

Liquid Biopsy Offers Advantages in the Modern Age of Oncology Research

Innovations in molecular assays enable comprehensive, precise analysis of cell-free DNA.

Introduction

Plasma-based assays have emerged for the purpose of detecting genetic variations. Cell-free DNA (cfDNA) circulating in the blood can arise from various tissues in the body. But because a disease-affected tissue only represents a portion of cfDNA, lack of sensitivity has prevented the widespread use of liquid biopsy until recently. However, molecular methods have evolved to enable detection of low-level molecular biomarkers in cfDNA with high specificity. Next-generation sequencing (NGS) provides the added benefit of assessing a large number of known biomarkers, or the discovery of new mutations, from a single sample.

Although liquid biopsy can involve many biological sample types, this application spotlight reviews analysis of cfDNA in blood. Cell-free DNA is particularly useful for assessing and monitoring certain diseases. It has been used to analyze genetic abnormalities in fetal DNA via sampling of maternal blood (noninvasive prenatal testing, or NIPT), monitoring organ rejection after transplantations, infectious diseases, and cancer.

Tumors can shed a significant amount of DNA, although the amount can vary according to tumor type. The DNA released from a dead tumor cell, also 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. Molecular methods have been developed for highly sensitive and specific detection of known mutations, and 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 such methods evolve, ctDNA analysis has been developed for various applications such as screening, therapy selection, monitoring, and identification of therapy resistance.

What advantages does liquid biopsy have over solid tissue biopsy?

As a relatively noninvasive assay, 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 in the analysis, liquid biopsy is more cost-effective, with a shorter turnaround time, and less chance of adverse associated events. Once the sample is acquired, DNA extraction methods for cfDNA analysis have also been optimized such that it is quicker and less expensive than dealing with formalin-fixed paraffin-embedded (FFPE) samples. Access to specific tissues can severely limit tissue biopsies either for initial assessments, or for repeat biopsies. Guidelines from NCCN and AMP 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 well enough to become a tool for assessing tumor heterogeneity and overcoming tissue sampling bias. During monitoring of disease progression, ctDNA analysis can monitor response to treatment in the original tumor location, and also assess metastasis to other locations in the body. With the use of sequencing methods that can identify new mutations, liquid biopsy can be especially useful for monitoring acquired resistance arising from new mutations. With the development of sensitive assays that assess numerous genes in a single assay, cfDNA can be used for comprehensive tumor profiling. Thus, liquid biopsy can not only identify new mutations during disease progression, but also mutations arising in new tissues outside of the original tumor source.

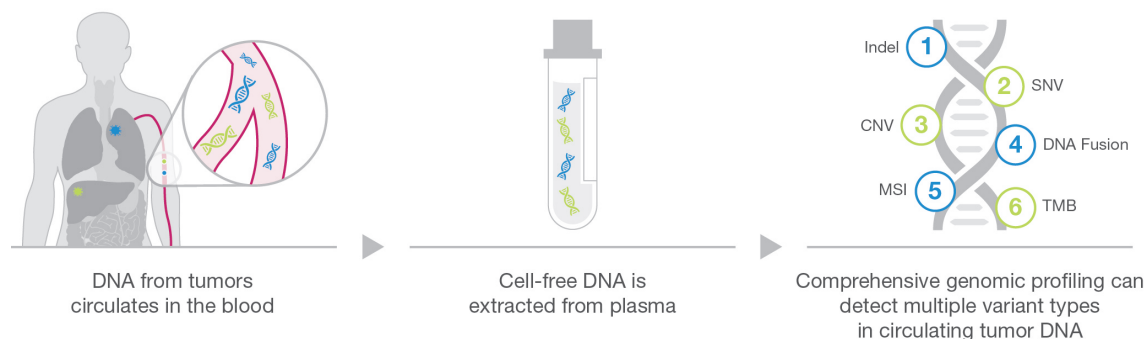


Figure 1: New molecular methods can assess numerous biomarkers and numerous tissues from plasma samples—Comprehensive genomic profiling, which is now possible from cfDNA, consolidates hundreds of markers into a single assay.

What technologies are 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 the data output is 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. Because multiple reads can be used to assess the number of target molecules present, quantitative measurements can also be done with NGS.

qPCR: Among the molecular methods discussed here, qPCR was developed the earliest. qPCR is sensitive enough for cfDNA analysis, and has the benefits of being inexpensive and 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.

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 not only assessment of multiple variant types, but also discovery of mutations in a new location of a gene. Thus, with a hypothesis-free approach, NGS provides high discovery value which can be useful for evaluation of any tumor with the potential to present new variations. 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).

What advantages does NGS offer for liquid biopsy?

NGS involves millions of DNA fragments being sequenced in parallel, followed by computational alignment of reads to the genome. Depending on the assay design, NGS can be highly comprehensive with both large numbers of gene targets and types of variants. 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 to be large enough to capture targets even when mutations exist in the hybridized regions. Subsequent sequencing of captured targets allow 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 to detect low-abundance variants accurately. Therefore, although NGS gene panels have existed with very comprehensive content, the application to liquid biopsy was limited until recently due to sensitivity issues.

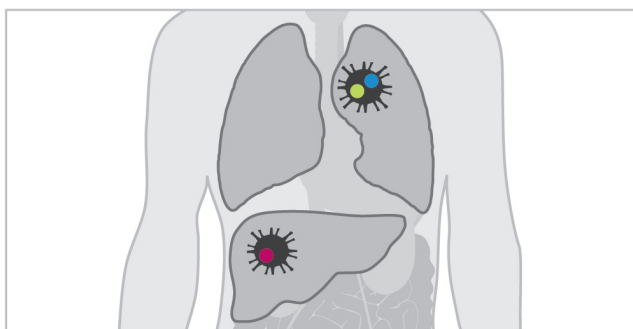
Table 1: Comparison of PCR and targeted NGS

Method	Benefits	Challenges
PCR	High sensitivity	Can only interrogate a limited set of variants
	Familiar workflow	Virtually no discovery power
	Capital equipment already available in most labs	Limited variant resolution
		Low scalability due to increasing sample input requirements
Targeted NGS	Higher sequencing depth enables higher sensitivity (down to 1%)	Not as cost-effective for sequencing low numbers of targets (< 20)
	Higher discovery power	Not as time-efficient for sequencing low numbers of targets (< 20)
	Higher variant resolution	
	Produce more data with the same amount of input DNA	
	Higher sample throughput with sample multiplexing	

NGS can provide specificity, sensitivity, and more data

Recent improvements in sequencing instrumentation provide options for sequencing samples at extremely high depth of sequencing coverage for large portions of the genome in a single sample. By providing comprehensive value combined with high sensitivity and specificity, NGS enables the analysis of hundreds of genes with the sequencing depth that is required for cfDNA analysis. These features not only provide assessment of a large number of known mutations, but also 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, thus enabling the identification of true variants with high specificity.



	Liquid biopsy	Tissue biopsy 1	Tissue biopsy 2	Tissue biopsy 3
Variant 1	✓	✓		
Variant 2	✓	✓	✓	
Variant 3	✓			✓

Figure 2: The power of liquid biopsy combined with NGS-enabled comprehensive genome profiling—Liquid biopsy combined with comprehensive genomic profiling increases the likelihood of detecting new mutations that arise during tumor evolution. The use of a comprehensive assay enables the detection of new mutations in the genome causing tissue heterogeneity (variant 1 and variant 2). Liquid biopsy also enables the detection of variants arising in new physical locations of the body (variant 3).

Using liquid biopsy combined with comprehensive molecular assays to assess somatic variants enables the 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 (Figure 2). 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 biomarkers³ and resistance alterations² not found in matched tissue biopsies.

Summary

An increasing number of biomarkers in cancer research has caused a corresponding increase in interest for molecular methods that can analyze numerous biomarkers in a single assay. 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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