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REVIEW ARTICLE

Current Status of Liquid Biopsy Biomarkers in Cancer

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Abstract: Liquid biopsy is a non-invasive diagnostic tool in cancer management, which offers real-time molecular information about tumor biology. The analysis of circulating biomarkers, including circulating tumor cells (CTCs), cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), exosomes, and microRNAs, provides valuable information about tumor heterogeneity, clonal evolution, and treatment response. These biomarkers enable early cancer detection, disease monitoring, and therapeutic decision-making while overcoming the limitations of traditional tissue biopsies. CTCs serve as direct representatives of tumor cells in circulation, while cfDNA and ctDNA analysis reveals genetic alterations and tumor burden. Exosomes and microRNAs play crucial roles in intercellular communication and disease progression. The clinical implementation of liquid biopsy has shown promising results in various cancer types, particularly in lung, breast, colorectal, and prostate cancers. Several challenges still continue to barricade the use of liquid biopsy, which includes standardization of isolation techniques, optimization of detection methods, and interpretation of results. The usage of multiple biomarker analyses and advanced molecular techniques has improved diagnostic accuracy and prognostic value.

Keywords: Liquid biopsy; Circulating tumor cells; Cell-free DNA; Exosomes; Cancer biomarkers.

1. Introduction

The usage of liquid biopsy for clinical oncology is a significant advancement in precision medicine, offering opportunities for real-time monitoring of cancer development [1]. Traditional tissue biopsies, while crucial for initial diagnosis, present several limitations including invasive nature, sampling bias due to tumor heterogeneity, and practical constraints in performing serial measurements during treatment [2]. Liquid biopsy addresses these limitations by enabling the analysis of tumor-derived components in biological fluids, predominantly blood, providing a comprehensive snapshot of tumor biology and evolution [3]. The molecular constituents analyzed in liquid biopsies encompass circulating tumor cells (CTCs), cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), exosomes, and microRNAs (listed out in Table 1). Each of these components offers unique insights into tumor characteristics, progression patterns, and treatment responses [4].

The technological foundation supporting liquid biopsy has experienced remarkable advancement over the past decade. The development of highly sensitive detection methods, including next-generation sequencing (NGS), digital PCR, and sophisticated isolation techniques, has dramatically improved the ability to detect and characterize rare tumor-derived components in circulation [5]. These technological innovations have expanded the utility of liquid biopsy from initial detection to monitoring disease progression, evaluating treatment efficacy, and identifying emerging resistance mechanisms [6]. The clinical significance of liquid biopsy extends across various cancer types, with particularly notable applications in lung, breast, colorectal, and prostate cancers [7]. In non-small cell lung cancer (NSCLC), liquid biopsy has demonstrated remarkable utility in detecting EGFR mutations, facilitating

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targeted therapy selection, and monitoring treatment response [8]. Similarly, in breast cancer, the enumeration of CTCs has shown strong prognostic value, while ctDNA analysis enables early detection of disease recurrence and treatment resistance [9].

Recent advances in molecular biology techniques have further enhanced the sensitivity and specificity of liquid biopsy applications. The integration of artificial intelligence and machine learning algorithms has improved data interpretation and clinical decision-making processes [10]. Moreover, the development of standardized protocols and quality control measures has increased the reliability and reproducibility of liquid biopsy results across different laboratory settings [11].

The molecular information obtained through liquid biopsy has profound implications for personalized cancer treatment. The ability to detect specific genetic alterations, monitor tumor burden, and identify resistance mechanisms in real-time allows for more precise therapeutic interventions and timely treatment modifications [12]. Moreover, the non-invasive nature of liquid biopsy facilitates longitudinal monitoring, enabling clinicians to track tumor evolution and adjust treatment strategies accordingly [13]. Several challenges remain in the widespread implementation of liquid biopsy in clinical practice. Technical considerations regarding sample collection, processing, and analysis standardization require careful attention [14]. Additionally, the interpretation of results, particularly in the context of tumor heterogeneity and clonal evolution, demands sophisticated analytical approaches and clinical validation [15].

Table 1. Major Liquid Biopsy markers used in Cancer Diagnosis

Biomarker Type	Size/Characteristics	Detection Methods	Clinical Applications	Advantages	Limitations
CTCs	12-25 μm	CellSearch System; Microfluidic devices; Size-based filtration	Prognostic monitoring; Metastasis prediction; Treatment response	Direct tumor cell analysis; Viable cells for culture; Molecular and functional studies	Low abundance; Short half-life; Complex isolation
ctDNA	160-180 bp	Digital PCR; NGS; BEAMing; Targeted sequencing	Early detection; Treatment monitoring; Resistance tracking	High sensitivity; Rapid turnover; Comprehensive mutation analysis	Variable abundance; Complex analysis; Cost barriers
Exosomes	30-150 nm	Ultracentrifugation; Size exclusion; Immunoaffinity	Early detection; Drug resistance; Disease monitoring	Stable in circulation; Multiple biomarkers; Protected cargo	Complex isolation; Poor standardization; Purity issues
microRNA	18-25 nucleotides	qRT-PCR; NGS; Microarray; Digital PCR	Diagnostic screening; Disease staging; Therapy response	High stability; Cancer-specific signatures; Non-invasive	Normalization issues; Technical variability; Complex analysis
Tumor-educated platelets	2-4 μm	RNA sequencing; Mass spectrometry; Flow cytometry	Cancer detection; Molecular subtyping; Response monitoring	Abundant in blood; Easy isolation; Stable processing	Limited specificity; Complex signatures; New technology

2. Circulating Tumor Cells (CTCs)

2.1. Biology and Origin

Circulating tumor cells represent a crucial component of liquid biopsy, serving as direct representatives of tumor tissue in the bloodstream. These cells detach from primary or metastatic tumor sites and enter circulation through complex biological processes, including epithelial-mesenchymal transition (EMT) [16]. During EMT, tumor cells undergo significant molecular and phenotypic alterations, losing their epithelial characteristics and acquiring mesenchymal properties that facilitate invasion and migration. The transformation enables these cells to breach the basement membrane, enter blood vessels through intravasation, and potentially establish metastatic sites through extravasation.

CTCs exist in various forms within the bloodstream, including single cells and cellular clusters. CTC clusters, also known as circulating tumor microemboli, demonstrate enhanced survival capabilities and increased metastatic potential compared to single CTCs [17]. The heterogeneous nature of CTCs reflects the molecular diversity of the primary tumor, providing valuable insights into tumor evolution and metastatic processes.

2.2. Molecular Characteristics

The molecular profile of CTCs encompasses diverse genetic and phenotypic features that distinguish them from normal blood cells. These cells typically express epithelial markers such as EpCAM, cytokeratins, and E-cadherin, while simultaneously exhibiting varying levels of mesenchymal markers including vimentin and N-cadherin [18]. The dynamic expression of these markers reflects the plasticity of CTCs and their ability to adapt to different microenvironments.

Genetic analysis of CTCs reveals significant heterogeneity in mutation profiles, copy number variations, and expression patterns. This genetic diversity often mirrors the heterogeneity observed in primary tumors and can provide crucial information about disease progression and treatment resistance mechanisms. Furthermore, CTCs may harbor unique genetic alterations not present in the primary tumor, offering insights into the evolution of metastatic disease.

2.3. Detection and Isolation Methods

2.3.1. Physical Properties-Based Isolation

Size-based isolation techniques exploit the typically larger size of CTCs compared to normal blood cells. These methods employ various technologies including:

Membrane Filtration: Utilizing specialized filters with precise pore sizes to capture CTCs while allowing smaller blood cells to pass through. Advanced filtration systems incorporate microfluidic principles to enhance separation efficiency and maintain cell viability [19].

Density Gradient Centrifugation: This technique separates CTCs based on their density differences compared to other blood components. Modified density gradient approaches have improved the recovery rates and purity of isolated CTCs.

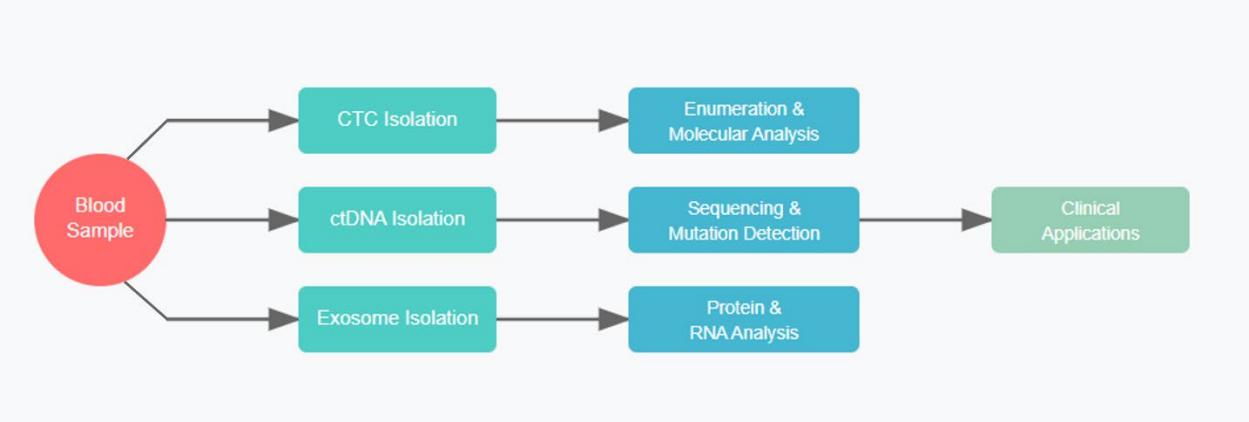

Figure 1. Isolation and analysis of liquid biopsy components

2.3.2. Biological Properties-Based Isolation

Immunoaffinity methods represent the most widely adopted approach for CTC isolation. The CellSearch system, as the only FDA-approved platform for CTC enumeration, utilizes anti-EpCAM antibodies conjugated to magnetic particles for positive selection of CTCs [20]. This system defines CTCs as nucleated cells expressing cytokeratins 8/18/19 while lacking CD45 expression.

Alternative immunoaffinity approaches include:

Microfluidic Devices: These systems combine physical and biological principles to achieve highly efficient CTC isolation. Advanced microfluidic platforms incorporate multiple capture mechanisms and can maintain cell viability for subsequent functional studies.

Negative Selection: This approach removes normal blood cells using specific markers, potentially capturing a broader spectrum of CTCs, including those that have undergone complete EMT.

2.4. Clinical Significance

The presence and quantity of CTCs serve as powerful prognostic indicators across multiple cancer types. In metastatic breast cancer, a threshold of ≥5 CTCs per 7.5 mL of blood correlates with reduced progression-free and overall survival [6]. Similar prognostic value has been demonstrated in prostate and colorectal cancers, where CTC enumeration helps predict treatment outcomes and disease progression.

3. Cell-free DNA (cfDNA) and Circulating Tumor DNA (ctDNA)

3.1. Origins and Characteristics

Cell-free DNA comprises short DNA fragments released into circulation through various cellular processes, including apoptosis, necrosis, and active secretion. In cancer patients, a fraction of cfDNA originates from tumor cells, termed circulating tumor DNA (ctDNA). The size distribution of cfDNA typically ranges from 160-180 base pairs, corresponding to DNA wrapped around a nucleosome plus linker [21]. The proportion of ctDNA within total cfDNA varies significantly among cancer types and stages, ranging from less than 0.1% to more than 50% in advanced disease.

The release of ctDNA into circulation occurs through multiple mechanisms. Apoptotic cell death results in characteristic DNA fragmentation patterns, while necrotic cells release longer DNA fragments. Active secretion of DNA by living tumor cells has also been documented, although its biological significance remains under investigation [22]. The half-life of cfDNA in circulation ranges from minutes to hours, making it an excellent marker for real-time monitoring of tumor dynamics.

3.2. Molecular Features and Analysis

3.2.1. Genetic Alterations in ctDNA

ctDNA harbors genetic alterations characteristic of the tumor of origin, including point mutations, copy number variations, chromosomal rearrangements, and methylation changes. These alterations serve as specific markers for tumor detection and monitoring. The genetic landscape of ctDNA reflects tumor heterogeneity and can reveal the presence of multiple tumor subclones [23].

3.2.2. Detection Technologies

The analysis of ctDNA requires highly sensitive and specific detection methods due to its often low abundance in circulation. Digital PCR technologies enable the detection of rare variants with sensitivity approaching 0.01%. This technique proves particularly valuable for monitoring known mutations and quantifying tumor burden. Next-generation sequencing approaches provide broader coverage of genetic alterations, allowing comprehensive genomic profiling. Targeted sequencing panels focus on clinically relevant mutations, while whole-genome sequencing enables the discovery of novel alterations [24].

3.3. Clinical Applications

3.3.1. Early Detection and Diagnosis

The presence of tumor-specific genetic alterations in ctDNA enables early cancer detection. Recent studies demonstrate the potential of ctDNA analysis for cancer screening in asymptomatic individuals. The detection sensitivity varies among cancer types, with particularly promising results in lung, colorectal, and breast cancers [25].

3.3.2. Disease Monitoring and Treatment Response

Serial monitoring of ctDNA levels provides real-time assessment of treatment efficacy. Decreasing ctDNA levels typically indicate treatment response, while increasing levels may signal disease progression. The rapid clearance of ctDNA enables earlier detection of treatment response compared to conventional imaging methods. Changes in ctDNA levels often precede clinical or radiological evidence of disease progression by several months [26].

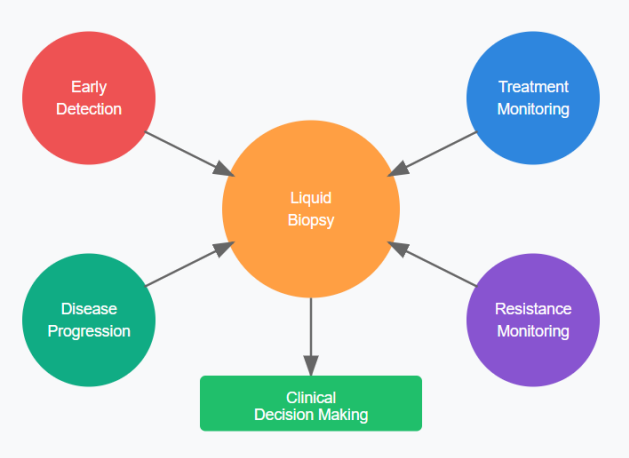

Figure 2. Clinical applications and outcomes of liquid biopsy in cancer management

3.3.3. Resistance

ctDNA analysis enables the detection of resistance mutations during treatment. In non-small cell lung cancer, the emergence of T790M mutations in EGFR can be detected in ctDNA before clinical progression becomes evident. Similar applications exist in other cancers, where specific resistance mutations guide therapeutic decision-making [27].

3.3.4. Quality Control

Sample collection and processing significantly impact ctDNA analysis. Standardized protocols for blood collection, processing, and storage are essential for reliable results. The choice of blood collection tubes, processing time, and storage conditions affects cfDNA yield and quality. Implementation of rigorous quality control measures ensures reproducibility and accuracy of ctDNA testing [28].

3.4. Quantification and Analysis

The accurate quantification of ctDNA requires sophisticated analytical approaches considering both technical and biological variables. Digital PCR platforms offer absolute quantification of specific mutations, while sequencing-based methods provide broader genomic coverage. The incorporation of unique molecular identifiers (UMIs) in sequencing protocols enhances detection sensitivity and reduces false-positive results by identifying PCR duplicates and sequencing artifacts [29].

3.5. Clinical Validation

The implementation of ctDNA analysis in clinical practice requires extensive validation across different cancer types and clinical scenarios. Large-scale clinical studies have demonstrated the utility of ctDNA monitoring in multiple settings. For instance, in colorectal cancer, post-surgical ctDNA detection predicts recurrence with high accuracy, enabling early intervention in high-risk patients [30].

Table 2. Clinical Validation Studies of Liquid Biopsy in Various Cancer Types

Cancer Type	Biomarker	Study Size	Clinical Outcome	Sensitivity	Specificity	Reference
Breast	CTCs ≥5/7.5mL	1944	OS, PFS	85%	90%	[8]
Lung	EGFR mutations in ctDNA	1128	Treatment response	92%	98%	[31]
Colorectal	ctDNA post-surgery	230	Recurrence prediction	88%	94%	[22]
Prostate	AR-V7 in CTCs	202	Treatment selection	83%	89%	[37]
Pancreatic	Exosomal GPC1	190	Early detection	86%	93%	[29]

3.6. Combination with Other Biomarkers

The combination of ctDNA analysis with other circulating biomarkers enhances diagnostic and prognostic accuracy. Integrated analysis of ctDNA with circulating tumor cells, protein markers, and imaging data provides complementary information about tumor biology and progression. This multi-modal approach improves clinical decision-making and patient stratification [31].

3.7. Advanced Applications

3.7.1. Tumor Mutational Burden Assessment

ctDNA analysis enables non-invasive assessment of tumor mutational burden, a predictor of immunotherapy response. The comprehensive genomic profiling of ctDNA provides insights into microsatellite instability status and other genomic signatures relevant to immunotherapy selection [32].

3.7.2. Minimal Residual Disease Detection

The high sensitivity of current ctDNA detection methods enables identification of minimal residual disease following curative treatment. This application proves particularly valuable in adjuvant therapy decision-making and early detection of disease recurrence. Studies across multiple cancer types demonstrate that ctDNA detection following surgery identifies patients at high risk of recurrence with greater sensitivity than conventional methods [33].

3.7.3. Clonal Evolution Monitoring

Serial ctDNA analysis reveals patterns of clonal evolution under therapeutic pressure. The emergence and disappearance of specific mutations reflect dynamic changes in tumor composition and adaptation to treatment. This information guides therapeutic strategies and helps understand resistance mechanisms [34].

3.8. Current Advances

3.8.1. Improved Detection

Recent technological developments focus on improving detection sensitivity and specificity. Novel molecular approaches, including error-suppression techniques and machine learning algorithms, enhance the detection of low-frequency variants. The development of targeted methylation analysis provides additional layers of information for cancer detection and classification [15].

3.8.2. Clinical Decision Support

Integration of ctDNA data with clinical information systems facilitates interpretation and clinical decision-making. Advanced computational approaches analyze complex genomic data and provide clinically actionable recommendations. These systems incorporate multiple parameters including mutation profiles, clonal dynamics, and clinical factors to optimize treatment selection [35].

3.8.3. Standardization

International initiatives aim to standardize ctDNA analysis and reporting. These efforts address pre-analytical variables, analytical validation, and clinical interpretation. The development of reference materials and quality metrics ensures reproducibility across laboratories and platforms [36].

4. Exosomes and microRNAs

4.1. Cancer-Associated Exosomes

Exosomes represent a specific subclass of extracellular vesicles, ranging from 30-150 nm in diameter, that play crucial roles in intercellular communication and cancer progression. These membrane-bound vesicles originate from the endosomal compartment through the formation of multivesicular bodies and subsequent release via fusion with the plasma membrane [37]. Cancer cells exhibit heightened exosome secretion compared to normal cells, with distinct molecular signatures reflecting their cell of origin.

4.2. Molecular Composition

The molecular architecture of cancer-derived exosomes encompasses a complex array of bioactive molecules. Their lipid bilayer membrane contains specific proteins, including tetraspanins (CD63, CD9, CD81) and membrane transport proteins. The internal cargo comprises nucleic acids (DNA, mRNA, microRNA), proteins, and metabolites that maintain functional stability during circulation [38]. The selective packaging of these molecules occurs through specific sorting mechanisms, resulting in unique molecular signatures that differ from their parent cells.

4.3. Role in Cancer Progression

Cancer-associated exosomes facilitate tumor progression through multiple mechanisms. They modify the local and distant tumor microenvironment by transferring oncogenic molecules between cells, establishing pre-metastatic niches, and modulating immune responses. The horizontal transfer of functional biomolecules through exosomes can induce phenotypic changes in recipient cells, promoting cancer cell survival, proliferation, and metastatic capability [39].

4.4. Isolation and Characterization Methods

4.4.1. Traditional Approaches

Ultracentrifugation remains a fundamental method for exosome isolation, employing sequential centrifugation steps to separate vesicles based on size and density. Size-exclusion chromatography provides an alternative approach, maintaining vesicle integrity while achieving high purity. Polymer-based precipitation methods offer simplified protocols but may co-isolate protein complexes and other contaminants [40].

4.4.2. Advanced Technologies

Microfluidic platforms have emerged as powerful tools for exosome isolation, offering advantages in processing speed, sample volume requirements, and automation potential. These systems often incorporate immunoaffinity capture, ensuring specific isolation of cancer-derived exosomes. Novel approaches utilizing acoustic waves or deterministic lateral displacement improve separation efficiency and maintain vesicle integrity [41, 42].

Table 3. Molecular Characterization Methods in Liquid Biopsy

Analysis Type	Technology Platform	Target Molecules	Detection Limit	Applications	Turnaround Time
Genomic	Digital PCR	Single mutations	0.01%	Mutation detection	24-48 hours
	Targeted NGS	Multiple genes	0.1%	Mutation profiling	5-7 days
	WGS	Genome-wide	1-5%	Global analysis	7-14 days
Transcriptomic	RNA-seq	RNA transcripts	1-10 copies	Expression analysis	5-7 days
	qRT-PCR	Specific transcripts	10-100 copies	Gene expression	24-48 hours
Proteomic	Mass spectrometry	Proteins	pg/mL	Protein profiling	3-5 days
Epigenetic	Methylation arrays	DNA methylation	0.1%	Epigenetic changes	5-7 days

4.5. microRNA Analysis in Cancer

4.5.1. Biological Significance

microRNAs represent crucial regulatory molecules in cancer development and progression. These small non-coding RNAs (18-25 nucleotides) modulate gene expression through post-transcriptional regulation. Cancer-specific microRNA signatures in circulation provide valuable diagnostic and prognostic information. The stability of exosomal microRNAs in circulation, protected by the vesicular membrane, makes them particularly suitable as biomarkers [43].

4.5.2. Detection and Quantification

Advanced molecular techniques enable comprehensive microRNA profiling. RNA sequencing platforms provide detailed analysis of microRNA expression patterns, while quantitative PCR methods offer targeted analysis of specific microRNAs. Novel detection platforms incorporating nanotechnology and molecular amplification schemes enhance sensitivity and specificity [44].

4.6. Clinical Applications

4.6.1. Diagnosis

Exosomal biomarkers demonstrate significant potential for early cancer detection. The combination of protein markers and microRNA signatures enhances diagnostic accuracy. Specific exosomal profiles have been identified for various cancer types, enabling non-invasive diagnosis and molecular classification [26].

4.6.2. Disease Monitoring

Serial analysis of exosomal markers provides real-time information about disease progression and treatment response. Changes in exosomal cargo composition reflect tumor evolution and therapeutic resistance development. The integration of exosomal analysis with other liquid biopsy components improves monitoring accuracy [26, 27].

4.7. Therapeutic Applications

The dual role of exosomes as diagnostic markers and therapeutic vehicles has garnered significant attention in cancer treatment. Engineered exosomes serve as natural delivery systems for therapeutic molecules, offering advantages over conventional drug delivery approaches. Their intrinsic ability to cross biological barriers, including the blood-brain barrier, combined with their natural targeting capabilities, makes them promising therapeutic carriers [11, 28]. Natural and modified exosomes demonstrate potential in various therapeutic strategies. In immunotherapy, dendritic cell-derived exosomes loaded with tumor antigens stimulate anti-tumor immune responses. The engineering of exosomes to carry specific therapeutic cargo, including small molecule drugs, siRNAs, and therapeutic proteins, has shown promising results in preclinical studies [45].

Table 4. Therapeutic Applications of Liquid Biopsy Components

Application	Type of Biomarker	Therapeutic Strategy	Development Stage	Findings	Challenges
Drug Delivery	Engineered exosomes	Small molecule transport	Phase I/II	Enhanced drug targeting	Manufacturing scale
Immunotherapy	Modified CTCs	Vaccine development	Preclinical	Immune response activation	Cell viability
Gene Therapy	Exosome-RNA	siRNA delivery	Phase I	Targeted gene silencing	Delivery efficiency
Combination Therapy	ctDNA guided	Resistance monitoring	Clinical validation	Improved response rates	Cost effectiveness
Personalized Medicine	Multi-marker	Treatment selection	Implementation	Better patient outcomes	Standardization

5. Conclusion

The usage of multiple circulating biomarkers, including CTCs, ctDNA, and exosomal markers, provides valuable information that enhances clinical decision-making. The molecular characterization of these biomarkers reveals complex patterns of tumor heterogeneity and adaptation, enabling more precise therapeutic interventions. The non-invasive nature and ability to perform serial measurements make liquid biopsy an invaluable tool for real-time monitoring of disease progression and treatment response. Standardization efforts and analytical validation have improved the reliability and reproducibility of liquid biopsy testing, facilitating its integration into routine clinical practice. Challenges for these techniques include the optimization of detection methods, standardization of analytical procedures, and interpretation of complex molecular data require ongoing attention. Nevertheless, the demonstrated clinical utility of liquid biopsy in cancer management, particularly in early detection, treatment monitoring, and resistance surveillance, establishes its essential role in modern oncology. The therapeutic applications of circulating biomarkers, especially in the context of engineered exosomes, represent a promising frontier in cancer treatment. The convergence of diagnostic and therapeutic applications through liquid biopsy components offers new opportunities for personalized medicine approaches, ultimately improving patient outcomes in cancer care.

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Mr. Santhosh Kumar Sorapalli is an undergraduate scholar pursuing his Bachelor of Pharmacy (B.Pharm) at K.G.R.L College of Pharmacy, Bhimavaram. During his academic journey, he has demonstrated keen interest in pharmaceutical sciences, particularly in the areas of pharmaceutical analysis and drug development. As an undergraduate researcher, he has actively participated in various academic projects and laboratory work under the guidance of faculty members. His commitment to learning is reflected in his academic performance and involvement in college activities.

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