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"LIQUID BIOPSIES: TOOL FOR CANCER DIAGNOSIS AND MONITORING"

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Abstract:

Liquid biopsy is a non-invasive technique that examines blood samples for circulating tumor indicators such as extracellular vesicles (EVs), cell-free DNA (cfDNA), and circulating tumor cells (CTCs). Liquid biopsy has several benefits over standard tissue biopsy, such as less invasiveness, the ability to detect tumor heterogeneity, and the ability to track the course of the disease in real time. While cfDNA conveys genetic variations for the purpose of discovering actionable mutations and tracking treatment response, CTCs shed from primary tumors and metastatic sites offer insights into tumor biology and metastatic potential and treatment response. EVs—exosomes in particular—contain molecular cargo indicative of the cell from which they originated, acting as extra biomarkers. However, there are obstacles in the way of liquid biopsy's extensive clinical application. Accurate analysis is hampered by technical constraints such low biomarker concentrations and the requirement for sensitive detection techniques. To guarantee repeatability across laboratories, pre- and analytical procedure standardization is crucial. Its usefulness in the early identification of cancer is still limited, necessitating meticulous assay validation to reduce the number of false-positive and false-negative results. Notwithstanding these obstacles, research is still being done to improve liquid biopsy methods. Extensive prospective studies are being conducted to verify its prognostic and predictive use in monitoring the course of the disease and directing treatment choices. Liquid biopsy has the potential to revolutionize individualized cancer therapy by offering real-time insights into tumor dynamics and therapeutic responses, provided that it is rigorously validated and advances technologically.

Key words: Liquid biopsy, Free DNA, Circulating tumor cells, tumor, diagnosis.

Introduction:

With the increasing incidence of cancer worldwide, there is a constant pursuit to improve cancer diagnosis procedures as well as treatment approaches. One of the biggest challenges in

the treatment of cancer is still early detection of tumors. Therefore, improving screening methods and early detection strategies together with putting in place efficient surveillance systems becomes critical to increasing treatment effectiveness and reducing cancer-related death rates. In this regard, precision medicine has become a central theme in the field of oncology. Genetic profiling plays a critical role in identifying the genetic abnormalities that drive tumor development, which in turn helps identify prognostic and diagnostic biomarkers and informs personalized treatment plans [1-2].

Even while tissue biopsies are now the gold standard for tumor profiling, they have a number of drawbacks, including the fact that they are intrusive, come with hazards, and can be difficult to get samples from particular anatomical regions. Additionally, they provide a limited view of the tumor environment. Because they consist of many cell subpopulations with different genetic and epigenetic modifications, tumors are by nature heterogeneous. In addition, tumor cells dynamically alter throughout time, especially in response to treatment stimulus, which exacerbates the heterogeneity and discrepancies between primary and metastatic lesions. As a result, tissue biopsies that are restricted in both space and time do not accurately depict the whole tumor profile, do not capture changes from various tumor sites, and are therefore not suitable for tracking the advancement of the illness [3-4].

Liquid biopsies, which identify cancer-derived components in patient biofluids such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), RNA, extracellular vesicles (EVs), and tumor-educated platelets (TEPs), have become the focus of recent oncological research due to these limitations. Genomic, epigenetic, transcriptomic, and proteomic insights into cancers and metastatic locations are provided via liquid biopsies. Their clinical integration holds the potential to improve heterogeneous entity classification, facilitate more stringent patient monitoring, and improve cancer screening, diagnosis, and prognosis. Treatment-resistant clones can be found, and treatment response can be evaluated more easily with the use of liquid biopsies. Because of how minimally intrusive they are, patients can undergo recurrent sampling as the condition progresses without experiencing undue stress. Liquid biopsies can also detect aggressive clones that are spreading and provide a more comprehensive genetic analysis of cancers, indicating their heterogeneity [5-7].

Liquid biopsy, a minimally invasive technique for detecting tumor cells and tumor-derived products in peripheral blood, is gaining traction in clinical settings due to its sensitivity, convenience, and cost-effectiveness. Current cancer diagnosis relies on tumor biomarker detection and tissue biopsy, but existing plasma protein biomarkers often lack sensitivity and positive predictive value, leading to false negatives in patients with low tumor burden. Tissue biopsy remains the gold standard for diagnosis, yet its invasiveness and subjective sampling pose challenges [8]. Despite its advantages, liquid biopsy faces obstacles such as technological limitations and tumor heterogeneity, which complicate biomarker interpretation. This review discusses the characteristics of CTCs, cfDNA, and EVs, outlines their applications in predictive and prognostic assessment in cancer, and addresses challenges and prospects for widespread clinical adoption [9-11].

In conclusion, the escalating global incidence of cancer underscores the urgent need for advancements in both diagnosis and treatment modalities. Early detection of tumors remains a formidable challenge, necessitating the refinement of screening techniques and the establishment of robust surveillance systems. Precision medicine has emerged as a cornerstone in oncological practice, leveraging genetic profiling to unveil the underlying molecular aberrations driving tumorigenesis. This approach not only facilitates the identification of diagnostic and prognostic biomarkers but also informs personalized treatment strategies tailored to individual patients.

While tissue biopsies have long served as the gold standard for tumor profiling, their invasive nature, and limitations in capturing tumor heterogeneity pose significant drawbacks. Liquid biopsies, on the other hand, offer a promising alternative by enabling the detection of cancer-derived components in biofluids, such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), RNA, extracellular vehicles (EVs), and tumor-educated platelets (TEPs). These liquid biopsies provide comprehensive molecular insights into tumors and metastatic sites, facilitating more accurate diagnosis, prognosis, and treatment monitoring.

Despite the potential of liquid biopsies, challenges such as technological limitations and tumor heterogeneity hinder their widespread clinical implementation. Addressing these obstacles will be crucial for realizing the full potential of liquid biopsies in improving cancer care. Overall, this study underscores the transformative potential of liquid biopsies in revolutionizing cancer diagnosis and management, paving the way for more precise and personalized therapeutic interventions in the fight against cancer.

Tumor and Circulating Tumor Cells:

First reported in the middle of the 1800s, circulating tumor cells (CTCs) are an essential component of tumor spread and contain protein, DNA, and RNA. Through active shedding or the epithelial–mesenchymal transition (EMT) from primary or metastatic lesions, these cells reach the peripheral blood circulation. Their existence permits thorough molecular analysis and facilitates functional studies in vivo and in vitro. CTCs are associated with unfavorable tumor features such advanced pathological staging and lymphatic invasion. They also function as possible triggers of metastatic recurrence, indicators of the burden of micrometastatic illness, and predictors of clinically relevant distant metastases [12].

Finding tumor-specific DNA abnormalities in CTCs provides a very specific way to identify them; research has shown that enriched CTC pools from patients with metastatic breast cancer contain mutations like PIK3A. Extensive examination encompassing DNA, RNA, and protein levels improves our comprehension of treatment resistance mechanisms and metastatic biology. Notably, predictive value for disease development has been revealed by whole-exome sequencing of CTCs collected from prostate cancer patients [13].

Due to their rarity, CTCs provide significant technological obstacles for enrichment, detection, and analysis in peripheral blood, despite their clinical value. As a result, improvements in sensitivity and specificity are required. Improved single-cell multimutation analysis may result from the development of novel approaches, such as the fusion of single-cell isolation methods

with whole genome amplification. Emerging technologies such as polydimethylsiloxane (PDMS) CTC chips and 3D tumor cell culture systems offer new avenues for CTC capture and pathophysiological assessment, facilitating tumor monitoring and prognosis, even though the FDA-approved CellSearchTM system is still the standard platform for CTC detection. To overcome current obstacles and improve CTC detection techniques for broad clinical use in tumor identification and prognosis evaluation, more research is necessary [14].

Free Circulating DNA:

DNA fragments that are circulating in the peripheral circulation and come from necrotic, apoptotic, or actively secreting cells are referred to as cell-free DNA (cfDNA). More research is being done to explore its potential as a diagnostic and monitoring tool for cancer patients. Several studies have indicated that it can be used to identify genetic abnormalities in a variety of cancer kinds and metastatic stages. When compared to non-cancer patients, cancer patients usually have higher plasma and serum levels of cfDNA, which can be attributed to primary tumors, circulating tumor cells (CTCs), micrometastatic deposits, and normal cells. Studies have shown somatic mutations in a variety of tumor types, indicating that genomic analysis of cfDNA mutations is a viable substitute for tissue-based research [15].

A subset of cfDNA derived from tumor cells called circulating tumor DNA (ctDNA) contains genetic and epigenetic information unique to tumors. Particularly, ctDNA analysis provides improved sensitivity for identifying tumor-specific abnormalities in biofluids such as cerebrospinal fluid (CSF) and urine. However, difficulties in distinguishing cancerous from non-tumorous DNA continue to exist, requiring improvements in isolation methods. Phase isolation, silicon membrane-based spin columns, or magnetic bead-based isolation are the main techniques used in current cfDNA extraction procedures. These techniques are then followed by analysis utilizing polymerase chain reaction (PCR) or next-generation sequencing (NGS) [16].

The analysis of ctDNA has been improved by technological advancements such as droplet digital PCR (ddPCR), digital PCR (dPCR), and cancer personalized profiling by deep sequencing (CAPP-Seq). This is especially useful in finding genomic aberrations linked to treatment resistance in patients with metastatic cancer. The cobas EGFR Mutation Test v2, which is approved by the FDA to identify EGFR mutations in plasma specimens from patients with non-small cell lung cancer (NSCLC), is one example of the growing clinical applications of cfDNA detection. To fully achieve the clinical promise of cfDNA analysis, more study is necessary as obstacles in enriching ctDNA and understanding its function in chromatin remodeling, treatment resistance, and metastasis persist despite advancements [17].

Extracellular Particles:

Extracellular vesicles (EVs) are membrane-bound particles that are secreted by cells. They include microvesicles (50–1000 nm) and exosomes (30–100 nm), which are prevalent in different body fluids. They carry messages to target cells in both healthy and unhealthy

circumstances, acting as intermediaries in intercellular communication. EVs have the potential to be innovative therapeutic delivery methods and noninvasive molecular diagnostics [18].

Nucleic acids, surface proteins, and intraluminal proteins are among the components of EVs that provide information on the origin of tumor cells and the site of metastatic spread. EVs use oncogenes and onco-microRNAs (miRNAs) to transfer signals that are involved in carcinogenesis, immune evasion, metastasis, and therapeutic response. Exosomes, for example, have the ability to control the invasiveness and viability of tumor cells as well as the behavior of endothelial cells to encourage angiogenesis. They may determine organ-specific niches, which may impact metastasis. Proteins, RNA, and DNA found in tumor-derived extracellular volatile matter (EV) can be analyzed to reveal pathophysiological circumstances and support cancer diagnosis [19].

EVs act as transporters of circulating tumor RNA, which has been identified as a potential cancer biomarker. This includes mRNAs, miRNAs, and long noncoding RNAs. Notably, certain patterns of miRNA expression in serum exosomes have demonstrated the ability to diagnose and predict the prognosis of malignancies, including esophageal squamous cell carcinoma (ESCC) [20].

Several techniques, including size exclusion, coprecipitation, immunoaffinity, and ultracentrifugation, are used to separate EVs according to their physical and biological properties. Size-selective separation of EVs and surface marker-based differentiation between cancer patients and breast cell lines have been made possible recently through the development of microfluidic-based separation techniques. These techniques make use of concepts such as viscous elasticity and aptamer-mediated approaches. Different exosome subpopulations and nonmembranous nanoparticles known as exomeres have been found by asymmetric flow field-flow fractionation (AF4), providing opportunities for enhanced EV analysis and use in cancer research and diagnosis [21].

Tumor Diagnosis and Profiling Using Liquid Biopsies:

The investigation of cell-free DNA (cfDNA) in liquid biopsies is an important field of research, demonstrating its utility in cancer monitoring and detection. When comparing cancer patients to healthy individuals, higher quantities, and higher quality of cfDNA have been found; these amounts are associated with advanced disease stages and metastasis. High cfDNA levels are non-specific, though, and they can also be present in a number of non-cancerous illnesses, which presents difficulties. However, more accurate cancer genotyping, and diagnosis can be made by identifying tumor-specific changes found in cfDNA, such as insertions, deletions, single nucleotide variants (SNVs), copy number variations (CNVs), and methylation alterations. This is referred to as circulating tumor DNA (ctDNA) [22].

The promise of liquid biopsies as an alternative or complement to tissue biopsies is highlighted by the concordance of (epi)genetic alterations between blood ctDNA and corresponding tumor tissues across a range of malignancies. Interestingly, the FDA has approved liquid biopsy-based tests to identify particular gene rearrangements and mutations in a variety of

malignancies, allowing for more focused therapy choices. However, the efficacy of liquid biopsies for early cancer identification is limited since the concordance of mutations between ctDNA and tumor tissues differs depending on cancer type and disease stage [23].

Other substances found in liquid biopsies, such as exosome DNA (exoDNA), messenger RNA (mRNA), microRNAs (miRNAs), and tumor-associated microparticles (taMPs), provide diagnostic and prognostic information in addition to ctDNA. Biofluids such as urine and cerebrospinal fluid (CSF) have become viable substitutes for liquid biopsies, offering benefits such as non-invasive sampling and enhanced patient compliance. Prognostic liquid biopsies are especially valuable because of the correlation between tumor burden and survival outcomes and the amounts of cfDNA and exoDNA. Circulating tumor cells (CTCs) also provide further prognostic data by indicating the course of the disease and forecasting survival rates [24].

Although liquid biopsies hold great potential, issues with sensitivity, specificity, and consistency still need to be resolved. To fully realize the clinical utility of liquid biopsies, more research and technological improvements are required. However, liquid biopsies' extensive molecular data hold great promise for revolutionizing cancer monitoring, diagnosis, and therapy approaches.

Tumor Follow-up Using Liquid Biopsies :

Liquid biopsies have great potential for tracking the course of cancer patients' disease and the effectiveness of their therapy because they are either non-invasive or minimally invasive, which makes them a better option for long-term follow-up than many invasive tissue biopsies. Conventional imaging modalities, including computed tomography (CT) scans, have drawbacks in terms of radiation exposure, expense, and sensitivity. They also frequently miss tiny lesions and don't provide information on genetic alterations brought on by medical interventions [25].

On the other hand, long-term biofluid collection—especially of cfDNA and ctDNA—allows for real-time cancer patient monitoring. Through the effective detection of resistant mutations in genes such as EGFR, ERBB2, PIK3CA, and RAS across a variety of malignancies, analysis of ctDNA has made it possible to predict therapy response and clinical outcomes early on. Interestingly, a post-treatment decline in ctDNA levels has been linked to a good prognosis; persistent or elevated levels, on the other hand, are indicative of disease progression, relapse, and a lower chance of survival. The dynamic nature of ctDNA mutations allows for quick modifications to treatment regimens and provides insights into therapy response [26].

In a similar vein, counting and characterizing circulating tumor cells (CTCs) has prognostic value and helps track the advancement of the tumor and the response to therapy. Following therapy, changes in CTC counts are correlated with prognosis; a decrease is linked to better results, and an increase indicates a higher likelihood of recurrence. Furthermore, CTCs provide early identification of genetic changes linked to treatment resistance, which may help in the selection of individualized treatment alternatives [27].

Furthermore, tracking circulating miRNA levels and analyzing transcriptome data from EVs and circulating RNA help with disease monitoring and therapy response evaluation.

Specifically, changes in EpCAM(+) tumor-associated microparticles (taMPs) and the assessment of postoperative biomarkers in different types of cancer are useful surveillance methods. Liquid biopsies have also been used to track and identify circulating cell-free Epstein-Barr virus (cfEBV) DNA in malignancies linked to EBV, which helps with treatment adaption and prognostic data. Liquid biopsies, when combined with current surveillance systems, provide a considerable improvement in patient management and treatment outcomes by providing early diagnosis of recurrence, residual disease, and resistance [28].

Monitoring Cancer Treatment:

In personalized medicine, the systematic identification of certain genetic alterations in tumor molecular drivers has become commonplace, with the goal of forecasting therapeutic resistance or response. During treatment follow-up, CTCs are essential for disease surveillance because they can detect chemoresistant subgroups and the response to chemotherapy. For example, the mRNA expression of AR-V7 in CTCs provides important information on medication sensitivity and resistance and predicts the failure of anti-androgen therapy in prostate cancer. Additionally, in patients with castration-resistant prostate cancer, CTC enumeration can confirm the anticancer effectiveness of novel medicines such abiraterone acetate [29].

Throughout tumor treatment, cfDNA monitoring allows for the early identification of mutations linked to resistance. Following PARP inhibition, changes in cfDNA concentration in prostate cancer are associated with a favorable prognosis by identifying novel mutations brought on by treatment-selective pressure-driven drug resistance. Since living tumor cells are what drive treatment variability and disease progression, ctDNA mostly represents dead tumor cells, analyzing mutations in cfDNA may yield more precise information about the efficacy of treatments than analyzing cfDNA levels alone [30].

The proteins found in extracellular vesicles (EVs) function as biomarkers to track the efficacy of treatment and identify drug resistance early on. It is now possible to understand drug resistance mechanisms by identifying certain proteins that are abundant in EVs derived from resistant cancer cells. EVs have the potential to serve as precision medicine drug carriers by supplying crucial signaling molecules that facilitate information transmission. With the use of liquid biopsies, doctors can adjust a patient's course of treatment in real time in response to changes in the tumor, guaranteeing that the patient gets the best possible care with the fewest possible side effects.

Advantages and Disadvantages of Liquid Biopsy:

By capturing the heterogeneous tumor profile at baseline and during follow-up, liquid biopsies have the potential to improve patient care without the dangers associated with standard tissue biopsies. This is demonstrated by the evidence that has been given. There has been a lot of research done on the use of liquid biopsies for cancer patient follow-up, and it looks like a wider clinical practice adoption is imminent. However, there are some obstacles to overcome before using liquid biopsies for cancer screening and early diagnosis. These arise mainly from the

frequently inadequate concentrations of analytes such ctDNA and CTCs, particularly in patients in the early stages of the disease [31].

Evaluating circulating analytes such as ctDNA, RNA, proteins, and metabolites is one way to improve the sensitivity of cancer detection. However, the cost-effectiveness and specificity of this method may be compromised. Liquid biopsy tests have a low specificity, which raises the possibility of false positive results that cause discomfort and needless invasive treatments for otherwise healthy people. For instance, changes from clonal hematopoiesis in peripheral blood cells may also be found in the cfDNA of people who do not have cancer; if this information is not thoroughly examined, this could result in a mistaken diagnosis [31].

Notwithstanding these obstacles, non-invasive liquid biopsy-based cancer screening and early detection remain highly desirable and anticipated applications, especially for cancer types that lack reliable screening instruments and are frequently detected at advanced stages of the disease. Future research on the usefulness of liquid biopsies for cancer screening and diagnosis is anticipated to come from developments in technology and growing interest in this field [31].

For applications later on, ensuring the number and quality of cfDNA samples is essential. It is advised to take precautions including avoiding genomic DNA contamination of samples and isolating cfDNA from plasma rather than serum. Additionally, very sensitive detection methods like ddPCR, NGS, or BEAMing are required because of the low amounts of ctDNA. The temporal heterogeneity of ctDNA, however, highlights the necessity of larger panels for follow-up investigation, which could reduce the sensitivity of detection. Notwithstanding these difficulties, quantifying total cfDNA has limited diagnostic utility but may be a straightforward and affordable substitute for predicting disease response and consequences [31].

Limitations of Liquid Biopsy:

Liquid biopsy has a wide range of possible uses in different tumor types, from early diagnosis and tumor monitoring to biomarker identification. However, a number of unresolved problems prevent widespread clinical implementation. Advances in technology are required to tackle issues including low quantities of biomarkers, contamination of cells, and the caliber of pre-analytical preservation techniques, all of which might affect the dependability of liquid biopsy outcomes [32].

For instance, poor detection rates make it difficult to identify circulating tumor cells (CTCs), especially in individuals who exhibit epithelial-mesenchymal transition (EMT) characteristics. With their increased sensitivity and specificity, novel biomarkers like Plastin3 may be able to help get around this restriction. Likewise, since next-generation sequencing (NGS) analysis of cell-free DNA (cfDNA) shows potential for mutation screening, assay standardization and validation are necessary to guarantee clinical efficacy and usability. Variations in sample quality, analytical platforms, and assay sensitivity can all contribute to discrepancies in reported results [32].

It is necessary to consider the features of the disease, the sample needs, the accessibility of analytical tools, and the availability of equipment while selecting the most practical and

effective liquid biopsy technique. The International Society for Extracellular Vesicles (ISEV) and other organizations are working to standardize processes, which will give exosome separation and enrichment methods crucial recommendations. Our knowledge of the biology of carcinogenesis and disease heterogeneity is still lacking, despite advances in technology. Because tumor heterogeneity is a major obstacle to liquid biopsy applications, it is crucial to assess the best assays for capturing CTC, ctDNA, and extracellular vesicle (EV) genotype changes. Liquid biopsy in conjunction with downstream molecular analysis presents a viable method for learning more about the biology of cancer. Reaching the full potential of liquid biopsy in clinical practice will depend on resolving these issues and expanding our knowledge of tumor heterogeneity [32].

Future Perspectives of Liquid Biopsy:

Although the clinical usefulness of liquid biopsy is still up for debate and there are still technical issues, the development of liquid biopsy has significantly raised awareness of CTCs and cfDNA as biomarkers. There is heterogeneity among patients with the same tumor type, and not all tumor forms lend themselves equally to the detection of CTCs and ctDNA. For example, even in individuals with a significant metastatic burden, the levels of CTCs and ctDNA in aggressive malignancies such as NSCLC may be lower than expected. The blood-brain barrier may be the reason why brain tumor detection presents special difficulties [33].

One difficulty is differentiating nucleic acids from tumors from those coming from dying normal cells, particularly when using cytotoxic cancer treatments. It is essential to comprehend these elements more fully in order to enhance diagnostic abilities. Prior to routine clinical usage, liquid biopsy test validation is crucial, highlighting the significance of quality control. Though their low concentration in many patients is a problem, CTCs allow a wide range of assays at the DNA, RNA, and protein levels, including functional studies like drug testing. In a similar vein, ctDNA analysis does not necessarily indicate therapy-resistant tumor cell clones, even when it offers insights regarding druggable mutations. To get around these restrictions, studies on tumor-associated platelets and tumor-derived exosomes are still being conducted. Clinical trials are being conducted to show the clinical usefulness of liquid biopsies in guiding cancer patients' therapy and predicting relapse. However, because of issues with assay specificity and the possibility of false-positive or false-negative results, its use for early cancer detection is still difficult [33].

In summary, various liquid biopsy systems might work well together to treat cancer patients. Personalized cancer medicine could be advanced by using real-time consecutive liquid biopsies to evaluate minimum residual illness following initial therapy and identify therapy resistance early. To confirm the therapeutic importance of discovered mutations and enhance the effectiveness of liquid biopsy in cancer treatment, large prospective cohort studies are required.

Conclusion:

The discourse surrounding liquid biopsy emphasizes how revolutionary it can be in the treatment of cancer, but it also draws attention to the difficulties and complications that come with putting it into practice. Although liquid biopsy provides a dynamic and minimally invasive way to monitor therapy response and understand tumor biology, especially when using CTCs and cfDNA analysis, there are a few important issues that need to be resolved before liquid biopsy is widely used in clinical settings. The variation in biomarker abundance and detectability throughout tumor types and even patients with the same cancer is one of the main obstacles. Technical constraints impede precise and trustworthy analysis, such as low biomarker concentrations and the requirement for sample purification and amplification. Moreover, accurate diagnosis and treatment planning depend on the ability to differentiate nucleic acids emanating from tumors from those coming from normal cells or modifications brought on by therapy. Despite these obstacles, research projects are still being undertaken to improve the therapeutic usefulness of liquid biopsy methods. Technological developments like next-generation sequencing and droplet digital PCR hold out hope for increasing sensitivity and specificity. Pre-analytical and analytical method standardization is essential to guarantee dependability and repeatability amongst laboratories. Clinical trials are currently being conducted to examine the predictive and prognostic utility of liquid biopsy in monitoring the course of the disease and guiding treatment options. Although encouraging findings have been noted, large-scale prospective studies are required for additional validation, especially in the areas of treatment response prediction and minimal residual illness detection. To sum up, liquid biopsy offers real-time insights into tumor dynamics and therapy responses, thereby representing a paradigm change in cancer diagnoses and management. But more research, cutting-edge technology, and thorough validation are necessary to reach its full potential. Liquid biopsy has the potential to transform individualized cancer therapy and enhance patient outcomes in the future with focused efforts to solve current issues and improve techniques.

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