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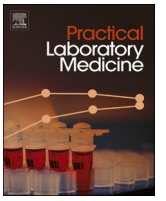

Liquid biopsy in cancer management: Integrating diagnostics and clinical applications

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ABSTRACT

Liquid biopsy is an innovative, minimally invasive diagnostic tool revolutionizing cancer management by enabling the detection and analysis of cancer-related biomarkers from bodily fluids such as blood, urine, or cerebrospinal fluid. Unlike traditional tissue biopsies, which require invasive procedures, liquid biopsy offers a more accessible and repeatable method for tracking cancer progression, detecting early-stage cancers, and monitoring therapeutic responses. The technology primarily focuses on analyzing circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and other cancer-derived genetic materials. These biomarkers provide critical information on tumor heterogeneity, mutation profiles, and potential drug resistance. In clinical practice, liquid biopsy has demonstrated its utility in identifying actionable mutations, guiding personalized treatment strategies, and assessing minimal residual disease (MRD). While liquid biopsy holds immense promise, challenges related to its sensitivity, specificity, and standardization remain. Efforts to optimize pre-analytical and analytical processes, along with the establishment of robust regulatory frameworks, are crucial for its widespread clinical adoption. This abstract highlights the transformative potential of liquid biopsy in cancer diagnosis, prognosis, and treatment monitoring, emphasizing its role in advancing personalized oncology. Further research, clinical trials, and regulatory harmonization will be vital in realizing its full potential in precision cancer care.

1. Introduction

Cancer is one of the most complex and lethal diseases faced by modern medicine. The need for accurate, timely, and non-invasive diagnostic tools has led to a transformative shift in how cancer is diagnosed and monitored. Traditional tissue biopsies, though effective in providing crucial information, are invasive, carry risks, and often fail to capture the full heterogeneity and dynamic nature of tumors over time. These limitations have paved the way for the development of liquid biopsy, a revolutionary approach that has the potential to reshape cancer diagnostics and clinical management [1].

Liquid biopsy refers to the analysis of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, and other tumor-derived components from easily accessible body fluids such as blood, urine, or cerebrospinal fluid (Fig. 1). This minimally invasive

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technique enables real-time monitoring of tumor evolution, providing key insights into cancer genetics and molecular changes without the need for repeated invasive procedures. As a result, liquid biopsy holds promise in multiple facets of cancer care, including early diagnosis, treatment monitoring, detection of minimal residual disease (MRD), and identification of therapeutic resistance [2–4]. The integration of liquid biopsy into clinical practice offers several advantages over conventional methods. It allows for the dynamic profiling of tumors, enabling personalized treatment approaches based on real-time molecular information. Additionally, it facilitates the detection of cancer recurrence or progression earlier than conventional imaging techniques, offering clinicians a powerful tool to make more informed treatment decisions. Despite the many benefits, liquid biopsy is not without its challenges. Issues surrounding sensitivity, specificity, standardization of techniques, and cost must be addressed to ensure its widespread clinical adoption [5]. However, with ongoing advancements in technology and research, liquid biopsy is rapidly evolving as a cornerstone of precision oncology.

This article will explore the fundamental principles of liquid biopsy, its clinical applications, and how it is transforming cancer management. By examining the latest advancements, technological innovations, and real-world applications, this introduction aims to provide a comprehensive overview of liquid biopsy's pivotal role in integrating diagnostics with clinical care in the fight against cancer.

1.1. *Background of cancer diagnostics*

Cancer diagnostics has traditionally relied on tissue biopsies, imaging techniques, and blood tests to identify the presence, type, and progression of cancer. Tissue biopsy, a gold standard for cancer diagnosis, involves the surgical removal of a sample from the tumor site for histopathological examination. This method provides essential information about tumor morphology, genetic mutations, and the molecular markers that drive cancer growth. However, tissue biopsies are often invasive, painful, and carry the risk of complications, such as infection, bleeding, or delayed recovery. Moreover, they are limited in their ability to capture the dynamic nature of cancer, which evolves over time and may not be fully represented by a single biopsy sample. The advent of imaging technologies such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) has further revolutionized cancer diagnostics [6–8]. These non-invasive methods allow for the visualization of tumors in great detail, enabling clinicians to assess tumor size, location, and metastatic spread. While imaging techniques have improved the detection and staging of cancer, they fall short in providing molecular-level information about the disease, which is crucial for personalized treatment strategies. Blood-based biomarkers, such as tumor markers (e.g., prostate-specific antigen [PSA], carcinoembryonic antigen [CEA]), have been utilized for cancer screening and monitoring treatment response. However, these markers often lack the specificity and sensitivity required for early detection or comprehensive understanding of the tumor's molecular profile. Furthermore, they cannot accurately reflect the genetic changes occurring within tumors over time, which limits their use in guiding targeted therapies or monitoring drug resistance [9–11].

In recent decades, the understanding of cancer biology has advanced significantly, revealing that tumors are not static entities but dynamic systems that continuously evolve. Tumors exhibit intratumoral heterogeneity, meaning that different regions of the same tumor may have distinct genetic mutations and molecular characteristics. This complexity makes cancer challenging to treat, as certain parts of the tumor may respond differently to therapies. Moreover, the emergence of treatment-resistant clones within the tumor can lead to relapse or disease progression. To address these challenges, there has been a growing need for diagnostic tools that are non-

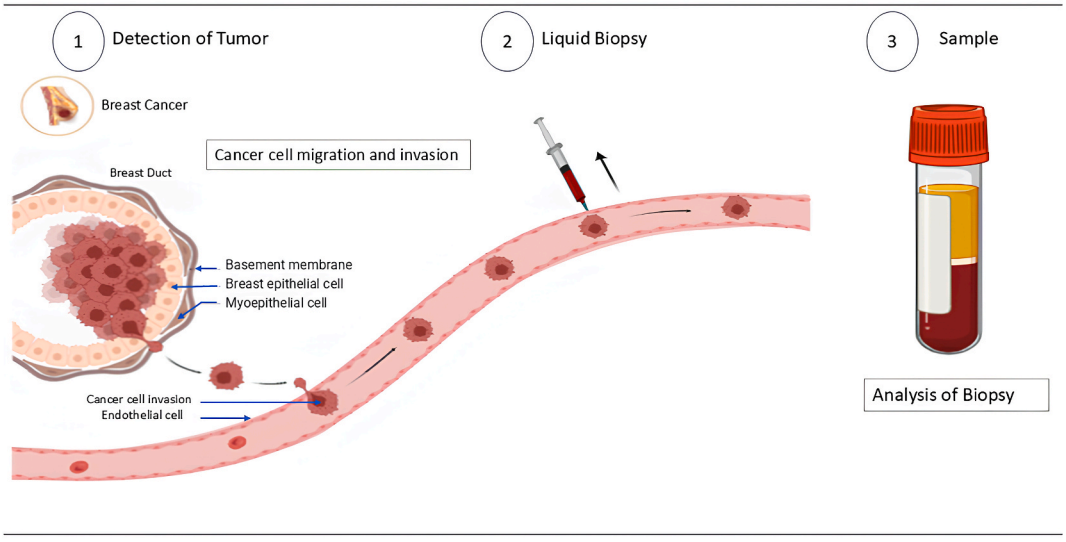

Fig. 1. Liquid Biopsy involving Cancer cells release into blood stream and further laboratory analysis.

invasive, able to capture the heterogeneity of tumors, and capable of providing real-time information about disease progression. This is where liquid biopsy emerges as a game-changing innovation in cancer diagnostics [12–14]. By analyzing tumor-derived components circulating in the blood or other bodily fluids, liquid biopsy offers a non-invasive means of tracking cancer in real time, paving the way for more personalized and adaptive cancer treatment strategies.

In summary, while traditional cancer diagnostics have been invaluable in improving survival rates and treatment outcomes, their limitations underscore the need for more advanced, comprehensive, and dynamic diagnostic tools like liquid biopsy. The rapid progress in cancer research and molecular diagnostics promises a future where precision oncology will be the standard of care, driven by innovations like liquid biopsy.

1.2. The evolution of liquid biopsy

The concept of liquid biopsy represents a significant evolution in cancer diagnostics, arising from the limitations of traditional tissue biopsy and the increasing need for more dynamic, non-invasive diagnostic methods. Over the past few decades, technological advancements in molecular biology and genomics have facilitated the development of liquid biopsy, transforming it into a promising tool for cancer management [15–17].

1.3. Early discoveries and foundation

The journey of liquid biopsy began in the 1970s when researchers first identified circulating tumor cells (CTCs) in the blood of cancer patients. These rare cells, shed from the primary tumor into the bloodstream, were initially considered anomalies, as their detection posed significant technical challenges due to their scarcity and the limitations of available technology. However, their presence hinted at the possibility of monitoring cancer progression through blood samples, laying the foundation for future liquid biopsy techniques. In the following years, the discovery of circulating tumor DNA (ctDNA) in the blood of cancer patients marked a critical milestone. Researchers found that tumors release fragmented DNA into the bloodstream, which contains the same genetic mutations as the primary tumor. This revelation opened new doors to studying cancer's genetic and molecular characteristics without the need for invasive tissue biopsies. As molecular detection techniques, such as polymerase chain reaction (PCR), improved, scientists gained the ability to detect ctDNA with greater sensitivity and accuracy [18]. The real breakthrough in liquid biopsy came with the advent of next-generation sequencing (NGS) and digital PCR technologies. These powerful tools allowed for the precise detection of genetic mutations, copy number variations, and other alterations in ctDNA, even in very small quantities. NGS, in particular, enabled the analysis of a wide range of genetic mutations and facilitated comprehensive tumor profiling through a simple blood draw. This paved the way for the practical application of liquid biopsy in cancer diagnosis, monitoring, and therapeutic decision-making.

Digital PCR also played a pivotal role in improving the sensitivity of ctDNA detection. Unlike conventional PCR, which may struggle with low-abundance DNA, digital PCR divides a sample into thousands of tiny partitions, amplifying and detecting even trace amounts of ctDNA with high specificity. These technological improvements dramatically enhanced the utility of liquid biopsy in capturing minute genetic alterations and tracking tumor evolution over time. In parallel with advances in CTC and ctDNA research, scientists discovered that tumors release extracellular vesicles, such as exosomes, into the bloodstream. These tiny vesicles carry proteins, RNA, and DNA that reflect the molecular profile of the tumor. Exosomes and other extracellular vesicles added another layer of complexity and richness to liquid biopsy, offering a non-invasive means to study tumor behavior, signaling pathways, and potential therapeutic targets. Exosome-based liquid biopsies hold particular promise for detecting cancers at early stages, as these vesicles are present in higher quantities and more accessible in blood samples than CTCs or ctDNA [19–21].

Furthermore, exosome analysis allows for the study of the tumor microenvironment, providing insights into cancer's interaction with surrounding tissues and immune cells, which is vital for developing novel therapeutic strategies.

2. Clinical integration and personalized medicine

As liquid biopsy technology evolved, its potential for real-time cancer monitoring and personalized treatment became clear. Unlike tissue biopsies, which capture a static snapshot of a tumor, liquid biopsy provides a dynamic view of the cancer's progression, detecting genetic mutations, resistance mechanisms, and minimal residual disease (MRD) after treatment. This capability aligns perfectly with the principles of precision oncology, where treatment decisions are tailored to the unique molecular characteristics of each patient's cancer. The integration of liquid biopsy into clinical practice gained momentum in the 2010s, with the U.S. Food and Drug Administration (FDA) approving the first liquid biopsy test in 2016 for detecting EGFR mutations in non-small cell lung cancer [22].

Since then, liquid biopsy has expanded to include a variety of cancer types, and its applications now extend to early detection, monitoring treatment response, assessing drug resistance, and predicting recurrence.

3. The future of liquid biopsy

Ongoing research aims to further refine liquid biopsy by improving sensitivity, specificity, and cost-effectiveness. Multi-omic approaches, which integrate data from ctDNA, CTCs, exosomes, and other biomarkers, are being explored to provide a more comprehensive understanding of cancer biology. Additionally, as new markers are discovered, the potential for liquid biopsy to detect cancers earlier, before symptoms arise, is growing, particularly in high-risk populations. The evolution of liquid biopsy is continuing at a rapid pace, driven by advances in molecular biology, genomics, and bioinformatics [23–25].

As these technologies mature, liquid biopsy is expected to become a cornerstone in cancer management, reducing reliance on invasive procedures and enabling a new era of personalized, precision oncology. In summary, liquid biopsy has evolved from a scientific curiosity to a revolutionary diagnostic tool with the potential to transform cancer care. By offering non-invasive, real-time insights into tumor biology, liquid biopsy not only addresses the limitations of traditional diagnostic methods but also opens new avenues for personalized medicine and improved patient outcomes [26].

4. Principles of liquid biopsy

Liquid biopsy is based on the principle that tumors shed various cellular and molecular components into the bloodstream and other bodily fluids, reflecting their genetic and biological characteristics (Fig. 2). By analyzing these components, clinicians can gain insights into tumor dynamics, treatment responses, and disease progression [27]. The primary principles of liquid biopsy include the following:

a) Tumor Shedding: Tumors release a variety of components into circulation through several mechanisms:
   - Circulating Tumor Cells (CTCs): As tumors grow, some cancer cells break off from the primary tumor and enter the bloodstream. The presence of CTCs can indicate metastasis and provide information about the tumor's characteristics.
   - Circulating Tumor DNA (ctDNA): Tumor cells undergo apoptosis (programmed cell death) and necrosis, releasing fragments of DNA into the bloodstream. ctDNA carries mutations and alterations specific to the tumor, making it a valuable biomarker for genetic profiling and monitoring.
   - Exosomes and Extracellular Vesicles: These are small membrane-bound vesicles secreted by tumor cells that contain proteins, lipids, RNA, and DNA. They reflect the molecular state of the tumor and can facilitate intercellular communication within the tumor microenvironment.

b) Non-Invasive Sampling: One of the key advantages of liquid biopsy is its non-invasive nature. A simple blood draw or collection of other bodily fluids allows for the analysis of tumor-derived components without the need for surgical interventions. This accessibility enhances patient comfort and reduces complications associated with traditional biopsies.

c) Multiplex Analysis: Liquid biopsy enables the simultaneous analysis of multiple biomarkers and components, providing a comprehensive overview of the tumor's molecular profile. Techniques such as next-generation sequencing (NGS) allow for the detection of a wide range of mutations, copy number variations, and epigenetic alterations in ctDNA, while CTCs and exosomes can be analyzed for proteins and RNA, offering insights into tumor heterogeneity.

d) Real-Time Monitoring: Liquid biopsy provides real-time insights into tumor dynamics. As tumors evolve in response to treatment or external factors, liquid biopsy can capture these changes, allowing for adaptive treatment strategies. For instance, detecting the emergence of resistant mutations in ctDNA can prompt adjustments in therapy to enhance effectiveness.

e) Personalized Medicine: The ability to analyze tumor-specific genetic alterations through liquid biopsy aligns with the principles of personalized medicine. By tailoring treatment strategies based on the molecular profile of a patient's cancer, clinicians can optimize therapeutic outcomes and minimize unnecessary side effects.

f) Sensitivity and Specificity: Liquid biopsy techniques are designed to achieve high sensitivity and specificity in detecting tumor-derived components. Advances in technology, such as digital PCR and advanced microfluidics, have significantly improved the detection limits for CTCs and ctDNA, allowing for the identification of low-abundance markers that can indicate early disease or treatment response.

g) Understanding Tumor Heterogeneity: Liquid biopsy provides a means to study intratumoral heterogeneity, as the components present in circulation can reflect the diversity of cancer cell populations within a tumor. This is crucial for understanding how different subclones may respond to treatment and for identifying potential resistance mechanisms.

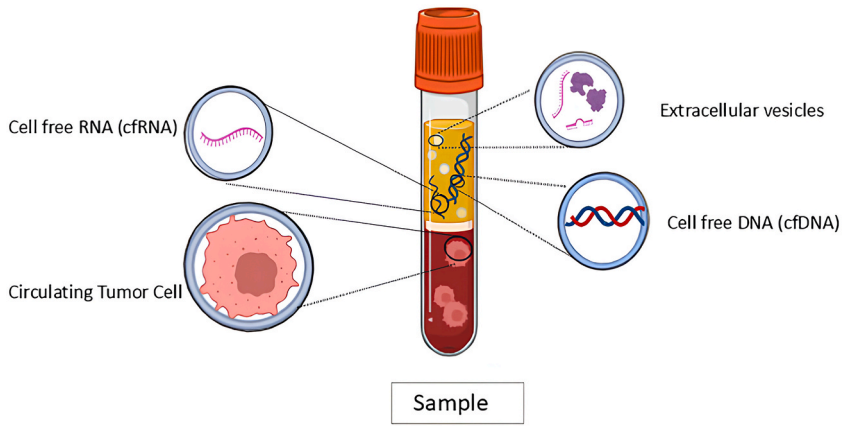

Fig. 2. Component of Liquid Biopsy sample containing cell-free DNA (cfDNA), cell-free RNA (cfRNA) extracellular vehicles (EVs), and tumors cell.

h) Applications in Multiple Cancer Types: The principles of liquid biopsy apply across various cancer types, making it a versatile tool in oncology. From detecting early-stage cancers to monitoring treatment efficacy in advanced disease, liquid biopsy offers a broad range of applications that can enhance patient management. The principles of liquid biopsy fundamentally revolve around the ability to obtain critical information about cancer biology from non-invasive samples. By harnessing the insights gained from analyzing CTCs, ctDNA, and exosomes, liquid biopsy stands at the forefront of cancer diagnostics and management, offering the potential to revolutionize the way clinicians approach cancer care.

5. Sample collection and processing methods

Effective sample collection and processing are critical components of liquid biopsy, as they directly influence the quality and reliability of the diagnostic information obtained. The following outlines the common methods for sample collection and processing in liquid biopsy [27–29].

5.1. Sample collection methods

5.1.1. Blood collection

- Peripheral Blood Draw: The most common method involves venipuncture to collect blood samples, typically using standard blood collection tubes. Blood samples can be drawn using either,
- EDTA or Citrate Tubes: Used for plasma separation.
- Cell-Free DNA (cfDNA) Tubes: Specialized tubes that stabilize ctDNA for subsequent analysis.
- Volume Considerations: Usually, a sample volume of 5–10 mL is sufficient for liquid biopsy analysis. However, larger volumes may be required for specific assays or technologies.

5.1.2. Other bodily fluids

- **Urine:** Liquid biopsy can also utilize urine samples containing ctDNA and exosomes. Urine collection is non-invasive and can provide insights into bladder and kidney cancers. Urine-based liquid biopsy is a promising and non-invasive method to detect and monitor various diseases, particularly bladder and kidney cancers and systemic conditions. Tumor-derived DNA fragments from apoptosis, necrosis, or active secretion of cancer cells cause tumor-specific genetic alterations (e.g., mutations, methylation changes). Monitoring bladder and kidney cancers due to proximity to the urinary tract. Potentially useful in detecting other cancers with systemic biomarker shedding. Exosomes and Extracellular Vesicles (EVs) are Small vesicles released by all cell types, including tumor cells, containing DNA, RNA, proteins, and lipids. It helps to identify tumor-related mutations, RNA transcripts, and protein markers that aid in distinguishing between different cancer types and subtypes. Proteins and Metabolites Secreted or filtered from cancerous cells or the tumor microenvironment into urine. Identifies cancer-specific protein biomarkers, such as NMP22 (bladder cancer) that analyses metabolic changes that occur during cancer progression. Cell-Free RNA (cfRNA) are RNA fragments shed into urine by tumor cells. Detection of non-coding RNAs, such as microRNAs (miRNAs), associated with cancer development. Urine-Based Liquid Biopsy Collection is painless and does not require specialized equipment or trained personnel. Easy to repeat, enabling dynamic tracking of disease progression or therapeutic response. Especially effective for urological cancers, as biomarkers are concentrated in the urine. RNA and some proteins in urine are prone to degradation, requiring careful sample handling. Urine might not always reflect tumors distant from the urinary tract.
- **Cerebrospinal Fluid (CSF):** CSF can be analyzed for tumor markers in cases of central nervous system cancers. However, collection is more invasive and requires a lumbar puncture. Cerebrospinal fluid (CSF) is an invaluable biofluid for analyzing tumor markers, particularly in cases of central nervous system (CNS) cancers such as gliomas, medulloblastomas, and metastatic brain tumors. CSF offers direct access to the brain and spinal cord, making it highly sensitive for detecting tumor-derived biomarkers. Components like circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), extracellular vesicles (e.g., exosomes), cell-free RNA (cfRNA), and tumor-specific proteins can be identified in CSF, providing critical insights into tumor genetics, molecular alterations, and the CNS microenvironment. These markers can aid in early diagnosis, prognosis, treatment monitoring, and detecting recurrence. However, the collection of CSF is more invasive than other liquid biopsy methods, requiring a lumbar puncture, which carries risks such as discomfort, infection, and post-procedure complications. Additionally, the limited volume of CSF available for testing and the low abundance of some biomarkers pose challenges.
- **Pleural Effusions and Ascitic Fluid**: Fluid collected from the pleural cavity or abdominal cavity can be analyzed, particularly in advanced-stage cancers with fluid accumulation. Pleural effusions and ascitic fluid, commonly seen in advanced-stage cancers, are valuable sources for liquid biopsy analysis. These fluids, collected from the pleural cavity or abdominal cavity, often accumulate due to malignancies such as lung, breast, ovarian, or gastrointestinal cancers. They contain tumor-derived components like circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), extracellular vesicles, and proteins that reflect the molecular characteristics of the underlying cancer. Analyzing these biomarkers can aid in diagnosing malignancy, identifying specific genetic or epigenetic alterations, and monitoring disease progression or therapeutic response. While the collection process involves minimally invasive techniques like thoracentesis for pleural effusion or paracentesis for ascitic fluid, it is typically performed only when clinically necessary. Challenges include variability in biomarker concentration and potential contamination from non-tumor cells.

- **Saliva**; Saliva is an emerging biofluid for liquid biopsy, offering a non-invasive and convenient method for cancer detection and monitoring. (Saliva as a potential non-invasive liquid biopsy for early and easy diagnosis/prognosis of head and neck cancer) It contains a variety of biomarkers, including circulating tumor DNA (ctDNA), cell-free RNA (cfRNA), proteins, metabolites, and extracellular vesicles such as exosomes, which can provide valuable insights into tumor biology. Saliva-based liquid biopsy is particularly promising for detecting head and neck cancers, as well as systemic cancers like lung and pancreatic cancer, where tumor-derived components may be present in salivary secretions. Advances in highly sensitive detection methods, such as digital PCR and next-generation sequencing (NGS), have enhanced the ability to identify tumor-specific mutations, epigenetic changes, and gene expression profiles from salivary samples. The simplicity of saliva collection allows for frequent sampling, making it ideal for real-time monitoring of disease progression or treatment response.

5.2. Sample processing methods

Separation of Components After collection, samples must be processed quickly to prevent degradation of biomolecules:

- Centrifugation: Blood samples are typically centrifuged to separate plasma from cellular components.
- Double Centrifugation: This method involves an initial low-speed centrifugation to remove cells, followed by a high-speed centrifugation to further clarify plasma and remove cell debris.

5.3. Isolation techniques once the plasma is obtained, the next step is to isolate specific components for analysis

5.3.1. Circulating tumor cells (CTCs)

Enrichment Techniques: CTCs can be isolated using various methods such as immunomagnetic separation (using antibodies against specific tumor markers), size-based filtration, or density gradient centrifugation [30–32].

5.3.2. Circulating tumor DNA (ctDNA)

- Extraction Methods: Several methods are available for isolating ctDNA from plasma, including silica-based extraction, magnetic bead-based extraction, and phenol-chloroform extraction. These methods aim to maximize yield while minimizing contamination.

5.3.3. Exosomes and Extracellular Vesicles

- Isolation Techniques: Exosomes can be isolated through ultracentrifugation, precipitation methods, or affinity-based methods that utilize specific surface markers. Ultrafiltration is also a commonly used technique.

5.3.4. Quality control and storage ensuring the integrity of the collected samples is crucial for accurate results

Assessing the quality of the extracted DNA or CTCs is essential, often done through qPCR or bioanalyzer techniques to measure size and concentration. Samples should be stored at −80 °C or in liquid nitrogen for long-term preservation [33–35]. Plasma samples should be processed within 2–4 h of collection to prevent degradation of ctDNA and other biomarkers.

5.4. Considerations for sample collection and processing

- Standardization: The standardization of protocols for collection, processing, and storage is critical to ensure reproducibility and comparability across studies and clinical applications.
- Minimizing Contamination: Care must be taken to avoid contamination from external sources or from the collection materials themselves, as this can lead to false-positive or false-negative results.
- Patient Factors: Factors such as the time of blood draw, patient health status, and pre-analytical conditions can impact the quality of liquid biopsy samples.

The methods of sample collection and processing in liquid biopsy are fundamental to obtaining high-quality, reliable data for cancer diagnostics and monitoring. By employing standardized protocols and effective isolation techniques, liquid biopsy can provide valuable insights into tumor dynamics, enabling improved patient management and personalized treatment strategies [36–38].

6. Technological approaches in liquid biopsy

Technological advancements have played a crucial role in the development and application of liquid biopsy. Various innovative techniques allow for the sensitive detection, isolation, and analysis of tumor-derived components from bodily fluids [39–41].

- Next-Generation Sequencing (NGS): NGS allows for the simultaneous sequencing of multiple genes and regions of interest in ctDNA, providing a comprehensive overview of genetic alterations within tumors. It is widely used for detecting mutations, copy number variations, and fusions that are critical for diagnosing cancer and monitoring treatment response. NGS offers high

throughput and sensitivity, enabling the detection of low-abundance ctDNA variants that may indicate disease progression or resistance to therapy.
- Digital PCR (dPCR): Digital PCR is a highly sensitive technique that partitions a sample into thousands of individual reactions, allowing for the quantification of rare genetic mutations in ctDNA. dPCR is particularly effective for detecting specific mutations or changes in ctDNA associated with cancer, making it valuable for monitoring treatment responses and detecting minimal residual disease (MRD). It provides absolute quantification of nucleic acids, minimizing variability and enhancing sensitivity compared to traditional PCR methods.
- Circulating Tumor Cell (CTC) Isolation Technologies: CTCs are isolated from blood using various enrichment techniques that target their unique properties.

6.1. Techniques

- Immunomagnetic Separation: Utilizes antibodies against specific tumor markers attached to magnetic beads to capture CTCs.
- Size-Based Filtration: Separates CTCs based on their larger size compared to normal blood cells.
- Microfluidics: Employs lab-on-a-chip devices that manipulate fluids at a microscale to isolate and analyze CTCs efficiently.
- Isolated CTCs can be characterized for genetic mutations, protein expression, and cellular behavior, providing insights into metastasis and treatment efficacy.

6.1.1. Exosome and extracellular vesicle analysis

- Exosomes are small vesicles released by cells that contain proteins, lipids, and nucleic acids, reflecting the molecular characteristics of the tumor.
- Isolation Techniques: Methods such as ultracentrifugation, precipitation, and size-exclusion chromatography are used to isolate exosomes from plasma or other fluids.
- Applications: Analysis of exosomes can provide information on tumor markers, genetic mutations, and microRNA profiles, contributing to early detection and monitoring of cancer.
- Technologies for Analysis: Techniques such as NGS, mass spectrometry, and PCR can be employed to analyze the content of exosomes.

6.1.2. Mass spectrometry

- Mass spectrometry is used to analyze proteins and metabolites present in biological samples, offering insights into tumor biology.
- Applications: It can identify protein biomarkers and metabolic signatures associated with cancer, aiding in diagnosis and monitoring treatment responses.
- Advantages: Mass spectrometry provides high sensitivity and specificity, allowing for the detection of low-abundance biomarkers.

6.1.3. Imaging technologies

- Advanced imaging techniques, such as liquid biopsy imaging and real-time PCR imaging, can visualize and quantify tumor-derived components in real-time.
- Applications: These techniques are useful for tracking the dynamics of CTCs and exosomes in circulation, providing insights into tumor behavior and treatment effects.

6.1.4. Microfluidics and lab-on-a-chip technologies

- Microfluidic devices manipulate small volumes of fluids, allowing for the rapid isolation and analysis of CTCs, ctDNA, and exosomes.
- Applications: These technologies facilitate high-throughput screening and multiplex assays, enabling simultaneous analysis of multiple biomarkers.
- Advantages: They require smaller sample volumes and provide quick results, making them suitable for clinical applications.

The technological approaches in liquid biopsy have revolutionized cancer diagnostics and management, enabling sensitive and specific detection of tumor-derived components. By integrating these advanced techniques, clinicians can gain valuable insights into tumor biology, monitor treatment responses, and make informed decisions in personalized cancer care. As technology continues to advance, the potential for liquid biopsy to transform oncology practice will only increase, paving the way for more effective and adaptive treatment strategies [42–45].

7. Emerging techniques and future trends in liquid biopsy

The field of liquid biopsy is rapidly evolving, driven by technological advancements and a deeper understanding of cancer biology. Emerging techniques and future trends are expected to enhance the accuracy, sensitivity, and applicability of liquid biopsy in clinical practice [46].

Some key developments and future directions in this field:

7.1. Single-cell analysis

- Single-cell sequencing and analysis technologies allow for the examination of individual cells, providing insights into tumor heterogeneity and the specific characteristics of CTCs or circulating immune cells.
- Applications: This approach can reveal the unique genetic mutations and expression profiles of individual tumor cells, helping to identify resistant clones and informing targeted therapies.
- Future Trend: Continued development in single-cell sequencing techniques will likely enhance the understanding of cancer evolution and treatment resistance.

7.2. Multi-omics integration

- Multi-omics approaches combine genomic, transcriptomic, proteomic, and metabolomic data to provide a comprehensive view of tumor biology.
- Applications: By integrating data from ctDNA, CTCs, and exosomes, clinicians can gain deeper insights into tumor dynamics, microenvironment interactions, and potential therapeutic targets.
- Future Trend: The development of standardized multi-omics platforms will facilitate more holistic cancer assessments and personalized treatment strategies.

7.3. Advanced liquid biopsy platforms

- New platforms are being developed to enhance the efficiency and effectiveness of liquid biopsy analyses, such as integrated microfluidic devices and automated processing systems.
- Applications: These platforms can streamline the workflow from sample collection to analysis, reducing time and variability in results.
- Future Trend: Enhanced automation and integration of various technologies will make liquid biopsy more accessible in clinical settings.

7.4. Artificial intelligence and machine learning

AI and machine learning algorithms are being utilized to analyze large datasets generated from liquid biopsy studies, improving diagnostic accuracy and predictive capabilities.

- Applications: These technologies can help identify patterns in genetic alterations, predict treatment responses, and refine risk assessments for cancer recurrence.
- Future Trend: The integration of AI-driven analytics into clinical decision-making will likely lead to more personalized and effective cancer management strategies.

7.5. Liquid biopsy for early detection

Research is ongoing to develop liquid biopsy methods for the early detection of cancer, including its application in screening high-risk populations.

- Applications: Early detection can significantly improve treatment outcomes, as interventions can be initiated at a more manageable stage of the disease.
- Future Trend: Continued efforts to validate and implement liquid biopsy for early cancer detection will enhance screening programs and reduce cancer mortality.

The emerging techniques and future trends in liquid biopsy promise to enhance its role in cancer management and diagnostics significantly. By advancing technologies and integrating new methodologies, the field is moving toward more personalized, efficient, and comprehensive approaches to cancer care. As these innovations are realized, liquid biopsy has the potential to transform how we diagnose, monitor, and treat cancer, ultimately improving patient outcomes and quality of life [47].

8. Clinical applications of liquid biopsy in cancer management

Liquid biopsy has emerged as a transformative tool in cancer management, offering various clinical applications that enhance diagnosis, treatment monitoring, and overall patient care. The key clinical applications of liquid biopsy in cancer management:

a) Early Detection of Cancer

Liquid biopsy can facilitate the early detection of cancer through the analysis of ctDNA, CTCs, and other biomarkers in blood samples.

Applications:

- Screening High-Risk Populations: Liquid biopsy tests can identify individuals at high risk for certain cancers, enabling early intervention and treatment.
- Detecting Minimal Residual Disease (MRD): The presence of ctDNA after treatment can indicate residual cancer cells, prompting closer monitoring and additional therapy.

b) Diagnosis and Tumor Profiling

Liquid biopsy allows for non-invasive tumor profiling, providing insights into the genetic and molecular characteristics of a patient's cancer.

Applications:

- Identifying Genetic Mutations: Liquid biopsy can detect specific mutations associated with various cancer types, aiding in diagnosis and treatment selection.
- Understanding Tumor Heterogeneity: Analysis of CTCs and ctDNA can reveal the diversity within a tumor, informing targeted therapy strategies.

c) Monitoring Treatment Response

Liquid biopsy offers a real-time assessment of treatment efficacy by tracking changes in tumor-derived components.

Applications:

- ctDNA Analysis: Decreases in ctDNA levels during treatment can indicate a positive response, while increases may signal disease progression or resistance.
- CTC Monitoring: Changes in the number or characteristics of CTCs can provide insights into how well a patient is responding to therapy.

d) Assessing Drug Resistance

Liquid biopsy can help identify emerging resistance mechanisms as tumors evolve during treatment.

Applications:

- Detecting Resistance Mutations: Liquid biopsy can reveal new mutations in ctDNA that confer resistance to targeted therapies, allowing for timely adjustments in treatment plans.
- Monitoring CTC Phenotypes: Changes in the characteristics of CTCs can indicate shifts in tumor biology and potential resistance to ongoing therapies.

e) Predicting Recurrence

Liquid biopsy can be utilized to assess the risk of cancer recurrence post-treatment.

Applications:

- ctDNA Monitoring: The presence of ctDNA after curative treatment can indicate a higher risk of recurrence, prompting closer surveillance and earlier intervention.
- Personalized Follow-Up Strategies: Patients can be stratified based on ctDNA levels, allowing for tailored follow-up protocols that are more sensitive to individual risk profiles.

f) Personalized Treatment Strategies

Liquid biopsy enables the development of personalized treatment plans based on the unique molecular characteristics of a patient's cancer.

Applications:

- Targeted Therapy Selection: Genetic profiling through liquid biopsy can identify actionable mutations, guiding the selection of targeted therapies that are most likely to be effective.
- Adaptive Treatment Approaches: As the tumor evolves, liquid biopsy can inform adjustments in therapy to optimize outcomes, ensuring treatment remains aligned with the tumor's current profile.

g) Clinical Trial Enrollment and Monitoring

Liquid biopsy can facilitate patient enrollment in clinical trials by identifying suitable candidates based on molecular

characteristics.

Applications:

- Biomarker-Driven Trials: Liquid biopsy can determine the presence of specific biomarkers required for entry into targeted therapy trials.
- Monitoring Trial Outcomes: Regular liquid biopsy assessments can provide real-time data on treatment efficacy and safety, enhancing trial management and outcomes analysis.

The clinical applications of liquid biopsy in cancer management represent a significant advancement in oncology, providing non-invasive methods for early detection, diagnosis, treatment monitoring, and personalized care. By harnessing the insights gained from analyzing tumor-derived components, liquid biopsy not only enhances patient management but also contributes to the overall goal of improving cancer outcomes and quality of life. As technology continues to evolve, the integration of liquid biopsy into routine clinical practice is likely to expand, further solidifying its role in modern oncology [48–51].

8.1. Detection of recurrence and metastasis

Liquid biopsy plays a pivotal role in the detection of cancer recurrence and metastasis, offering real-time insights into tumor dynamics without the need for invasive procedures [52,53]. The key aspects of how liquid biopsy contributes to monitoring recurrence and metastasis:

a) Monitoring Circulating Tumor DNA (ctDNA)

ctDNA, which consists of small fragments of DNA released into the bloodstream by dying tumor cells, serves as a biomarker for cancer recurrence. Measuring ctDNA levels after curative treatment can help identify residual disease. Rising ctDNA levels may indicate impending recurrence, allowing for early intervention. The concentration of ctDNA can correlate with tumor burden, making it a useful tool for assessing disease progression or response to therapy.

b) Detecting Circulating Tumor Cells (CTCs)

CTCs are cancer cells that have shed from the primary tumor into the bloodstream, serving as indicators of metastasis and recurrence. The presence and characteristics of CTCs can provide information about metastatic potential and help in assessing disease stage. Changes in CTC counts during treatment can reflect response rates and help in identifying cases of relapse.

c) Profiling Genetic Mutations

Liquid biopsy allows for the detection of specific mutations associated with tumor recurrence or metastasis. Mutations in ctDNA that emerge during treatment can signify the development of resistance, guiding clinicians in adjusting therapeutic strategies. Regular analysis of ctDNA can help track changes in the tumor's genetic landscape, revealing how it adapts over time.

d) Clinical Implications

Liquid biopsy enables more frequent and less invasive monitoring, facilitating timely interventions and reducing the need for repeated tissue biopsies. By stratifying patients based on ctDNA levels or CTC presence, clinicians can tailor follow-up and treatment protocols, enhancing patient care.

8.2. Liquid biopsy in personalized therapy

Liquid biopsy is revolutionizing personalized therapy in oncology, allowing for tailored treatment strategies based on the unique molecular profile of a patient's cancer [54]. Liquid biopsy contributes to personalized therapy:

a) Genomic Profiling for Targeted Therapy

Liquid biopsy enables the analysis of genetic mutations and alterations in ctDNA, guiding the selection of targeted therapies. Detection of specific genetic alterations can direct the use of targeted therapies, improving the likelihood of treatment success. As tumors evolve, liquid biopsy can provide updated genomic profiles, allowing for adjustments to treatment plans based on current tumor characteristics.

b) Monitoring Treatment Responses

Liquid biopsy offers real-time insights into how well a patient is responding to therapy. A decrease in ctDNA levels can indicate a positive treatment response, while rising levels may signal disease progression or inadequate response, prompting adjustments to the therapeutic approach. Monitoring changes in CTC counts and characteristics can provide additional insights into treatment efficacy

and disease progression.

c) Identifying Minimal Residual Disease (MRD)

Liquid biopsy can detect minimal residual disease post-treatment, which is crucial for predicting recurrence. The presence of ctDNA indicating MRD can trigger early interventions, such as additional therapy, to prevent recurrence. Patients with detectable MRD can be subjected to more intensive follow-up and monitoring strategies to catch recurrence early.

d) Assessing Patient-Specific Treatment Options

Liquid biopsy allows for the identification of patient-specific biomarkers that may influence treatment choices. Liquid biopsy can aid in understanding how a patient's genetic makeup influences their response to specific drugs, facilitating personalized treatment decisions. As the tumor evolves, liquid biopsy can guide adaptive therapy strategies, ensuring treatment remains aligned with the tumor's current profile.

e) Clinical Implications

By enabling personalized therapy, liquid biopsy has the potential to improve treatment outcomes and minimize unnecessary side effects. Clinicians can make more informed decisions about treatment strategies based on the dynamic information provided by liquid biopsy analyses.

Liquid biopsy significantly enhances the detection of recurrence and metastasis while facilitating personalized therapy in cancer management. By providing real-time insights into tumor dynamics and molecular characteristics, liquid biopsy supports informed clinical decisions and tailored treatment strategies, ultimately improving patient outcomes in oncology. As technology advances, the integration of liquid biopsy into routine clinical practice is likely to expand, offering new avenues for personalized cancer care [55–58].

9. Comparative analysis: liquid biopsy vs. tissue biopsy

Both liquid biopsy and tissue biopsy are valuable tools in cancer diagnosis and management, each with distinct advantages and limitations. This comparative analysis highlights the key differences, benefits, and challenges associated with each approach.

a) Methodology

- Liquid Biopsy:
  Sample Type: Involves the analysis of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, and other components found in bodily fluids, primarily blood.
  Collection Process: Non-invasive; typically involves a simple blood draw.
  Analysis Techniques: Utilizes methods such as next-generation sequencing (NGS), digital PCR, and mass spectrometry to analyze tumor-derived components.
- Tissue Biopsy:
  Sample Type: Involves the collection of tissue samples directly from a tumor, which can be obtained through various methods, such as needle aspiration, core biopsy, or surgical resection.
  Collection Process: Invasive; may require surgical procedures, which can carry risks and complications.
  Analysis Techniques: Involves histopathological examination, immunohistochemistry, and genomic profiling.

b) Diagnostic Information

- Liquid Biopsy:
  Dynamic Monitoring: Provides real-time insights into tumor biology, enabling the detection of changes over time, including treatment response and disease progression.
  Tumor Heterogeneity: Captures a broader representation of tumor heterogeneity, as it analyzes components shed by multiple tumor cells.
  Molecular Profiling: Can identify specific mutations, gene amplifications, and other biomarkers relevant for targeted therapies.
- Tissue Biopsy:
  Comprehensive Histology: Provides detailed histological information and architecture of the tumor, including the tumor microenvironment.
  Specificity: Often considered the gold standard for confirming cancer diagnosis, especially for specific subtypes or rare cancers.
  Biomarker Analysis: Can provide information on protein expression and other histological markers critical for prognosis and treatment decisions.

c) Clinical Applications

•Liquid Biopsy:

Early Detection: Useful for early detection of cancer, monitoring minimal residual disease (MRD), and tracking recurrence.

Treatment Monitoring: Allows for real-time assessment of treatment efficacy and detection of emerging resistance mutations.

Patient Management: Facilitates non-invasive follow-up and monitoring strategies, enhancing patient comfort.

•Tissue Biopsy:

Initial Diagnosis: Typically, the first-line approach for confirming cancer diagnosis and obtaining tissue for molecular profiling.

Comprehensive Evaluation: Provides a thorough evaluation of tumor characteristics, which is essential for determining treatment strategies.

Tumor Staging: Offers critical information for staging and grading tumors, which are essential for prognosis and treatment planning.

d) Limitations

•Liquid Biopsy:

Sensitivity and Specificity: While improving, some liquid biopsy assays may still have limitations in sensitivity for certain cancer types or stages.

Interpretation Challenges: The presence of ctDNA or CTCs does not always correlate with active disease, leading to potential false positives or negatives.

Standardization Needs: There is a need for standardized protocols for sample collection, processing, and analysis to ensure reproducibility.

•Tissue Biopsy:

Invasiveness: Invasive procedures can lead to complications, discomfort, and longer recovery times for patients.

Limited Temporal Insight: A single tissue sample may not fully capture tumor heterogeneity or temporal changes, potentially missing evolving mutations.

Accessibility Issues: In some cases, tumors may be difficult to access, and not all patients may be eligible for surgical biopsy.

10. Future directions

Integration of Both Approaches: The complementary nature of liquid and tissue biopsies suggests that an integrated approach may provide the most comprehensive insights into cancer diagnosis and management. Continued advancements in liquid biopsy technologies and methodologies may enhance their sensitivity, specificity, and clinical utility, making them more widely applicable in oncology.

Liquid biopsy and tissue biopsy each play critical roles in cancer diagnosis and management, with unique advantages and limitations. Liquid biopsy offers a non-invasive, real-time approach to monitoring tumor dynamics, while tissue biopsy provides detailed histological and molecular insights. Understanding the strengths and weaknesses of each method is essential for optimizing cancer care and tailoring treatment strategies to individual patients. As research and technology continue to evolve, the integration of both approaches may pave the way for more effective and personalized cancer management [59,60].

10.1. Liquid biopsy in specific cancer types

Liquid biopsy has demonstrated significant potential across various cancer types, providing insights into tumor dynamics, aiding in early detection, and facilitating personalized treatment strategies [61–63]. Below is an overview of how liquid biopsy is applied in specific cancer types.

a) Lung Cancer
- ctDNA Analysis: Liquid biopsy can detect mutations in the EGFR gene, which are crucial for guiding targeted therapies. Monitoring ctDNA levels can help assess treatment response and identify resistance mutations.
- CTC Monitoring: The presence and characteristics of CTCs can provide information about metastatic potential and overall prognosis.

b) Breast Cancer
- Early Detection: Liquid biopsy can aid in the early detection of breast cancer by identifying specific mutations or elevated levels of ctDNA.
- Monitoring Recurrence: Regular ctDNA analysis post-treatment can help detect minimal residual disease (MRD) and predict recurrence, enabling timely interventions.
- Genomic Profiling: Analysis of biomarkers such as HER2/neu in circulating components can guide targeted therapy decisions.

c) Colorectal Cancer
- Molecular Profiling: Liquid biopsy can identify mutations in key genes (e.g., KRAS, NRAS) that inform treatment options and predict response to targeted therapies.
- Monitoring Response: ctDNA levels can be used to monitor treatment efficacy, allowing for early detection of disease progression or recurrence.

d) Prostate Cancer

- Androgen Receptor Analysis: Liquid biopsy can detect mutations and amplifications in the androgen receptor gene, informing treatment strategies for advanced disease.
- ctDNA for Monitoring: Assessing ctDNA can provide insights into tumor burden and treatment response, helping to manage the disease more effectively.

e) Ovarian Cancer
- Biomarker Identification: Liquid biopsy can detect biomarkers such as CA-125 and genetic alterations in BRCA1/2, which are critical for diagnosis and treatment planning.
- Monitoring Recurrence: Regular ctDNA analysis can help track recurrence, providing an opportunity for earlier interventions.

f) Melanoma
- Targeted Therapy Monitoring: Liquid biopsy can detect mutations in the BRAF gene, essential for guiding targeted therapy in melanoma patients.
- ctDNA Analysis: Monitoring ctDNA can provide insights into treatment efficacy and potential resistance, allowing for timely adjustments in therapy.

g) Pancreatic Cancer
- Early Detection Challenges: Liquid biopsy shows promise for detecting specific mutations (e.g., KRAS) associated with pancreatic cancer, potentially improving early diagnosis.
- Monitoring Disease Progression: Regular ctDNA analysis can help track disease progression and response to therapy, providing critical information for patient management.

h) Head and Neck Cancers
- Biomarker Detection: Liquid biopsy can identify HPV DNA and other biomarkers relevant for diagnosis and treatment planning.
- Monitoring Treatment Response: Assessing ctDNA can provide insights into treatment efficacy and disease recurrence.

Liquid biopsy has established itself as a valuable tool across various cancer types, enabling early detection, monitoring of treatment response, and personalized therapy strategies. By harnessing the information derived from circulating tumor components, clinicians can make more informed decisions, improving patient outcomes and quality of life. As research continues to advance in this area, the role of liquid biopsy in cancer management is expected to expand, further enhancing its impact in oncology.

11. Standardization and regulatory frameworks for liquid biopsy in cancer management

Liquid biopsy is a rapidly emerging technology in cancer management that allows for the detection of cancer-related biomarkers, such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and other genetic materials, from a simple blood sample. However, its widespread clinical use requires standardization and a robust regulatory framework to ensure its reliability, accuracy, and safety [64–66]. Here is an overview of the standardization and regulatory frameworks for liquid biopsy in cancer management:

11.1. Key areas of standardization in liquid biopsy

a) Pre-analytical Factors: The collection, handling, and storage of blood or other body fluids (like plasma or urine) need standardization to prevent degradation or loss of biomarkers. Factors such as anticoagulant use, time to sample processing, and storage temperature are crucial. Standard operating procedures (SOPs) must be established for processing samples, including centrifugation methods and the isolation of ctDNA or CTCs.
- Analytical Validity: Methods used for detecting biomarkers (e.g., next-generation sequencing, PCR-based techniques) must be validated for accuracy, sensitivity, specificity, and reproducibility across laboratories. Standardized assays must be developed for quantifying ctDNA, CTCs, and other relevant markers, including thresholds for positive and negative results.

Clinical Utility: Clinical trials and studies should demonstrate how liquid biopsy impacts treatment decisions, such as guiding targeted therapies or monitoring disease progression. Guidelines should be developed to define which cancers and clinical situations liquid biopsy is appropriate for, alongside its comparison to tissue biopsies [67].

b) Regulatory Frameworks
- International Regulatory Bodies: FDA (U.S.): The U.S. Food and Drug Administration (FDA) oversees the approval and regulation of liquid biopsy devices under the Medical Devices category. They ensure that the liquid biopsy tests meet standards for clinical performance. In 2020, the FDA approved the first liquid biopsy companion diagnostic, Guardant360 CDx, for identifying mutations in non-small cell lung cancer (NSCLC) patients.
- EMA (Europe): The European Medicines Agency (EMA) has developed regulatory guidelines for in vitro diagnostics (IVD), including liquid biopsy. Liquid biopsies in Europe are regulated under the In Vitro Diagnostic Medical Devices Regulation (IVDR), which came into effect in 2022. This regulation is stricter in terms of clinical evidence and performance evaluation.
- WHO: The World Health Organization (WHO) provides global guidance and frameworks for cancer diagnostics, including the use of liquid biopsy technologies in cancer control programs.
- Clinical Laboratory Improvement Amendments (CLIA): In the U.S., clinical labs performing liquid biopsy tests must be certified under the CLIA program, which ensures quality standards for testing performed on human samples.

- Companion Diagnostics (CDx): Liquid biopsy tests that identify biomarkers to guide targeted therapy often function as companion diagnostics. Regulatory agencies (like the FDA and EMA) require evidence demonstrating the utility of these tests in identifying patients who may benefit from specific treatments.
- Data Privacy and Ethical Standards: As liquid biopsy relies on genetic information, frameworks like the General Data Protection Regulation (GDPR) in Europe and HIPAA in the U.S. regulate how patient data is handled, ensuring privacy and security in genetic testing.

Ethical guidelines must also address patient consent, particularly when analyzing genetic material that might have broader implications for family members.

c) Challenges in Standardization and Regulation
- Inter-laboratory Variability: Different labs may have variable results due to differing protocols, which necessitates international collaboration to standardize techniques and outcomes.
- Evolving Technologies: Rapid advances in sequencing and molecular biology techniques mean that regulatory frameworks must keep up with innovation while ensuring patient safety.
- Harmonization of Guidelines: Globally, there is a need to harmonize liquid biopsy standards and regulatory guidelines to allow for the international application of liquid biopsy tests.

d) Key Organizations Influencing Standardization
- International Organization for Standardization (ISO): ISO develops global standards that may apply to various aspects of liquid biopsy, such as the quality management systems for diagnostic laboratories (e.g., ISO 15189 for medical laboratories).
- Clinical and Laboratory Standards Institute (CLSI): CLSI works on developing clinical standards and guidelines, which may include protocols for molecular diagnostics and sample processing relevant to liquid biopsy.
- European Society for Medical Oncology (ESMO): ESMO provides clinical guidelines for the use of biomarkers in oncology, including liquid biopsies.

To fully integrate liquid biopsy into clinical practice, regulatory frameworks must ensure consistent and reliable performance across different settings and patient populations. Collaborative efforts between regulatory agencies, clinical laboratories, and manufacturers are essential for the ongoing development of robust standards and guidelines. The global harmonization of these efforts will be key to unlocking the full potential of liquid biopsy for cancer management [68–70].

11.2. Regulatory and ethical considerations in liquid biopsy

As liquid biopsy technologies advance and become more integrated into clinical practice, several regulatory and ethical considerations must be addressed to ensure their safe and effective use. Below are the key aspects to consider:

a) Regulatory Frameworks
- Approval Processes: Liquid biopsy tests must undergo rigorous evaluation and approval processes by regulatory bodies (e.g., the FDA in the United States) to ensure their safety, efficacy, and reliability. This includes demonstrating clinical validity and utility.
- Standardization of Testing: There is a need for standardized protocols regarding sample collection, processing, and analysis to enhance reproducibility and reliability across laboratories.
- Classification of Tests: Regulatory bodies may need to develop specific classifications for liquid biopsy tests (e.g., companion diagnostics vs. general screening tests) to streamline approval and oversight processes.

b) Clinical Validation and Utility
- Demonstrating Clinical Relevance: Manufacturers must provide evidence of the clinical relevance of liquid biopsy results, including how these results will impact patient management, treatment decisions, and outcomes.
- Longitudinal Studies: Ongoing research and longitudinal studies are essential to establish the long-term effectiveness and reliability of liquid biopsy tests in diverse populations.

c) Informed Consent
- Transparency in Testing: Patients should be fully informed about the purpose, benefits, risks, and limitations of liquid biopsy tests. This includes discussions about the implications of potential false positives or negatives.
- Understanding Results: Patients must be educated on how to interpret results and the potential impact on their treatment and prognosis, fostering informed decision-making.

d) Privacy and Data Security
- Genomic Data Protection: The collection and analysis of genetic information raise significant privacy concerns. It is essential to establish robust measures to protect patients' genomic data from unauthorized access and misuse.
- Data Sharing Ethics: Considerations regarding data sharing among research institutions, healthcare providers, and regulatory agencies must prioritize patient privacy and consent.

e) Ethical Implications of Findings
- Incidental Findings: Liquid biopsy tests may reveal unexpected or incidental findings unrelated to the primary cancer diagnosis. Clinicians must navigate how to communicate these findings and their implications to patients.

- Psychological Impact: The use of liquid biopsy for monitoring disease progression may induce anxiety or distress among patients. Addressing these psychological aspects is crucial for comprehensive patient care.

f) Equity and Accessibility
- Access to Technology: Ensuring equitable access to liquid biopsy technologies is vital, particularly for underserved populations. Disparities in access could exacerbate existing health inequities.
- Cost Considerations: The affordability of liquid biopsy tests is a significant barrier to widespread adoption. Efforts must be made to keep costs manageable and provide coverage through insurance.

The integration of liquid biopsy into clinical practice presents various regulatory and ethical challenges that must be carefully navigated. Establishing robust regulatory frameworks, ensuring informed consent, protecting patient privacy, and addressing the psychological and ethical implications of findings are essential for fostering trust and acceptance of this innovative technology. As the field evolves, ongoing dialogue among stakeholders—clinicians, patients, regulatory bodies, and researchers—will be crucial for shaping the future of liquid biopsy in oncology [71–73].

12. Challenges and limitations of liquid biopsy

While liquid biopsy holds great promise in cancer diagnosis and management, several challenges and limitations must be addressed to fully realize its potential. The key challenges associated with liquid biopsy:

a) Sensitivity and Specificity
- Detection Limitations: Liquid biopsy assays may have lower sensitivity for detecting ctDNA or CTCs, especially in early-stage cancers or when tumor burden is low. This can lead to false negatives.
- False Positives: The presence of ctDNA from non-malignant conditions or benign tumors may result in false positives, complicating clinical decision-making.

b) Tumor Heterogeneity
- Genetic Variability: Tumors often exhibit significant genetic heterogeneity, which may not be fully captured in a liquid biopsy sample. This can lead to incomplete profiling and potentially ineffective treatment strategies.
- Dynamic Changes: Tumor mutations and profiles can change over time, and a single liquid biopsy may not represent the tumor's evolving landscape.

c) Standardization and Protocols
- Lack of Standardization: Variability in sample collection, processing, and analysis methods can affect the reproducibility and reliability of results. Establishing standardized protocols is crucial for clinical implementation.
- Regulatory Hurdles: As a relatively new field, liquid biopsy faces regulatory challenges that may slow the approval and integration of these tests into clinical practice.

d) Interpretation of Results
- Complexity of Data: The analysis of ctDNA and CTCs generates complex datasets that require sophisticated bioinformatics tools for interpretation. This complexity can lead to challenges in deriving clinically actionable insights.
- Clinical Relevance: Determining the clinical significance of specific mutations or alterations detected in liquid biopsies can be challenging, especially for rare or novel mutations.

e) Cost and Accessibility
- Cost Considerations: While liquid biopsy can reduce the need for invasive procedures, the costs associated with advanced testing and analysis can be a barrier to widespread adoption.
- Accessibility Issues: Access to liquid biopsy tests may vary by region, with some patients facing barriers to obtaining these diagnostic tools.

f) Ethical and Psychological Concerns
- Patient Anxiety: The prospect of using liquid biopsy for monitoring disease can lead to increased anxiety among patients regarding potential recurrence or treatment changes.
- Informed Consent: Ensuring patients understand the implications of liquid biopsy results and the potential for incidental findings is crucial for ethical practice.

Despite its potential benefits, liquid biopsy faces several challenges and limitations that need to be addressed to optimize its use in clinical oncology [74–77]. Enhancing sensitivity and specificity, establishing standardized protocols, and improving data interpretation are essential steps for overcoming these obstacles. As research progresses and technology advances, it is expected that many of these challenges will be mitigated, paving the way for more widespread adoption and integration of liquid biopsy into cancer care [78–80].

13. Future perspectives of liquid biopsy

The field of liquid biopsy is rapidly evolving, offering promising avenues for advancing cancer diagnosis, treatment, and monitoring. Continued innovations in detection technologies, such as ultra-sensitive NGS and advanced imaging techniques, are expected to enhance the sensitivity and specificity of liquid biopsy assays, allowing for earlier detection of cancers and more accurate monitoring

of disease progression [79–81]. Future liquid biopsy tests may enable the simultaneous analysis of multiple biomarkers, including ctDNA, CTCs, and exosomal RNA, providing a comprehensive view of tumor dynamics and enhancing the ability to tailor treatments [82,83]. A hybrid approach that integrates liquid biopsy with traditional tissue biopsy could provide a more complete understanding of tumor biology, enhancing treatment decisions and patient outcomes. The combination of liquid biopsy with advanced imaging techniques (e.g., PET scans, MRI) may facilitate real-time monitoring of tumor response and progression [84–86]. Liquid biopsy could play a central role in real-time monitoring of treatment responses, allowing clinicians to tailor therapies based on dynamic changes in the tumor profile, optimizing patient outcomes [87–89].

The use of liquid biopsy in clinical trials may help identify suitable candidates for targeted therapies based on their unique molecular profiles, facilitating the development of more personalized treatment protocols. Liquid biopsy has the potential to become a standard tool for early cancer detection in high-risk populations, allowing for earlier intervention and improved prognoses [90–93]. Liquid biopsy could be integrated into routine follow-up care to monitor for recurrence in cancer survivors, enabling timely intervention and improved long-term outcomes. Efforts to standardize liquid biopsy testing and reduce costs could lead to wider adoption in various healthcare settings, including low-resource environments [94–97].

Ensuring that all patients, regardless of socioeconomic status or geographic location, have access to liquid biopsy technologies will be crucial for improving cancer care globally [98–100]. As liquid biopsy technologies continue to advance, regulatory bodies will need to adapt their frameworks to ensure the safety, efficacy, and reliability of these tests while fostering innovation. Ongoing discussions about patient consent, data privacy, and the handling of incidental findings will be essential to address the ethical implications of liquid biopsy [101,102].

14. Conclusion

Liquid biopsy is poised to revolutionize cancer care by providing a non-invasive, dynamic approach to diagnostics and treatment monitoring. As the field continues to evolve, addressing existing challenges and enhancing integration into clinical practice will be crucial for maximizing its benefits. With ongoing research and innovation, liquid biopsy is set to play an increasingly vital role in the future of oncology, ultimately improving patient outcomes and transforming cancer management strategies. The future of liquid biopsy holds tremendous potential for transforming cancer diagnosis, treatment, and monitoring. As technological advancements continue to evolve, liquid biopsy is expected to play a pivotal role in personalized medicine, early detection, and comprehensive cancer care. Addressing regulatory, ethical, and accessibility challenges will be crucial to fully realize the benefits of liquid biopsy and ensure its successful integration into clinical practice. With continued research and innovation, liquid biopsy may revolutionize the way we approach cancer management in the years to come.

CRediT authorship contribution statement

Shashwat Pandey: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Preeti Yadav:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization.

Ethics approval and consent to participate

Ethical approval or individual consent is not applicable.

Declaration of competing interest

Authors declare that we have no conflict of interest.
(Corresponding Author)

Data availability

No data was used for the research described in the article.

References

[1] E. Ricciardi, E. Giordani, G. Zicchedda, I. Falcone, P. Giacomini, M. Fanciulli, M. Russillo, M. Cerro, G. Ciliberto, A. Morrone, A. Guerrisi, Metastatic melanoma: liquid biopsy as a new precision medicine approach, Int. J. Mol. Sci. 24 (4) (2023 Feb 16) 4014.
[2] P. Kamińska, K. Buszka, M. Zabel, M. Nowicki, C. Alix-Panabieres, J. Budna-Tukan, Liquid biopsy in melanoma: significance in diagnostics, prediction and treatment monitoring, Int. J. Mol. Sci. 22 (18) (2021 Sep 8) 9714.
[3] M. Boyer, L. Cayrefourcq, O. Dereure, L. Meunier, O. Becquart, C. Alix-Panabières, Clinical relevance of liquid biopsy in melanoma and merkel cell carcinoma, Cancers 12 (4) (2020 Apr 13) 960.
[4] S.K. Huang, D.S. Hoon, Liquid biopsy utility for the surveillance of cutaneous malignant melanoma patients, Mol. Oncol. 10 (3) (2016 Mar 1) 450–463.
[5] V. Aleotti, C. Catoni, C. Poggiana, A. Rosato, A. Facchinetti, M.C. Scaini, Methylation markers in cutaneous melanoma: unravelling the potential utility of their tracking by liquid biopsy, Cancers 13 (24) (2021 Dec 10) 6217.
[6] L. Fattore, C.F. Ruggiero, D. Liguoro, V. Castaldo, A. Catizone, G. Ciliberto, R. Mancini, The promise of liquid biopsy to predict response to immunotherapy in metastatic melanoma, Front. Oncol. 11 (2021 Mar 18) 645069.
[7] N. Slusher, N. Jones, T. Nonaka, Liquid biopsy for diagnostic and prognostic evaluation of melanoma, Front. Cell Dev. Biol. 12 (2024 Aug 2) 1420360.

[8] E.G. Dobre, C. Constantin, M. Neagu, Skin cancer research goes digital: looking for biomarkers within the droplets, J. Personalized Med. 12 (7) (2022 Jul 13) 1136.
[9] G. Durante, E. Broseghini, F. Comito, M. Naddeo, M. Milani, I. Salamon, E. Campione, E. Dika, M. Ferracin, Circulating microRNA biomarkers in melanoma and non-melanoma skin cancer, Expert Rev. Mol. Diagn. 22 (3) (2022 Mar 4) 305–318.
[10] A. Mohammadpour, M. Derakhshan, H. Darabi, P. Hedayat, M. Momeni, Melanoma: where we are and where we go, J. Cell. Physiol. 234 (4) (2019 Apr) 3307–3320.
[11] P.P. Naik, Cutaneous malignant melanoma: a review of early diagnosis and management, World J. Oncol. 12 (1) (2021 Feb) 7.
[12] D. Fernández-Lázaro, J.L. García Hernández, A. Caballero García, A. Caballero del Castillo, M. Villaverde Hueso, J.J. Cruz-Hernández, Clinical perspective and translational oncology of liquid biopsy, Diagnostics 10 (7) (2020 Jun 30) 443.
[13] E.G. Dobre, M. Surcel, C. Constantin, M.A. Ilie, A. Caruntu, C. Caruntu, M. Neagu, Skin cancer pathobiology at a glance: a focus on imaging techniques and their potential for improved diagnosis and surveillance in clinical cohorts, Int. J. Mol. Sci. 24 (2) (2023 Jan 5) 1079.
[14] T.M. Wu, J.B. Liu, Y. Liu, Y. Shi, W. Li, G.R. Wang, Y.S. Ma, D. Fu, Power and promise of next-generation sequencing in liquid biopsies and cancer control, Cancer Control 27 (3) (2020 Aug 14) 1073274820934805.
[15] L.B. Medhin, A.B. Beasley, L. Warburton, B. Amanuel, E.S. Gray, Extracellular Vesicles as a Liquid Biopsy for Melanoma: InSeminars in Cancer Biology, vol. 89, Academic Press, 2023 Feb 1, pp. 92–98.
[16] Y.A. Hagyousif, B.M. Sharaf, R.A. Zenati, W. El-Huneidi, Y. Bustanji, E. Abu-Gharbieh, M.A. Alqudah, A.D. Giddey, A.Y. Abuhelwa, K.H. Alzoubi, N.C. Soares, Skin cancer metabolic profile assessed by different analytical platforms, Int. J. Mol. Sci. 24 (2) (2023 Jan 13) 1604.
[17] L.M. Gosman, D.A. Ţăpoi, M. Costache, Cutaneous melanoma: a review of multifactorial pathogenesis, immunohistochemistry, and emerging biomarkers for early detection and management, Int. J. Mol. Sci. 24 (21) (2023 Nov 1) 15881.
[18] I. Heidrich, B. Deitert, S. Werner, K. Pantel, Liquid biopsy for monitoring of tumor dormancy and early detection of disease recurrence in solid tumors, Cancer Metastasis Rev. 42 (1) (2023 Mar) 161–182.
[19] N. Nataren, M. Yamada, T. Prow, Molecular skin cancer diagnosis: promise and limitations, J. Mol. Diagn. 25 (1) (2023 Jan 1) 17–35.
[20] A. Eisenstein, E.C. Gonzalez, R. Raghunathan, X. Xu, M. Wu, E.O. McLean, J. McGee, B. Ryu, R.M. Alani, Emerging biomarkers in cutaneous melanoma, Mol. Diagn. Ther. 22 (2018 Apr) 203–218.
[21] Z. Eslami-S, L.E. Cortés-Hernández, C. Alix-Panabières, The metastatic cascade as the basis for liquid biopsy development, Front. Oncol. 10 (2020 Jul 21) 1055.
[22] A. Liskova, M. Samec, L. Koklesova, F.A. Giordano, P. Kubatka, O. Golubnitschaja, Liquid biopsy is instrumental for 3PM dimensional solutions in cancer management, J. Clin. Med. 9 (9) (2020 Aug 25) 2749.
[23] B. Arneth, Update on the types and usage of liquid biopsies in the clinical setting: a systematic review, BMC Cancer 18 (2018 Dec) 1–2.
[24] N. Hasan, A. Nadaf, M. Imran, U. Jiba, A. Sheikh, W.H. Almalki, S.S. Almujri, Y.H. Mohammed, P. Kesharwani, F.J. Ahmad, Skin cancer: understanding the journey of transformation from conventional to advanced treatment approaches, Mol. Cancer 22 (1) (2023 Oct 6) 168.
[25] P.P. Naik, Cutaneous malignant melanoma: a review of early diagnosis and management, World J. Oncol. 12 (1) (2021 Feb) 7.
[26] E.G. Dobre, M. Surcel, C. Constantin, M.A. Ilie, A. Caruntu, C. Caruntu, M. Neagu, Skin cancer pathobiology at a glance: a focus on imaging techniques and their potential for improved diagnosis and surveillance in clinical cohorts, Int. J. Mol. Sci. 24 (2) (2023 Jan 5) 1079.
[27] Fath M. Karami, A. Azargoonjahromi, N. Jafari, M. Mehdi, F. Alavi, M. Daraei, N. Mohammadkhani, A.L. Mueller, A. Brockmueller, M. Shakibaei, Z. Payandeh, Exosome application in tumorigenesis: diagnosis and treatment of melanoma, Med. Oncol. 39 (2) (2022 Feb) 19.
[28] Nkosana-Nyawata ID. Understanding delay: a grounded theory examination of the pre-diagnostic journey of individuals with malignant melanoma. *An Analysis of the Experiences of Individuals Subsequently Diagnosed with High Risk Malignant Melanoma from Problem Identification through to Initial Specialist Treatment* (Doctoral Dissertation, University of Bradford).
[29] S. Madheswaran, N. Mungra, F.A. Biteghe, J. De la Croix Ndong, A.T. Arowolo, H.A. Adeola, D. Ramamurthy, K. Naran, N.P. Khumalo, S. Barth, Antibody-based targeted interventions for the diagnosis and treatment of skin cancers, Anti Cancer Agents Med. Chem. 21 (2) (2021 Jan 1) 162–186.
[30] Onosetale MI, Srivastava N, Samyuktha S, Shetty SU, Samrin S, Nadaf SF. UNDERSTANDING SKIN CANCER: A REVIEW.
[31] Karanja F. Clinicopathological Features of Malignant Melanoma of the Skin Among Patients Seen at Kenyatta National Hospital.
[32] E.G. Dobre, C. Constantin, M. Neagu, Skin cancer research goes digital: looking for biomarkers within the droplets, J. Personalized Med. 12 (7) (2022 Jul 13) 1136.
[33] D.M. Aboulafia, Kaposi's sarcoma, Clin. Dermatol. 19 (3) (2001 May 1) 269–283.
[34] N. Dupin, A. Jary, S. Boussouar, C. Syrykh, A. Gandjbakhche, S. Bergeret, R. Palich, Current and future tools for diagnosis of Kaposi's sarcoma, Cancers 13 (23) (2021 Nov 25) 5927.
[35] G. Ficarra, A.M. Berson, Jr S. Silverman, J.M. Quivey, F. Lozada-Nur, D.D. Sooy, C.A. Migliorati, Kaposi's sarcoma of the oral cavity: a study of 134 patients with a review of the pathogenesis, epidemiology, clinical aspects, and treatment, Oral Surg. Oral Med. Oral Pathol. 66 (5) (1988 Nov 1) 543–550.
[36] S. Sweetser, Gastrointestinal manifestations of systemic diseases, Yamada's Textbook of Gastroenterology 15 (2022 Apr) 2231–2273.
[37] M. Garcia-Pardo, K. Czarnecka, J.H. Law, A. Salvarrey, R. Fernandes, J. Fan, L. Corke, T.K. Waddell, K. Yasufuku, L.L. Donahoe, A. Pierre, Plasma-first: accelerating lung cancer diagnosis and molecular profiling through liquid biopsy, Therapeutic Advances in Medical Oncology 14 (2022 Sep) 17588359221126151.
[38] C. Luchini, N. Veronese, A. Nottegar, V. Cappelletti, M.G. Daidone, L. Smith, C. Parris, L.A. Brosens, M.G. Caruso, L. Cheng, C.L. Wolfgang, Liquid biopsy as surrogate for tissue for molecular profiling in pancreatic cancer: a meta-analysis towards precision medicine, Cancers 11 (8) (2019 Aug 10) 1152.
[39] M. Dankner, A.A. Rose, S. Rajkumar, P.M. Siegel, I.R. Watson, Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations, Oncogene 37 (24) (2018 Jun 14) 3183–3199.
[40] K. Khaddour, L. Maahs, A.M. Avila-Rodriguez, Y. Maamar, S. Samaan, G. Ansstas, Melanoma targeted therapies beyond BRAF-mutant melanoma: potential druggable mutations and novel treatment approaches, Cancers 13 (22) (2021 Nov 22) 5847.
[41] H. Chen, R. Luthra, M.J. Routbort, K.P. Patel, M.E. Cabanillas, R.R. Broaddus, M.D. Williams, Molecular profile of advanced thyroid carcinomas by next-generation sequencing: characterizing tumors beyond diagnosis for targeted therapy, Mol. Cancer Therapeut. 17 (7) (2018 Jul 1) 1575–1584.
[42] R. Nussinov, H. Jang, C.J. Tsai, F. Cheng, Precision medicine and driver mutations: computational methods, functional assays and conformational principles for interpreting cancer drivers, PLoS Comput. Biol. 15 (3) (2019 Mar 28) e1006658.
[43] D. Planchard, Identification of driver mutations in lung cancer: first step in personalized cancer, Targeted Oncol. 8 (2013 Mar) 3–14.
[44] N.S. Kiran, C. Yashaswini, R. Maheshwari, S. Bhattacharya, B.G. Prajapati, Advances in precision medicine approaches for colorectal cancer: from molecular profiling to targeted therapies, ACS Pharmacol. Transl. Sci. 7 (4) (2024 Mar 19) 967–990.
[45] M. Colombino, M. Casula, P. Paliogiannis, A. Manca, M.C. Sini, M. Pisano, D.A. Santeufemia, A. Cossu, G. Palmieri, Heterogeneous pathogenesis of melanoma: BRAF mutations and beyond, Crit. Rev. Oncol. Hematol. (2024 Jul 6) 104435.
[46] H. Mechahougui, J. Gutmans, G. Colarusso, R. Gouasmi, A. Friedlaender, Advances in personalized oncology, Cancers 16 (16) (2024 Aug 16) 2862.
[47] T. Stricker, D.V. Catenacci, T.Y. Seiwert, Molecular profiling of cancer—the future of personalized cancer medicine: a primer on cancer biology and the tools necessary to bring molecular testing to the clinic, InSeminars in oncology 38 (2) (2011 Apr 1) 173–185. WB Saunders.
[48] K.C. Kurnit, E.E. Dumbrava, B. Litzenburger, Y.B. Khotskaya, A.M. Johnson, T.A. Yap, J. Rodon, J. Zeng, M.A. Shufean, A.M. Bailey, N.S. Sánchez, Precision oncology decision support: current approaches and strategies for the future, Clin. Cancer Res. 24 (12) (2018 Jun 15) 2719–2731.
[49] H. Mechahougui, J. Gutmans, G. Colarusso, R. Gouasmi, A. Friedlaender, Advances in personalized oncology, Cancers 16 (16) (2024 Aug 16) 2862.
[50] A.C. Ilie-Petrov, D.A. Cristian, A.S. Diaconescu, A. Chitul, A. Blajin, A. Popa, D.M. Mandi, R. Negreanu, C. Vieru, R. Vrîncianu, C.M. Ardeleanu, Molecular deciphering of colorectal cancer: exploring molecular classifications and analyzing the interplay among molecular biomarkers MMR/MSI, KRAS, NRAS, BRAF and CDX2–A comprehensive literature review, Chirurgia (Bucharest, Romania: 1990) 119 (2) (2024) 136–155.
[51] H. Soehnge, A. Ouhtit, O.N. Ananthaswamy, Mechanisms of induction of skin cancer by UV radiation, Front. Biosci. 2 (1) (1997 Nov 1) 538, 1.

[52] I. Kim, Y.Y. He, Ultraviolet radiation-induced non-melanoma skin cancer: regulation of DNA damage repair and inflammation, Genes & diseases 1 (2) (2014 Dec 1) 188–198.
[53] V.O. Melnikova, H.N. Ananthaswamy, Cellular and molecular events leading to the development of skin cancer, Mutation research/fundamental and molecular mechanisms of mutagenesis 571 (1–2) (2005 Apr 1) 91–106.
[54] F.R. De Gruijl, Skin cancer and solar UV radiation, European journal of cancer 35 (14) (1999 Dec 1) 2003–2009.
[55] A.C. Chen, G.M. Halliday, D.L. Damian, Non-melanoma skin cancer: carcinogenesis and chemoprevention, Pathology-Journal of the RCPA. 45 (3) (2013 Apr 1) 331–341.
[56] F. Liu-Smith, J. Jia, Y. Zheng, UV-induced molecular signaling differences in melanoma and non-melanoma skin cancer. Ultraviolet light in human health, diseases and environment (2017) 27–40.
[57] S.N. Lone, S. Nisar, T. Masoodi, M. Singh, A. Rizwan, S. Hashem, W. El-Rifai, D. Bedognetti, S.K. Batra, M. Haris, A.A. Bhat, Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments, Mol. Cancer 21 (1) (2022 Mar 18) 79.
[58] J. Feng, B. Li, J. Ying, W. Pan, C. Liu, T. Luo, H. Lin, L. Zheng, Liquid biopsy: application in early diagnosis and monitoring of cancer, Small structures 1 (3) (2020 Dec) 2000063.
[59] S.K. Huang, D.S. Hoon, Liquid biopsy utility for the surveillance of cutaneous malignant melanoma patients, Mol. Oncol. 10 (3) (2016 Mar 1) 450–463.
[60] H.Y. Ho, K.S. Chung, C.M. Kan, S.C. Wong, Liquid biopsy in the clinical management of cancers, Int. J. Mol. Sci. 25 (16) (2024 Aug 6) 8594.
[61] D. Horgan, T. Čufer, F. Gatto, I. Lugowska, D. Verbanac, Â. Carvalho, J.A. Lal, M. Kozaric, S. Toomey, H.Y. Ivanov, J. Longshore, Accelerating the development and validation of liquid biopsy for early cancer screening and treatment tailoring, InHealthcare 10 (9) (2022 Sep 7) 1714. MDPI.
[62] G. De Rubis, S.R. Krishnan, M. Bebawy, Liquid biopsies in cancer diagnosis, monitoring, and prognosis, Trends in pharmacological sciences 40 (3) (2019 Mar 1) 172–186.
[63] G. Poulet, J. Massias, V. Taly, Liquid biopsy: general concepts, Acta Cytol. 63 (6) (2019 Oct 8) 449–455.
[64] B. Arneth, Update on the types and usage of liquid biopsies in the clinical setting: a systematic review, BMC Cancer 18 (2018 Dec) 1–2.
[65] S.N. Lone, S. Nisar, T. Masoodi, M. Singh, A. Rizwan, S. Hashem, W. El-Rifai, D. Bedognetti, S.K. Batra, M. Haris, A.A. Bhat, Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments, Mol. Cancer 21 (1) (2022 Mar 18) 79.
[66] C. Alix-Panabieres, Perspective: the future of liquid biopsy, Nature 579 (7800) (2020 Mar 26) S9.
[67] R. Palmirotta, D. Lovero, P. Cafforio, C. Felici, F. Mannavola, E. Pellè, D. Quaresmini, M. Tucci, F. Silvestris, Liquid biopsy of cancer: a multimodal diagnostic tool in clinical oncology, Therapeutic advances in medical oncology 10 (2018 Aug) 1758835918794630.
[68] M. Nikanjam, S. Kato, R. Kurzrock, Liquid biopsy: current technology and clinical applications, J. Hematol. Oncol. 15 (1) (2022 Sep 12) 131.
[69] M. Nikanjam, S. Kato, R. Kurzrock, Liquid biopsy: current technology and clinical applications, J. Hematol. Oncol. 15 (2022) 131.
[70] L. Sivapalan, J.C. Murray, J.V. Canzoniero, B. Landon, J. Jackson, S. Scott, V. Lam, B.P. Levy, M. Sausen, V. Anagnostou, Liquid biopsy approaches to capture tumor evolution and clinical outcomes during cancer immunotherapy, Journal for immunotherapy of cancer 11 (1) (2023).
[71] D. Horgan, G. Ciliberto, P. Conte, D. Baldwin, L. Seijo, L.M. Montuenga, L. Paz-Ares, M. Garassino, F. Penault-Llorca, F. Galli, I. Ray-Coquard, Bringing greater accuracy to Europe's Healthcare Systems: the unexploited potential of biomarker testing in oncology, Biomed. Hub 5 (3) (2020 Sep 14) 1–42.
[72] O.A. Bamodu, C.C. Chung, I.I.T.R. Pisanic, Harnessing liquid biopsies: exosomes and ctDNA as minimally invasive biomarkers for precision cancer medicine, The Journal of Liquid Biopsy 7 (2023 Nov) 100126.
[73] N. Normanno, K. Apostolidis, F. de Lorenzo, P.A. Beer, R. Henderson, R. Sullivan, A.V. Biankin, D. Horgan, M. Lawler, Cancer Biomarkers in the era of precision oncology: addressing the needs of patients and health systems, InSeminars in cancer biology 84 (1) (2022 Sep) 293–301. Academic Press.
[74] C. Frascarelli, G. Bonizzi, C.R. Musico, E. Mane, C. Cassi, E. Guerini Rocco, A. Farina, A. Scarpa, R. Lawlor, L. Reggiani Bonetti, S. Caramaschi, Revolutionizing cancer research: the impact of artificial intelligence in digital biobanking, J. Personalized Med. 13 (9) (2023 Sep 16) 1390.
[75] A.J. Bronkhorst, S. Holdenrieder, The changing face of circulating tumor DNA (ctDNA) profiling: factors that shape the landscape of methodologies, technologies, and commercialization, Med. Genet. 35 (4) (2023 Nov 29) 201–235.
[76] D. Schreyer, J.P. Neoptolemos, S.T. Barry, P. Bailey, Deconstructing pancreatic cancer using next generation-omic technologies–from discovery to knowledge-guided platforms for better patient management, Front. Cell Dev. Biol. 9 (2022 Jan 13) 795735.
[77] B. Pastò, G. Buzzatti, C. Schettino, U. Malapelle, A. Bergamini, C. De Angelis, L. Musacchio, M.V. Dieci, E. Kuhn, M. Lambertini, A. Passarelli, Unlocking the potential of Molecular Tumor Boards: from cutting-edge data interpretation to innovative clinical pathways, Crit. Rev. Oncol. Hematol. (2024 May 7) 104379.
[78] R. Alkhatib, K.I. Gaede, Data management in biobanking: strategies, challenges, and future directions, BioTech 13 (3) (2024 Sep 2) 34.
[79] B.H. Jasani, C.R. Taylor, Precision Cancer Medicine, Springer International Publishing, 2021.
[80] S.E. Kalloger, J.M. Karasinska, C. Warren, D.J. Renouf, D.F. Schaeffer, Advancing the care of pancreatic cancer patients: moving beyond just tumour tissue, in: Biomarker Insights, vol. 16, 2021 Oct 11772719211049852.
[81] S. Figiel, A. Bates, D.A. Braun, R. Eapen, M. Eckstein, B.J. Manley, M.I. Milowsky, T.J. Mitchell, R.J. Bryant, J.P. Sfakianos, A.D. Lamb, Clinical implications of basic research: exploring the transformative potential of Spatial'Omics in uro-oncology, Eur. Urol. 87 (1) (2024 Sep 2) 8–14.
[82] K.B. Goldberg, G.M. Blumenthal, A.E. McKee, R. Pazdur, The FDA oncology center of excellence and precision medicine, Exp. Biol. Med. 243 (3) (2018 Feb) 308–312.
[83] N. Lyu, A. Hassanzadeh-Barforoushi, L.M. Rey Gomez, W. Zhang, Y. Wang, SERS biosensors for liquid biopsy towards cancer diagnosis by detection of various circulating biomarkers: current progress and perspectives, Nano Convergence 11 (1) (2024 May 29) 22.
[84] C. Loy, L. Ahmann, I. De Vlaminck, W. Gu, Liquid biopsy based on cell-free DNA and RNA, Annu. Rev. Biomed. Eng. (2024 Feb 12) 26.
[85] T. Fioretos, V. Wirta, L. Cavelier, E. Berglund, M. Friedman, M. Akhras, J. Botling, H. Ehrencrona, L. Engstrand, G. Helenius, T. Fagerqvist, Implementing precision medicine in a regionally organized healthcare system in Sweden, Nat. Med. 28 (10) (2022 Oct) 1980–1982.
[86] H. Adeola, R.W. Goosen, P. Goldberg, J. Blackburn, Prospects of 'omics based molecular approaches in colorectal cancer diagnosis and treatment in the developing world: a case study in Cape Town, South Africa, Colorectal Cancer–Surg Diagn Treat. 12 (2014 Mar) 346–401.
[87] E. Anklam, M.I. Bahl, R. Ball, R.D. Beger, J. Cohen, S. Fitzpatrick, P. Girard, B. Halamoda-Kenzaoui, D. Hinton, A. Hirose, A. Hoeveler, Emerging technologies and their impact on regulatory science, Exp. Biol. Med. 247 (1) (2022 Jan) 1–75.
[88] D. Horgan, Y. Hamdi, J.A. Lal, T. Nyawira, S. Meyer, D. Kondji, N.M. Francisco, R. De Guzman, A. Paul, K.R. Nallamalla, W.Y. Park, Empowering quality data–the Gordian knot of bringing real innovation into healthcare system, Diagnosis 10 (2) (2023 May 15) 140–157.
[89] S.S. Beniwal, P. Lamo, A. Kaushik, D.L. Lorenzo-Villegas, Y. Liu, A. MohanaSundaram, Current status and emerging trends in colorectal cancer screening and diagnostics, Biosensors 13 (10) (2023 Oct 13) 926.
[90] N. Rajewsky, G. Almouzni, S.A. Gorski, S. Aerts, I. Amit, M.G. Bertero, C. Bock, A.L. Bredenoord, G. Cavalli, S. Chiocca, H. Clevers, LifeTime and improving European healthcare through cell-based interceptive medicine, Nature 587 (7834) (2020 Nov 19) 377–386.
[91] C. Ladbury, A. Amini, A. Govindarajan, I. Mambetsariev, D.J. Raz, E. Massarelli, T. Williams, A. Rodin, R. Salgia, Integration of artificial intelligence in lung cancer: rise of the machine, Cell Reports Medicine 4 (2) (2023 Feb 21).
[92] J. Krasic, L. Skara, A.K. Bojanac, M. Ulamec, D. Jezek, T. Kulis, N. Sincic, The utility of cfDNA in TGCT patient management: a systematic review, Therapeutic Advances in Medical Oncology 14 (2022 May) 17588359221090365.
[93] Y. Derbal, Can artificial intelligence improve cancer treatments? Health Inf. J. 28 (2) (2022 May 7) 14604582221102314.
[94] M. Sorokin, E. Rabushko, J.M. Rozenberg, T. Mohammad, A. Seryakov, M. Sekacheva, A. Buzdin, Clinically relevant fusion oncogenes: detection and practical implications, Therapeutic Advances in Medical Oncology 14 (2022 Dec) 17588359221144108.
[95] F. Momen-Heravi, S.J. Getting, S.A. Moschos, Extracellular vesicles and their nucleic acids for biomarker discovery, Pharmacol. Therapeut. 192 (2018 Dec 1) 170–187.
[96] R.J. Leary, I. Kinde, F. Diehl, K. Schmidt, C. Clouser, C. Duncan, A. Antipova, C. Lee, K. McKernan, F.M. De La Vega, K.W. Kinzler, Development of personalized tumor biomarkers using massively parallel sequencing, Sci. Transl. Med. 2 (20) (2010 Feb 24) 20ra14.
[97] M. Cieślik, A.M. Chinnaiyan, Cancer transcriptome profiling at the juncture of clinical translation, Nat. Rev. Genet. 19 (2) (2018 Feb) 93–109.

[98] D.T. Chung, D.S. Tung, T.N. Dung, Harnessing immune checkpoint inhibitors against gastric cancer: charting the course to expanded therapeutic benefit, Biomedical Research and Therapy 11 (4) (2024 Apr 30) 6305–6325.
[99] B.N. Thaddi, V.B. Dabbada, B. Ambati, E.K. Kilari, Decoding cancer insights: recent progress and strategies in proteomics for biomarker discovery, J. Protein Proteonomics 15 (1) (2024 Mar) 67–87.
[100] A. Rehaman, Precision medicine: discovering the future of personalized healthcare, Open Horizon Scientific Review 1 (1) (2023 Jun 16) 18–27.
[101] L.A. Levit, J.M. Peppercorn, A.L. Tam, J.M. Marron, D.J. Mathews, K. Levit, N. Roach, M.J. Ratain, Ethical framework for including research biopsies in oncology clinical trials: American Society of Clinical Oncology research statement, J. Clin. Oncol. 37 (26) (2019 Sep 10) 2368–2377.
[102] M. Basik, A. Aguilar-Mahecha, C. Rousseau, Z. Diaz, S. Tejpar, A. Spatz, C.M. Greenwood, G. Batist, Biopsies: next-generation biospecimens for tailoring therapy, Nat. Rev. Clin. Oncol. 10 (8) (2013 Aug) 437–450.