GUARDANTREVEAL™

Quantifying ctDNA Using a Tissue-Free Test for Minimal Residual Disease (MRD) Detection

Important Note: Guardant Reveal on Infinity was developed, and its performance characteristics were determined, by the Guardant Health Clinical Laboratory in Redwood City, CA, USA, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. Guardant Reveal is a Laboratory Developed Test.

Background

Minimal Residual Disease (MRD) describes the presence of tumor cells after a curative intent surgery or therapy. Genomic and/or epigenomic MRD assays have been developed to identify circulating tumor DNA (ctDNA) in blood from patients with early-stage solid tumors. The presence of ctDNA after the completion of curative intent treatment is prognostic, indicating a high likelihood of cancer recurrence.[1,2] Additionally, emerging data indicate that initiating adjuvant treatment may reduce the risk of recurrence for patients with ctDNA detected after surgery.[3]

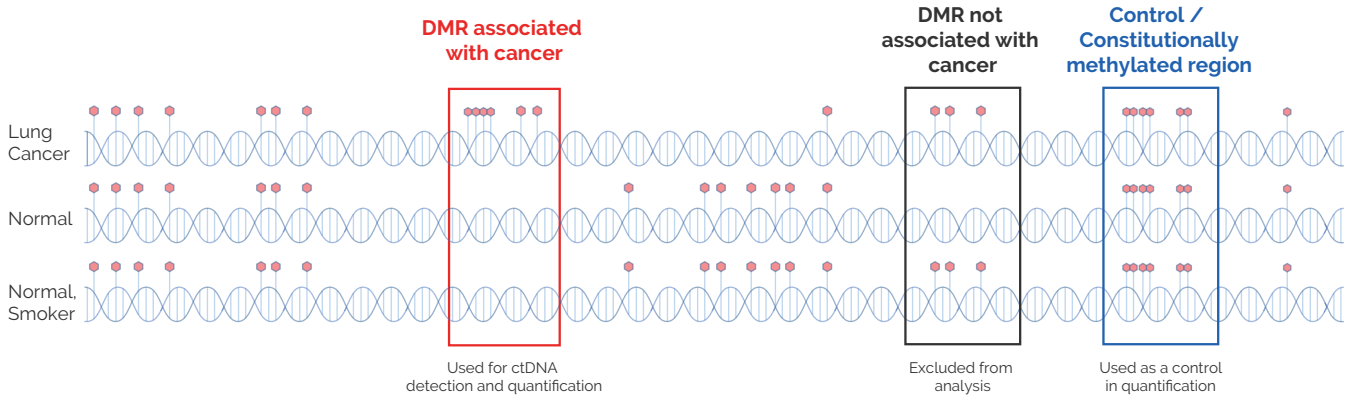
Guardant Infinity is the first commercially available platform that integrates epigenomics and genomics to transform precision oncology. In this context, we describe how Guardant Reveal, a tissue-free, MRD monitoring test that analyzes thousands of epigenetic signals, detects and quantifies ctDNA in patients with early-stage cancer using the Guardant Infinity platform.

Methylation and Cancer

Genomic alterations significantly contribute to carcinogenesis by activating oncogenes, inactivating tumor suppressor genes, and inducing genomic instability. Epigenomic alterations also play a significant role in carcinogenesis by affecting gene expression.[4] Methylation, a chemical modification of DNA, is one type of epigenomic alteration.[4] Methylation can serve as an early driver of tumorigenesis by either silencing tumor suppressor genes or activating oncogenes.[5]

Compared to normal cells, tumor cells exhibit a distinct methylation pattern.[6] Analyzing differentially methylated regions (DMRs) associated with specific solid tumors can differentiate normal cell-free DNA (cfDNA) from cancer-derived ctDNA (Figure 1).

Figure 1. Identification of DMRs to detect ctDNA



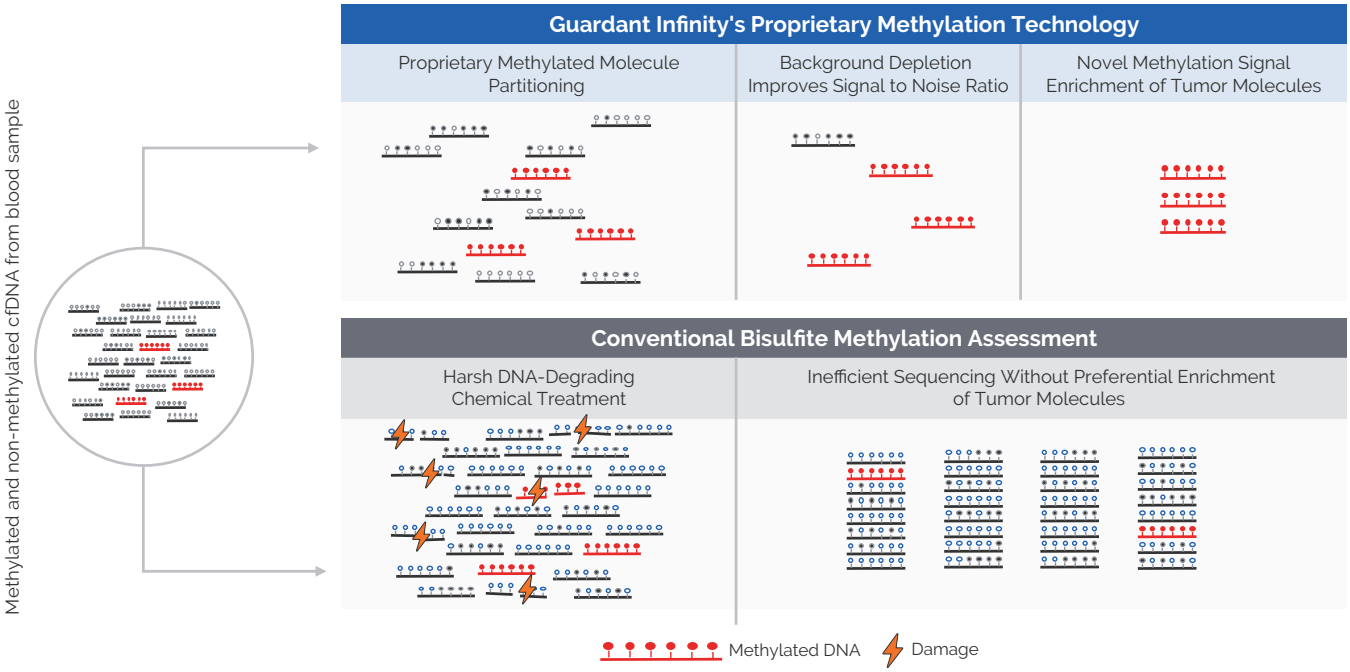
Informative methylation regions were identified by comparing normal vs. cancer samples from large cohorts

Guardant Reveal evaluates DMRs to identify ctDNA. To develop this approach, Guardant Health analyzed nearly 5,000 samples from both cancer-free donors and from patients with cancer to identify DMRs that accurately and reliably identify ctDNA molecules in plasma samples. Regions of hypermethylation distinctive to cancer samples and absent in cancer-free samples were identified from over 1,700 patients with colorectal, breast, lung, or bladder cancer and 3,000 cancer-free donors, including patients with common comorbidities. These distinctive regions are utilized to identify and detect ctDNA with Guardant Reveal.

Guardant Reveal Employs a Novel Method for Methylation Analysis and ctDNA detection

Bisulfite sequencing is the most common method for analyzing DNA methylation, but requires harsh chemical treatment, leading to significant molecule loss and low sequencing efficiency.[7] Molecule loss impairs assay performance in the context of MRD, where low tumor burden is typical. To achieve highly sensitive detection of ctDNA through methylation analysis, Guardant Health developed the Guardant Infinity platform. Guardant Infinity's proprietary technology separates methylated from non-methylated cfDNA molecules and preferentially enriches methylated tumor DNA, minimizing molecule loss and reducing background noise (Figure 2). Guardant Reveal utilizes this technology for MRD detection.

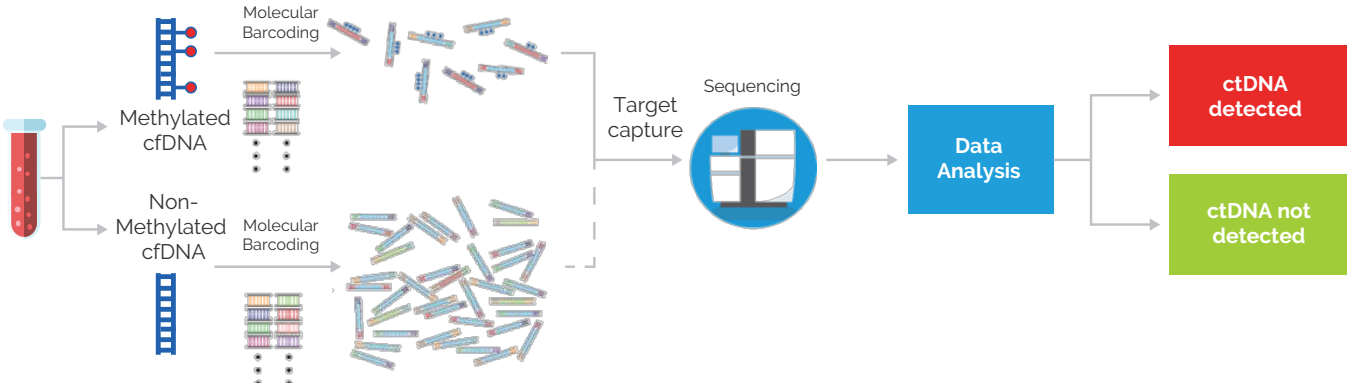
Figure 2. Methylation analysis approaches



Guardant Reveal analyzes cell-free DNA (cfDNA) extracted from plasma to determine the presence and quantity of tumor-associated cfDNA, eliminating the need for tumor tissue or separate sequencing of peripheral blood mononuclear cells (PBMCs). Briefly, the process is as follows:

1. Using automated methods, cfDNA is extracted from plasma, partitioned based on its methylation state, repaired, ligated with custom-made adapters for molecular tracking, and then amplified to create libraries.
2. The libraries are subsequently enriched for specific regions using an epigenomic panel that targets tumor-associated DNA methylation signatures.
3. The enriched libraries undergo next-generation sequencing. The sequencing data is analyzed using Guardant Health's proprietary bioinformatics pipeline software (BIP), which is trained to detect the presence of ctDNA based on epigenomic signals.
4. Following the bioinformatics analysis, Guardant Reveal reports either "ctDNA detected" along with an estimated tumor fraction, or "ctDNA not detected".

Figure 3. Guardant Reveal workflow



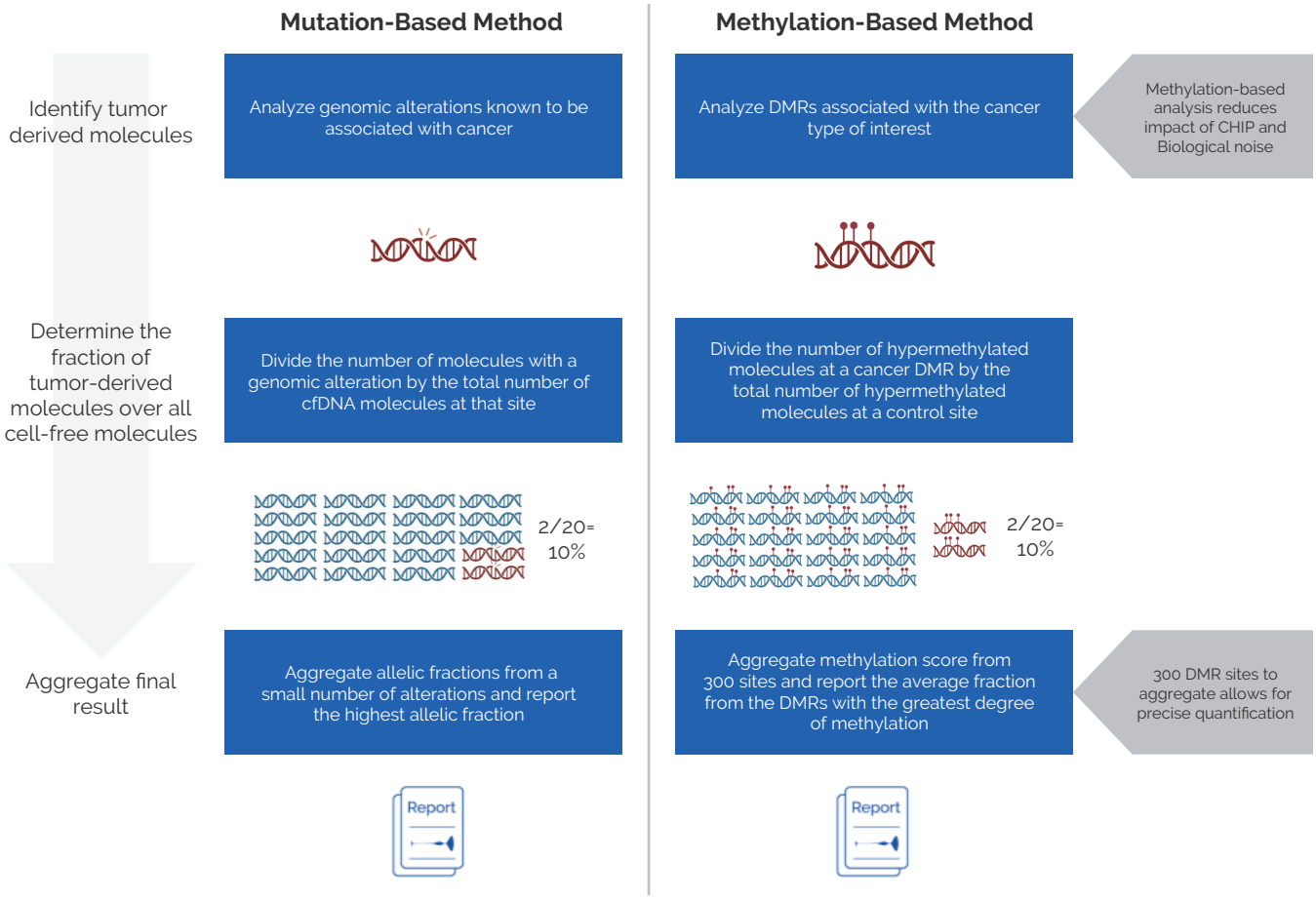
Quantification of Tumor Fraction on Guardant Reveal Powered by Guardant Infinity

The presence or absence of ctDNA after completion of curative intent therapy has been shown to predict cancer recurrence. Quantifying ctDNA to monitor tumor burden and ctDNA dynamics is increasingly important. A patient's tumor burden can be estimated by comparing the fraction of ctDNA to the total cfDNA molecules in a sample. The maximum genomic allele fraction (maxMAF) of a genomic assay can be used to quantify tumor burden.

However, this method may have limited precision and accuracy in the early-stage setting, where tumor shedding is lower than in the metastatic setting. Tumor fraction can also be derived from methylation assays, such as Guardant Reveal, by assessing the ratio of methylated molecules in differentially methylated regions compared to the total number of methylated molecules in constitutively methylated regions (Figure 4). Tumor fraction derived from methylation reduces the impact of clonal hematopoiesis of indeterminate potential (CHIP) and biological noise, thus improving precision and accuracy.

Figure 4. Process of deriving tumor fraction on Guardant Reveal

Methylation-based quantification method is similar to the mutation-based method but reduces impact of CHIP and biological noise, thus improving precision and accuracy.



Briefly, the process of generating tumor fraction through a methylation-based method is as follows:

- In a patient's sample, cancer specific DMRs are normalized with appropriately matched control regions. This normalization is achieved by dividing the methylated molecule count for each cancer DMR by the methylated molecule count of the matched control region. The matched control region is identified as a region with similar opportunity for methylation across samples and patients.
- A methylation percentage is calculated for each cancer-specific DMR, derived from the count of methylated molecules.
- The DMRs with the highest scores are selected and averaged to calculate the tumor fraction.

Early Insights: Guardant Reveal and Tumor Fraction

In an interim analysis of COSMOS*-CRC-01 utilizing Guardant Reveal[8], which included over 130 patients and more than 700 samples, all patients were evaluable for MRD analysis without the need for tumor tissue. This cohort included patients with R0 stage II or III colon cancer. When assessing outcomes at the 28-day, post-operative time point, a significant difference was observed in 24-month recurrence-free survival between patients with detected ctDNA and those without (Figure 5A).

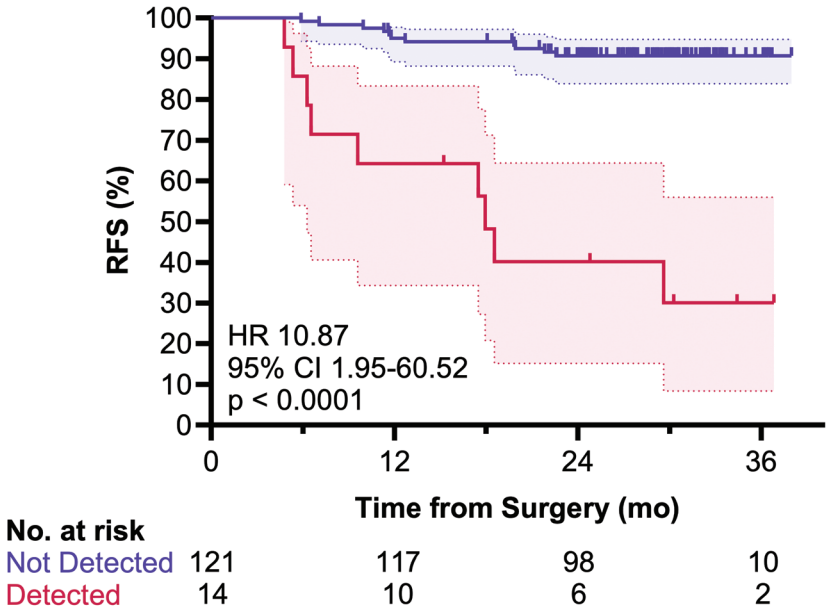
Additionally, surveillance monitoring, which involved patients with two or more post-surgery samples, was conducted in 134 patients, providing early insights into tumor fraction relative to treatment and recurrence. Among 12 patients who experienced recurrence, each having at least two post-surgery samples, the estimated tumor fraction increased as the time of recurrence approached (Figure 5B). Tumor fraction was also influenced by adjuvant therapy, as evidenced in select patient examples below.

For patients who had more than two post-surgery samples and were included in the surveillance analysis, a high sensitivity of 80% for detecting recurrence was achieved, with a specificity of 98%**. This interim analysis from a large, prospective ctDNA monitoring study demonstrates sensitive and specific MRD detection and tumor fraction quantification in stage II-III colon cancer using a tissue-free epigenomic assay.

* The COSMOS acronym stands for Conquer Solid Malignancies by blood screening
** Among samples for those with at least one year of follow-up.

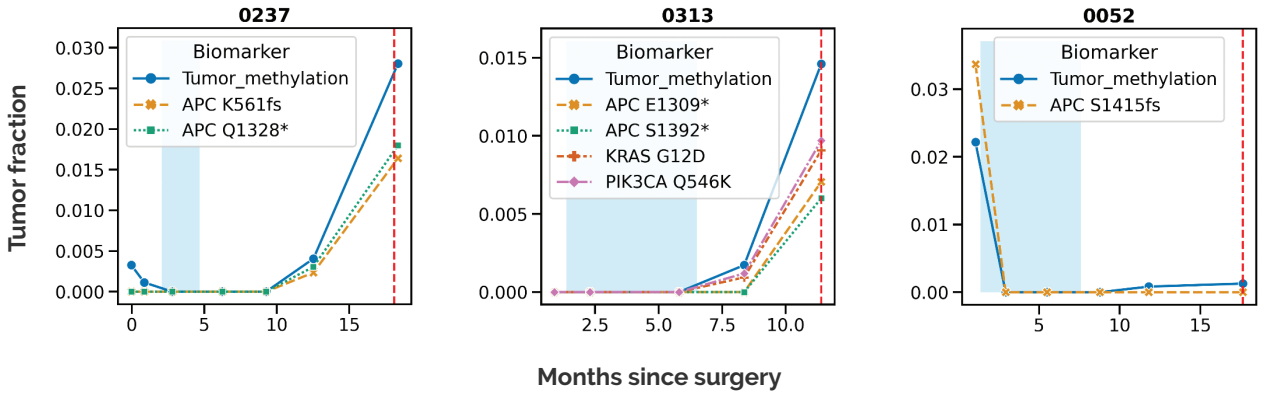
Figure 5. Recurrence-free survival (RFS) and Tumor Fraction from clinical validation cohort

A. Recurrence-free survival from single, post-operative time point prior to ACT, if given (n=135)



ctDNA Status	Not Detected	Detected
No. of Events / Total No.	11 / 121	9 / 14
Median RFS	Not Reported	18.0 mo
24M RFS (95% CI)	90.7% (83.9-94.8)	40.2% (15.1-64.4)

B. Tumor Fraction trends concordant with genomics, treatment, and recurrence



Notes: "Tumor_methylation" is tumor fraction and can be tracked by the blue line. The blue box defines when patient received adjuvant therapy. The red, dotted, vertical line is when recurrence occurred.*Denotes mutation type and means the protein is now a stop codon, which is annotated here as an asterisk.

Summary

- Guardant Reveal leverages the Guardant Infinity platform to preserve methylated regions in cell-free DNA, enabling highly efficient detection of minimal residual disease.
- Guardant Reveal calculates ctDNA tumor fraction from the Guardant Infinity platform using an epigenomics approach, determined by comparing the degree of methylation across thousands of DMRs compared with that in controls.
- Early analysis demonstrates the ability to detect and report tumor fraction clinically using an epigenomics-based assay.
- Guardant Health continues to lead liquid biopsy technology with the Guardant Infinity platform, including accelerating advancements in the field of minimal residual disease with Guardant Reveal.

References

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