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Liquid Biopsy and NGS: Driving translational clinical research to the next level

Cell-free DNA holds the potential to unravel tumor heterogeneity.

Introduction

Cell-free DNA (cfDNA) circulating in the blood can arise from various tissues, tumors, or other microorganisms present in the body. Liquid biopsy analysis of cfDNA in blood is particularly useful for assessing and monitoring certain diseases, including cancer.

Tumors can shed a significant amount of DNA. The DNA released from a dead tumor cell, referred to as circulating tumor DNA (ctDNA), represents a small fraction of the total cfDNA in the blood. Therefore, a robust assay is required to detect somatic mutations at low frequencies.

Next-generation sequencing (NGS) methods enable highly sensitive and specific detection of known mutations. More recently, comprehensive assays are being developed to analyze a wider range of candidate genes and variant types for new tumors that do not have known variants. As these methods evolve, ctDNA analysis for various applications such as screening, therapy selection, monitoring, and identification of therapy resistance is gaining prominence as a method for monitoring disease state.

Advantages of liquid biopsy over tissue biopsy

As a relatively noninvasive method for sample aquisition, liquid biopsies are especially valuable when the tissue of interest is inaccessible. Even when diseased tissue can be accessed, rebiopsy for monitoring is desired for many diseases for which cfDNA analysis offers several advantages.

Liquid biopsy sample acquisition can be done through common phlebotomy methods for which properly trained professionals are abundant. Tissue biopsy, on the other hand, often requires specialized skills from qualified technicians or surgeons. For this essential step, liquid biopsy is more cost effective, offers a shorter turnaround time, and has less chance of adverse associated events. After the sample is acquired, DNA extraction methods optimized for cfDNA analysis are quicker and less expensive than dealing with formalin-fixed paraffin-embedded (FFPE) samples. Access to specific tissues can severely limit tissue biopsies for initial assessments or repeat biopsies. Guidelines from the National Comprehensive Cancer Network (NCCN) have been revised to recommend liquid biopsy for certain tumor types, especially in cases where tissue biopsy is not an option, such as non-small cell lung cancer.[1]

Although ctDNA coming from a specific tissue source represents only a small fraction of total cfDNA, cfDNA analysis has been refined to become a tool for assessing tumor heterogeneity and overcoming tissue sampling bias. ctDNA analysis can be used to monitor disease progression and response to treatment. With sequencing methods that can identify new mutations, liquid biopsy can be especially useful for monitoring acquired resistance arising from new alterations. As sensitivity increases and assays that assess numerous genes simultaneously are developed, cfDNA can be used for comprehensive tumor profiling (Figure 1). Thus, liquid biopsy can identify new mutations during disease progression and mutations arising in new tissues outside of the original tumor source.

Technologies used to analyze ctDNA

The most commonly used molecular methods for analyzing cfDNA are quantitative PCR (qPCR), droplet digital PCR (ddPCR), and NGS. Both PCR methods involve using specific DNA probes to target specific genes and output a quantitative measurement of the number of targets in the sample. NGS also involves using probes to capture specific DNA fragments, but the data output is the sequence of the captured DNA.

qPCR: qPCR is efficient when analyzing a small number of variants. However, qPCR assays are limited to the relatively few targets that are specified and assess only specified variant types, thus offering little discovery value.

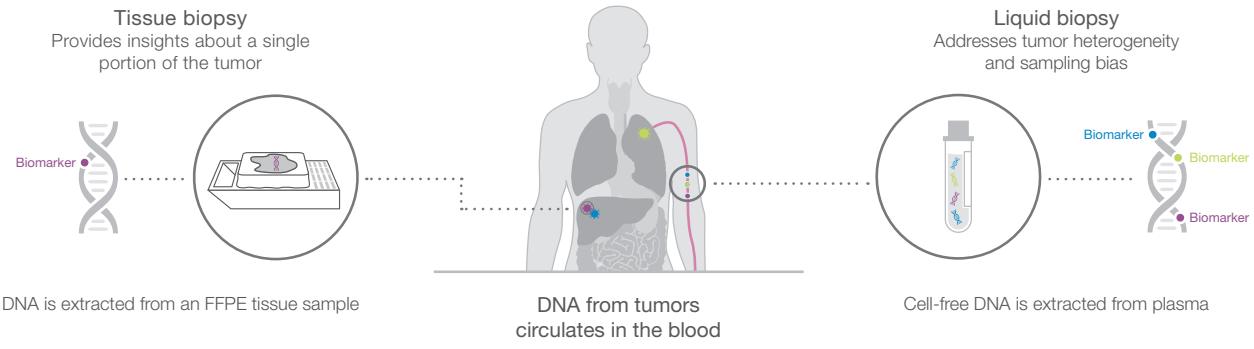

Figure 1: New molecular methods can assess numerous tissues and biomarkers from plasma samples—Comprehensive genomic profiling using cfDNA consolidates hundreds of markers into a single assay.

Table 1: Comparison of PCR and targeted NGS

Method	Benefits	Challenges
PCR	High sensitivity Familiar workflow Capital equipment already available in most labs	Can only interrogate a limited set of variants with low resolution Virtually no discovery power Low scalability due to increasing sample input requirements
Targeted NGS	Higher sequencing depth enables higher sensitivity (down to 0.5%) Higher discovery power, ideal for addressing tumor heterogeneity Higher variant resolution Higher sample throughput with sample multiplexing	Not as cost effective for sequencing low numbers of targets (< 20) Not as time efficient for sequencing low numbers of targets (< 20)

ddPCR: ddPCR is superior to qPCR in accuracy, although there is additional cost and technical expertise associated. ddPCR also lacks discovery value as the number of targets and variant types are limited to the design of the specific assay.

NGS: Because NGS involves single-nucleotide resolution of DNA sequences, the ability to discover new variants is possible without prior knowledge included in the assay design. This enables assessment of multiple variant types and discovery of mutations in a new location of a gene. NGS is more expensive and time-consuming when analyzing a small number of variants or samples. But, when assays are designed to cover more molecular targets, the comprehensive nature of NGS can provide value in efficiency and cost-savings (Table 1).

Advantages NGS offers for liquid biopsy

NGS sequences millions of DNA fragments in parallel, followed by computational alignment of reads to the genome. Depending on the assay design, NGS can be highly comprehensive, covering large numbers of gene targets and variant types. As the number of actionable biomarkers in cancer treatments continues to grow, the ability to consolidate a large number of biomarkers into one test will likely become more valuable in both research and clinical settings by reducing the number of tests needed to find meaningful answers. NGS has the potential to enable savings of sample, time, and money by avoiding iterative testing.

Options for sequencing library preparation, such as hybrid–capture chemistry, enable large fragments of targeted genes to be pulled out of cfDNA samples. Hybridization probes can be designed large enough to capture targets even when mutations exist in the hybridized regions. Subsequent sequencing of captured targets allows discovery of new mutations for which prior knowledge is not required during assay design. NGS assays can be designed to either target a large number of genes with low sequencing depth (more comprehensive, less sensitive), or a relatively small number of genes with higher sequencing depth (less comprehensive, more sensitive). In the case of liquid biopsy, in which the fraction of ctDNA within a cfDNA sample is potentially low, high sequencing depth is necessary to provide the sensitivity needed to detect low-abundance variants accurately. Therefore, although NGS gene panels exist with comprehensive content, the application to liquid biopsy was limited until recently due to sensitivity challenges.

NGS enables comprehensive genomic profiling

Recent improvements in sequencing instrumentation provide options for sequencing samples at extremely high depth of coverage for large portions of the genome in a single sample. By providing comprehensive value combined with high sensitivity and specificity, NGS enables analysis of hundreds of genes with the sequencing depth required for cfDNA analysis. These features provide assessment of a large number of known mutations and enable discovery of new driver mutations in cancer research. For improved accuracy, new molecular tools and bioinformatic methods are available. Unique molecular identifiers (UMIs) can be integrated into DNA library preparations to tag individual DNA molecules prior to amplification steps and later used during data analysis to identify PCR-introduced errors. Sophisticated algorithms identify sequencing artifacts and reduce error-inducing background noise, facilitating identification of true variants with high specificity.

Liquid biopsy combined with comprehensive molecular assays to assess somatic variants enables detection of new mutations arising from tumor evolution, drug resistance, and metastasis. Cancer is an unpredictable disease in which driver genes are not always known, or correctly estimated by tissue type. With the simultaneous ability to assess numerous genes and numerous tissues, the synergy of liquid biopsy with NGS-enabled comprehensive genomic profiling offers high value. Recent studies that performed liquid biopsy paired with corresponding tissue biopsy from tumor samples have demonstrated that, when comprehensive assays are used, cfDNA analysis detected a significant number of guideline-recommended biomarkers and resistance alterations not found in matched tissue biopsies.[2,3]

Summary

An increasing number of biomarkers in the precision medicine era is driving a corresponding increase in interest for molecular methods that can consolidate numerous biomarkers in a single test. On the other hand, studies have shown that liquid biopsy, with a broader view of systemic tumor evolution, can yield valuable information for certain tumor types that might be missed with localized tissue biopsy. With recent advancements in NGS technology, both of these goals can be accomplished, enabling comprehensive genomic profiling combined with the sensitivity and specificity required for liquid biopsy applications.

References

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