ISSN: 2457-0400

Impact Factor: 6.711

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

www.wjahr.com

Volume: 9, Issue: 11

Page N. 169-176

Year: 2025

Review Article

Coden USA: WJAMA3

LIQUID BIOPSY IS A NON-INVASIVE THAT ANALYSE LIQUID TISSUE FOR CIRCULATING TUMOUR CELLS/TUMOUR DNA TO DETECT CANCER

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Article Received: 05 October 2025 **Article Revised:** 25 October 2025 **Article Published:** 01 November 2025

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How to cite this Article: 1*Preetam Datta, 1Dr. Dhrubo Jyoti Sen, 2Dr. Pruthviraj K. Chaudhary, 2Shloka V. Chaudhari, 3Honey R. Parmar, 4Dr. Devanshi V. Chaudhary and 5Dushyant P. Chaudhary (2025). Liquid Biopsy Is A Non-Invasive That Analyse Liquid Tissue For Circulating Tumour Cells/Tumour Dna To Detect Cancer. World Journal of Advance Healthcare Research, 9(11), 169–176.

ABSTRACT

Liquid biopsy is an emerging, minimally invasive diagnostic technique that enables the detection and analysis of tumour-derived biomarkers such as circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), extracellular vesicles (EVs), and other cell-free nucleic acids present in body fluids, primarily blood. It offers significant advantages over traditional tissue biopsy, which is invasive, limited in frequency, and may not adequately represent tumour heterogeneity. Liquid biopsy provides dynamic insights into cancer development, progression, treatment response, and minimal residual disease (MRD). Advanced analytical technologies, including next-generation sequencing (NGS) and digital PCR, enhance the sensitivity and specificity of biomarker detection, enabling precision oncology and personalized treatment strategies. Clinically, liquid biopsy is gaining increasing application in early cancer diagnosis, targeted therapy selection, prognosis assessment, and relapse monitoring. Despite its promising potential, challenges such as low biomarker abundance in early-stage disease, variability in detection methods, cost constraints, and lack of standardized protocols still limit widespread adoption. Ongoing research aims to improve detection accuracy and expand applications, including multi-cancer early detection tests and AI-supported data interpretation. As technology continues to evolve, liquid biopsy is expected to revolutionize cancer management by enabling earlier intervention, improved patient monitoring, and better therapeutic outcomes.

KEYWORDS: CTC, MRD, DNA, ctDNA, NGS, PCR.

INTRODUCTION

A liquid biopsy is a non-invasive test that analyses a blood or other bodily fluid sample for circulating tumour cells (CTCs) or circulating tumour DNA (ctDNA) to detect cancer. It is used to detect cancer early, monitor treatment effectiveness, and track recurrence, sometimes as an alternative to traditional surgical biopsies. The procedure is minimally invasive, often causing only mild discomfort at the blood draw site. **Catherine Alix-Panabières** [8 October 1970, Strasbourg, France] and **Klaus Pantel** [He is the founder and chairman of the Institute for Tumour Biology at the Medical School of the University of Hamburg.] coined the term "liquid biopsy" in 2010, and are widely credited with its introduction as a diagnostic concept. While others have made significant contributions to its development, Alix-Panabières and Pantel are recognized for establishing the

foundational term and concept for analysing circulating tumour cells and DNA in a patient's blood.[1-3]

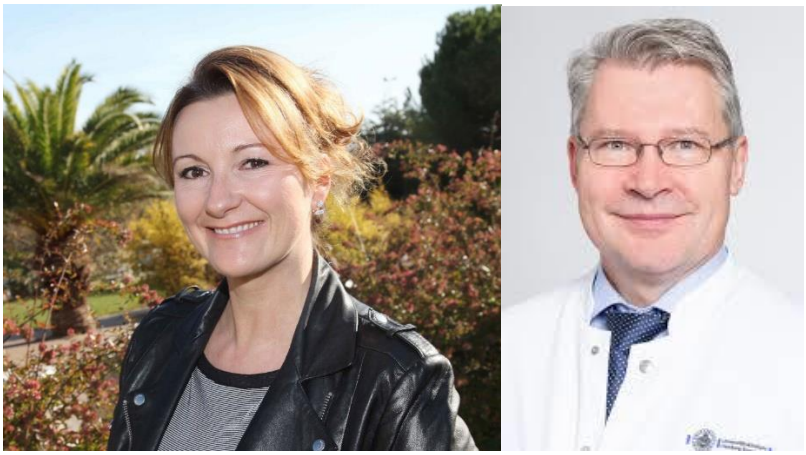

Figure-1: Catherine Alix-Panabières and Klaus Pantel [Founder of liquid biopsy].

A **biopsy** is a medical procedure to remove a sample of tissue or cells for examination to diagnose a disease. It is most often used to determine if a growth is cancerous, but can also identify other issues like inflammation or infection. The type of biopsy varies depending on the location and type of sample needed, with common methods including needle, endoscopic, and surgical removal.

What a biopsy is used for

- **Cancer diagnosis:** To confirm if a suspicious lump or area is cancerous and to identify the specific type, grade, and other characteristics that can guide treatment decisions.
- **Identifying other conditions:** To diagnose the cause of unexplained lesions, inflammation, moles, or infections.
- **Diagnosing blood/bone marrow diseases:** A bone marrow biopsy can help diagnose various blood-related conditions and cancers.

How a biopsy is performed

- **Needle biopsy:** A needle is used to extract a tissue or fluid sample.
- **Core biopsy:** A wide needle is used to get a larger sample.
- **Fine-needle aspiration (FNA) biopsy:** A thin needle is used to remove a sample.
- **Endoscopic biopsy:** An endoscope—a thin, flexible tube with a light and camera—is inserted through a natural opening (like the mouth or rectum) or a small incision to view an organ and remove tissue from a suspicious area.

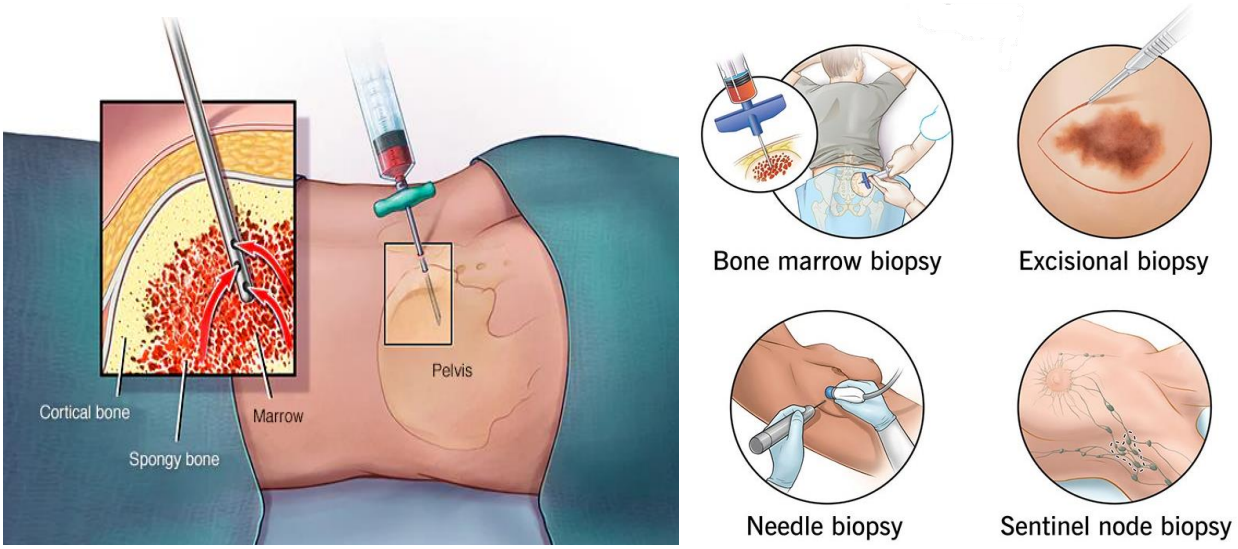

Figure-2: Biopsy.

- **Surgical biopsy:** A more invasive procedure that may involve removing a whole lump or a larger portion of a suspicious area.
- **Incisional biopsy:** Only a sample of the lump is removed.
- **Excisional biopsy:** The entire lump is removed.

- **Anaesthesia:** Some biopsies, like those in a doctor's office, can be done with a local anaesthetic to numb the area. More extensive procedures may require sedation or general anaesthesia.
- **Results:** A pathologist examines the sample under a microscope. Final, detailed results can take a week or longer, but some initial findings can be available much sooner, especially during surgery.
- **Risks:** Risks are generally small and may include bleeding, bruising, and infection. Some pain is expected, and a scar may form. It is important to contact your doctor if you experience severe pain, fever, or excessive bleeding.

A **liquid biopsy** is a non-invasive test that analyses a blood or other bodily fluid sample for circulating tumour cells (CTCs) or circulating tumour DNA (ctDNA) to detect cancer. It is used to detect cancer early, monitor treatment effectiveness, and track recurrence, sometimes as an alternative to traditional surgical biopsies. The procedure is minimally invasive, often causing only mild discomfort at the blood draw site.[4-6] A test performed on a sample of blood, urine, or other body fluid. It looks for cancer cells or fragments of DNA, RNA, and other molecules released by tumours into the body fluids. A liquid biopsy is a non-invasive medical test that analyses a bodily fluid, most commonly blood, to detect and study diseases like cancer. It works by finding and examining traces of cancer cells (circulating tumour cells) or genetic material (circulating tumour DNA) that have broken off from a tumour and are circulating in the bloodstream. Liquid biopsies can be used to detect cancer early, determine treatment options, monitor how well treatment is working, and check for cancer recurrence. A sample of blood, or sometimes other fluids like urine or cerebrospinal fluid, is taken from the patient. The sample is analysed in a lab for the presence of circulating tumour cells (CTCs) or circulating tumour DNA (ctDNA). These markers provide information about the tumour's genetic makeup, which can help guide treatment decisions. Unlike traditional surgical biopsies, liquid biopsies are minimally invasive and require only a simple blood draw, which is less painful and can be repeated more easily over time.[7-9]

Uses of liquid biopsies

- **Early detection:** Researchers are developing liquid biopsies to find cancer at its earliest stages, when it may be easier to treat.
- **Treatment planning:** They can help doctors choose the most effective treatment by identifying specific genetic mutations in the cancer.
- **Monitoring effectiveness:** Doctors can use liquid biopsies to see how well a patient's treatment is working.
- **Detecting recurrence:** A liquid biopsy can be used to check if cancer has come back after treatment.

Complementary to other tests

- They can be used to get more information when a traditional tissue biopsy isn't feasible or possible.
- **Circulating tumour cells (CTCs):** Cancer cells from a tumour that have broken off and are traveling in the bloodstream.
- **Circulating tumour DNA (ctDNA):** Fragments of DNA from tumour cells that are circulating in the blood.
- **Other molecules:** Other biomaterials like proteins and microRNAs can also be analysed.
- **Early detection:** May help find cancer at an early stage.
- **Treatment planning:** Provides information about the tumour's genetic makeup to help doctors choose the most effective treatments.
- **Monitoring:** Can track how well a treatment is working or check if cancer has returned after treatment.

When traditional biopsies are difficult: It is an option when a surgical biopsy is not feasible or is too invasive.

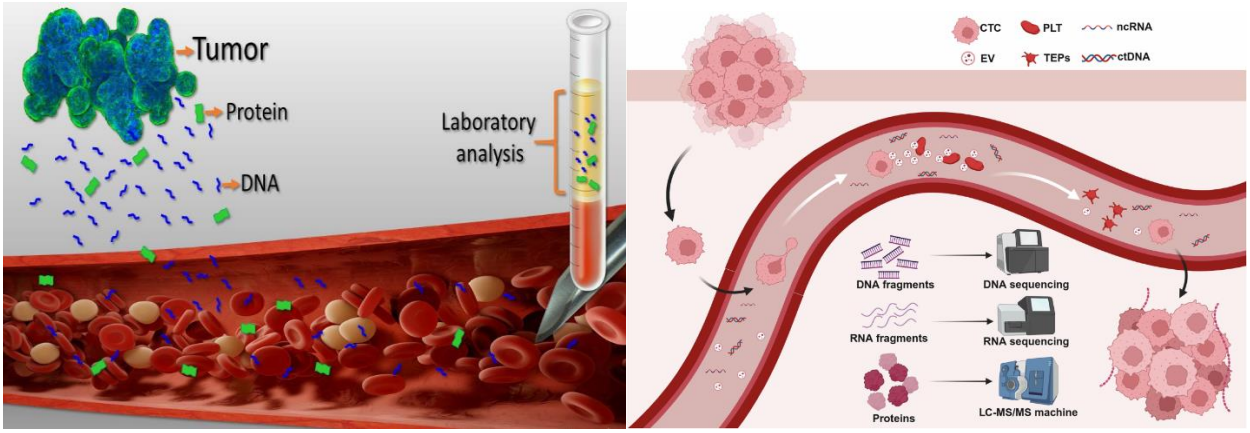

Figure-3: Liquid Biopsy.

A sample of blood, urine, or another bodily fluid is collected. The sample is sent to a lab for analysis. The procedure is minimally invasive and is often performed with a simple blood draw. Liquid biopsy is a test for

analysing biomaterials (proteins, microRNAs (miRNAs), circulating tumour cells (CTCs), etc.) in body fluids, such as blood, saliva, breast milk, and urine. Cancer cells and stromal cells within the tumour tissues can secrete various molecules in body fluids. For instance, liquid biopsy has demonstrated a sensitivity of roughly 70% to 90% in lung cancer cases, which is quite promising for a non-invasive diagnostic tool. Liquid biopsies have not shown to be more accurate than tumour biopsies. However, in theory, they may be able to provide much of the needed information about a person's cancer from a single blood draw. And getting a blood sample is safe, inexpensive, and easy to repeat. It is a pain free.[10-12]

Liquid biopsy's main advantages are its non-invasive nature, the ability to capture a more comprehensive picture of tumour heterogeneity by analysing material from multiple tumour sites, and the potential for serial monitoring over time. The primary disadvantages are its lower sensitivity in detecting very low concentrations of tumour DNA, the potential for false positives and negatives, and the challenges in isolating rare tumour components.

Advantages: In recent years, liquid biopsy has gained traction because it provides easy access to tumour-derived biomarkers with fewer potential complications for the patient compared to a surgical biopsy.

- **Minimally invasive:** Unlike traditional tissue biopsies, liquid biopsies typically involve a simple blood draw or the collection of other bodily fluids (like urine or cerebrospinal fluid), making the procedure less painful and with lower risk.
- **Comprehensive tumour analysis:** Liquid biopsies can provide a more complete picture of tumour genetics since they collect material from all tumour sites, potentially revealing tumour heterogeneity that a single tissue biopsy might miss.
- **Serial monitoring:** The non-invasive nature allows for frequent sampling, which makes it easier to monitor how a tumour responds to treatment over time and to detect minimal residual disease (MRD) or cancer recurrence.
- **Faster results:** Liquid biopsies can sometimes provide faster results compared to traditional biopsies.

Disadvantages: Disadvantages of a biopsy include risks such as bleeding, infection, and pain, as well as the possibility of damaging nearby tissue or organs. Other drawbacks are that biopsies can be expensive, cause stress, may result in misdiagnosis if the sample is not representative, and some tumours are not accessible for a biopsy. Additionally, a biopsy provides only a single snapshot of the tumour's genetic and cellular makeup and may not capture its full heterogeneity.[13-15]

Potential complications and risks

- **Bleeding:** A risk, particularly with large needles or biopsies in difficult-to-reach areas.
- **Infection:** A risk at the biopsy site.
- **Pain:** Mild pain is common after a needle biopsy and can usually be managed with pain relievers.
- **Damage to nearby tissue or organs:** There is a risk of puncture damage during the procedure.
- **Scarring:** A small scar at the biopsy site is a potential outcome.
- **Allergic reaction:** An allergic reaction to the numbing medication is a minor risk.

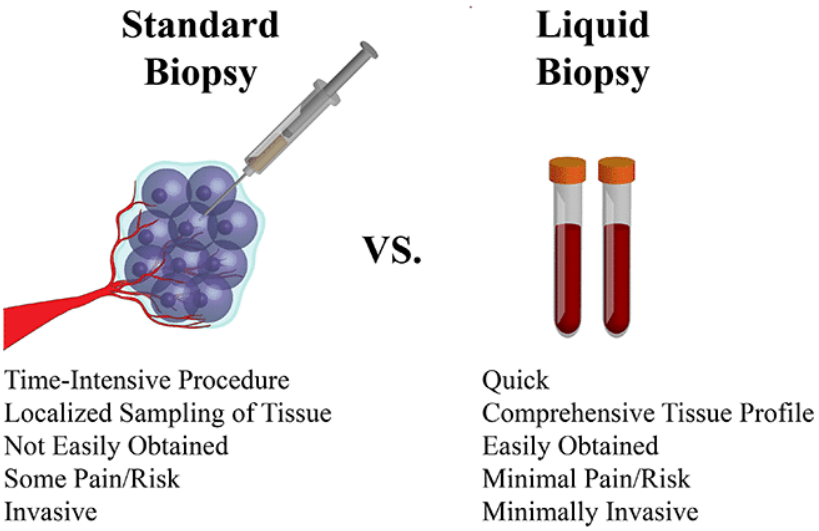

Figure-4: Differentiate between Standard Biopsy & Liquid Biopsy.

Limitations of the procedure

- **Invasiveness and cost:** Biopsies are invasive and can be resource-intensive, requiring specialized skills and equipment.
- **Limited accessibility:** Some tumours in sensitive or inaccessible areas cannot be biopsied.
- **Incomplete representation:** A biopsy sample is only a snapshot of the tumour at a single point in

time, which may not represent the full tumour heterogeneity.

- **Misdiagnosis risk:** The biopsy may miss cancer, especially if the sample is not fully representative of the entire tumour.
- **Mental stress:** The procedure and waiting for results can cause mental stress for the patient.
- **Needle tract seeding:** In rare cases, cancer cells could theoretically spread along the needle's path, though this is rare and the immune system often clears the cells.
- **Lower sensitivity:** It can be challenging to detect the very low amounts of circulating tumour DNA (ctDNA) or tumour cells in a patient's blood, especially in early-stage cancers, which can lead to false-negative results.
- **Potential for false positives:** Non-tumour genetic mutations from other sources, such as clonal hematopoietic mutations of indeterminate potential (CHIP), can be mistaken for cancer-related mutations, leading to a false positive.
- **Tumour heterogeneity challenges:** While liquid biopsy can capture a broad view of heterogeneity, the amount of tumour material shed can vary between different metastatic sites, potentially skewing results.
- **Technical limitations:** The required technology for analysis is highly sensitive and can be susceptible to technical artifacts, making result interpretation difficult in some cases.
- **Clinical utility still evolving:** While promising, the clinical utility of liquid biopsies still needs to be validated in many settings, and results require careful interpretation and sometimes confirmatory testing.[16-18]

- **Core Analytes of the Liquid Biopsy**
- The effectiveness of liquid biopsy stems from its ability to isolate and analyse specific tumour-derived materials circulating in the blood. The three primary analytes are.

1. Circulating Tumour Cells (CTCs): CTCs are intact cancer cells that have detached from the primary or metastatic tumour site and entered the bloodstream. They are the physical agents responsible for metastasis.

- **Significance:** CTC analysis provides information on cell morphology, protein expression, and epithelial-to-mesenchymal transition (EMT) status. Their count is often prognostic, correlating with disease burden and patient survival.
- **Methodology:** Isolation is technically challenging due to their extreme rarity (as few as 1–10 cells per billion normal blood cells). Common techniques include immunomagnetic separation (e.g., Cell Search system, the only FDA-approved platform) and size- or density-based filtration.

2. Circulating Tumour DNA (ctDNA): ctDNA refers to fragmented DNA released into the bloodstream primarily from apoptotic (dying) or necrotic (rupturing) tumour cells. These fragments typically range from 150 to 200 base pairs.

- **Significance:** ctDNA carries the specific genetic and epigenetic alterations (mutations, amplifications, deletions) of the tumour, making it a highly valuable biomarker for identifying actionable drug targets and monitoring the effectiveness of therapy.
- **Methodology:** Highly sensitive techniques are required to detect ctDNA, which usually constitutes less than 1% of the total cell-free DNA (cfDNA) in the blood. These include Next-Generation Sequencing (NGS), Digital PCR (dPCR), and BEAMing (Beads, Emulsions, Amplification, and Magnetics).

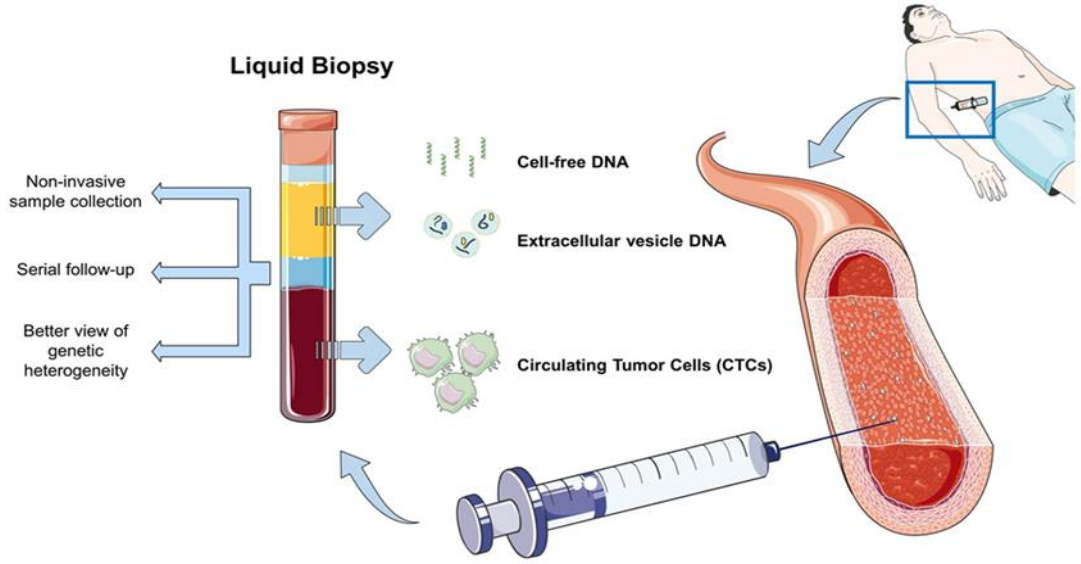

Figure-5: Mechanism of Liquid Biopsy.

3. Exosomes and Extracellular Vesicles (EVs): Exosomes are nanosized lipid bilayer vesicles (30–150 nm) secreted by nearly all cells, including tumour cells. They play a critical role in intercellular communication.

• **Significance:** Tumour-derived exosomes carry a payload of proteins, lipids, mRNA, microRNAs (miRNAs), and double-stranded DNA fragments, reflecting the molecular status of the parent tumour. They contribute to metastasis and immune suppression.

• **Methodology:** Isolation typically involves ultracentrifugation, size-exclusion chromatography, or specialized microfluidic platforms.

Clinical Applications and Impact: Liquid biopsy is poised to redefine several aspects of cancer management across the patient journey:

1. Early Detection and Screening: In patients at high risk (e.g., strong family history), liquid biopsy holds promise for multi-cancer early detection (MCED). By detecting tumour-specific genomic changes or methylation patterns in cfDNA long before symptoms appear, it offers a non-invasive tool for screening, potentially improving outcomes through earlier intervention.

2. Monitoring Minimal Residual Disease (MRD): Following curative treatment (surgery or chemotherapy), residual microscopic disease, or MRD, is a major cause of relapse. Liquid biopsy can detect ultra-low levels of ctDNA with patient-specific mutations, providing a highly sensitive measure of MRD status. A positive ctDNA result post-treatment can signal impending relapse months before radiological evidence appears.

3. Guiding Personalized Treatment and Detecting Resistance: Perhaps the most crucial clinical application is in real-time therapy selection for patients with advanced disease.

• **Targeted Therapy Selection:** ctDNA analysis can identify mutations that make a tumour susceptible to specific targeted drugs (e.g., EGFR mutations in lung cancer).

• **Resistance Monitoring:** As tumours evolve under drug pressure, they frequently develop new mutations leading to treatment resistance (e.g., EGFR T790M resistance mutation). Liquid biopsy allows for timely and repeated sampling to identify these resistance mechanisms, enabling clinicians to pivot to a second-line therapy without the delay and risk of a repeat tissue biopsy.

4. Prognosis and Assessment of Disease Burden: The concentration or specific characteristics of CTCs and ctDNA can serve as prognostic indicators, correlating with overall survival and progression-free survival in various cancers, including breast, prostate, and colorectal cancers.

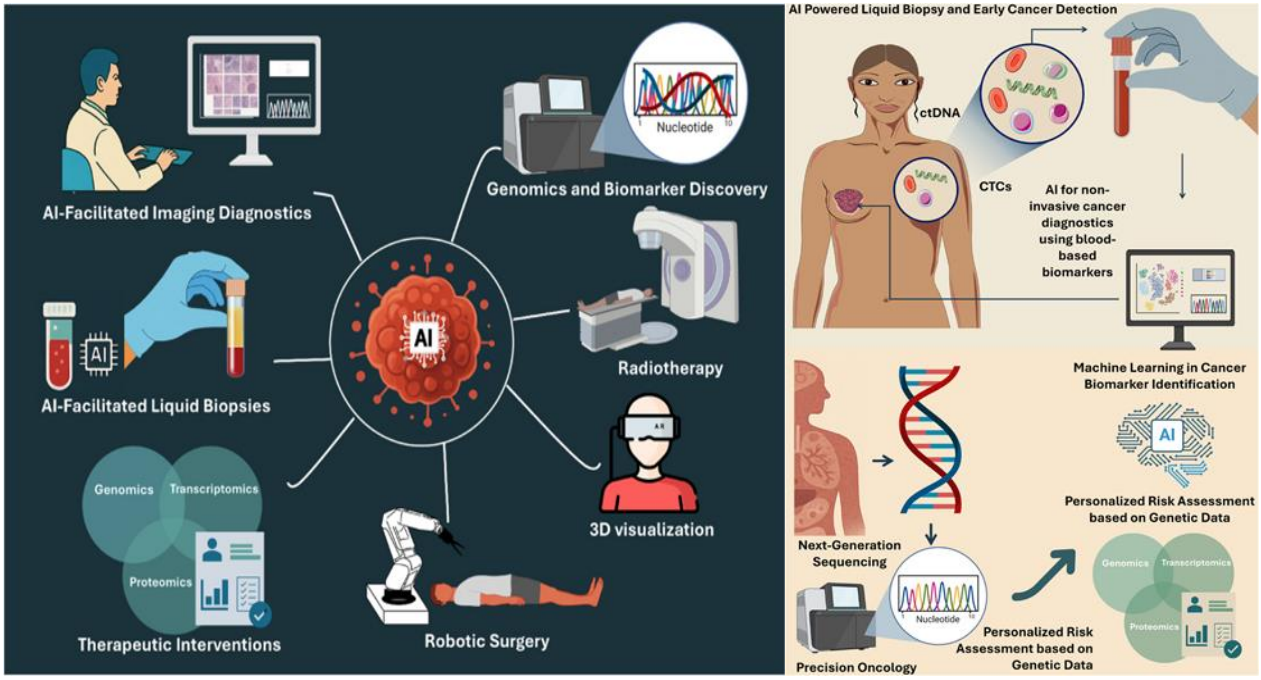

Figure-6: AI in Liquid Biopsy.

Advantages over Traditional Biopsy: Minimally invasive: usually just a blood draw — less risk, less discomfort.

Repeatable: can be done multiple times to monitor disease progression or response.

Captures tumour heterogeneity and dynamics: Since tumour cells shed into circulation continuously, one may capture evolving mutations and metastasis potential which tissue biopsy at one site may miss.

Faster turnaround: In some contexts, liquid biopsy may offer faster results (depending on assay).

Limitations & Challenges: Sensitivity & specificity: Not all tumours shed detectable biomarkers into circulation; false negatives and false positives remain a challenge.
Low biomarker levels in early disease: Detection in early-stage cancer may be harder because biomarker concentrations are low.
Standardization and validation: Methods vary widely; there is a need for standardized protocols, validated assays, and robust clinical trials.
Interpretation and clinical utility: Even when biomarkers are detected, linking them to specific actionable decisions is not always straightforward.
Cost and access: Advanced molecular assays may be expensive and not available everywhere, especially in resource-limited settings.
Cannot always pinpoint tumour location: Liquid biopsy may indicate that tumour-derived DNA is present but not exactly where the tumour is located.[19,20]

Future Directions
Early cancer screening: Researchers are working on multi-cancer early detection (MCED) tests using liquid biopsy to screen asymptomatic populations.
Improved sensitivity and new biomarkers: Discovery of novel biomarkers (e.g., methylation patterns, fragmentomics) that enhance detection of minimal disease.
Integration with AI/data analytics: Using machine learning to interpret complex biomarker signatures for better prediction.
Wider clinical implementation & cost reduction: As technology matures, it may become more accessible.
Liquid biopsy for non-cancer uses: While most work is in oncology, similar approaches may extend to other diseases (e.g., prenatal diagnostics, transplant monitoring).
Artificial Intelligence enhances liquid biopsy by analysing large, complex datasets to improve diagnostic accuracy and support clinical decisions. It uses machine learning and other algorithms to find subtle patterns in biomarkers like circulating tumour DNA (ctDNA), cell-free RNA (cfRNA), and exosomes, enabling earlier cancer detection, real-time disease monitoring, and prediction of treatment response.

Key roles of AI in liquid biopsy
Enhanced early detection: AI algorithms can identify low-abundance biomarkers in blood, which significantly improves the sensitivity and specificity of liquid biopsy for detecting cancer at its earliest stages.
Improved accuracy: By analysing multi-omics data (such as genomic, transcriptomic, and proteomic information), AI can create more comprehensive profiles and distinguish between cancerous and benign samples with higher accuracy.
Biomarker discovery and profiling: AI is used to discover new cancer biomarkers and to more effectively profile existing ones, such as identifying mutations in ctDNA or analysing the cargo of extracellular vesicles.
Data interpretation and integration: AI can integrate diverse and complex data, including molecular data from liquid biopsy and unstructured clinical information from electronic health records, to provide a more complete picture of a patient's condition.
Predictive modelling: AI can build models to predict a patient's response to specific treatments and assess the risk of cancer recurrence.
Real-time monitoring and minimal residual disease detection: By continuously analysing liquid biopsy data, AI can support real-time monitoring of disease progression and help detect minimal residual disease after treatment.
Personalized medicine: Ultimately, the insights from AI-powered liquid biopsy lead to more personalized and effective cancer management strategies.

CONCLUSION
Liquid biopsy represents a disruptive technology capable of transforming cancer diagnostics and treatment. By providing a safe, repeatable, and comprehensive molecular snapshot of a patient's cancer burden, it facilitates early detection, personalized drug selection, and dynamic monitoring of therapeutic response and resistance. While challenges related to sensitivity and standardization persist, ongoing research and technological advancements suggest that liquid biopsy will soon move from the research lab to routine clinical practice, significantly improving patient outcomes in the fight against cancer.

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