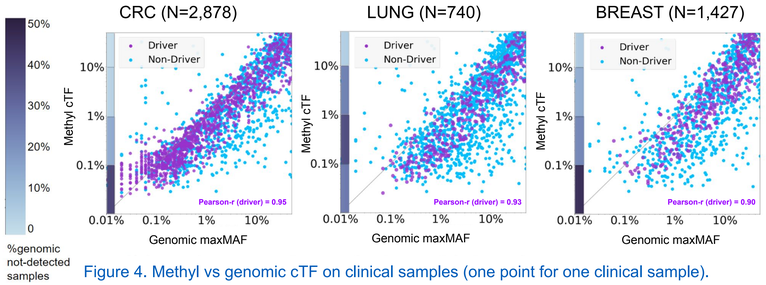


Figure 4. Methyl vs genomic cTF on clinical samples (one point for one clinical sample).

“Driver” = genomic maxMAF from predefined “driver” genes (more accurate representation of true cTF).
Color of left side bars show methyl cTF of samples that do not have detectable somatic mutations

In a cohort of 5,045 clinical samples (CRC, lung, and breast cancer patients, (N=522, 909, 696 and 784 for stage I to IV, together with 2,656 of unknown stage), 64% had somatic mutations, and 90% showed evidence of the ctDNA presence based on methylation analysis. Notably, in Figure 4 with Pearson-r(driver) >0.9, methyl cTF is highly consistent with somatic MAFs from “driver” mutations, which may be a more accurate representation of cTF than “non-driver” mutations.

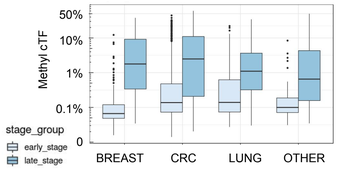


Figure 5. The methyl cTF distribution in early and late stage cancer patients

Upon analyzing 231 additional samples from various cancer types, we found that the majority of samples lacking detectable somatic mutations had epigenomics tumor fractions below 0.1% (side bars of Figure 4). Samples from early-stage cancer has a significantly lower cTF than late-stage cancer (Figure 5, paired t-test p<0.01). In these early-stage samples, somatic mutation-based methods are unable to detect evidence of ctDNA.

Conclusions

- With methylome sequencing, GuardantINFINITY enables accurate quantification of ctDNA level with a liquid-only approach, providing advantages compared to traditional quantification with somatic VAF:
  - GuardantINFINITY accurately detects and quantifies cTF in patients without detectable somatic mutations, offering more patients easy-to-access longitudinal ctDNA monitoring tools.
  - Based on thousands of differentially methylated regions, the quantitation from GuardantINFINITY provides greater precision in repeated measurement compared to somatic VAF.