Applications of AI-Assisted Liquid Biopsy for Early Cancer Detection and Treatment Monitoring: A Systematic Review of Current Evidence

Mohammad Delowar Hossain Gazi[1], Tania Yeasmin[2], Iftiaz Ahmed Alfi[3], Mohd Abdullah Al Mamun[4*], Md Rubel Mia[5], Elton Bicalho do Carmo[6], Niladry Chowdhury[7], Md Ahnaf Tajwar Kamal[8], Nawfat Kamal Munifa[9], Md Rokibul Hasan[10]

[1]College of Graduate and Professional Studies, Trine University, Indiana, USA.
[2]Department of Computer Science and Engineering, American International University-Bangladesh, Dhaka, Bangladesh.
[3]Tagliatela College of Engineering, University of New Haven, Connecticut, USA.
[4]MBA in Information Technology Management, Westcliff University, Irvine, California, USA.
Email: mamun.westcliffuniversity.usa@gmail.com (Corresponding Author)
[5]Department of Comparative Studies, Florida Atlantic University, Florida, USA.
[6]Department of Software Engineering, University of Maryland, Adelphi, MD, USA.
[7]College of Graduate and Professional Studies, Trine University, Indiana, USA.
[8]MSc in Applied Statistics and Data Science, The University of Texas at Arlington, Texas, USA.
[9]Department of Biomedical Engineering, The University of Texas at Arlington, Texas, USA.
[10]Harrison College of Business and Computing and Management, Southeast Missouri State University, Cape Girardeau, MO, USA.

***Corresponding author: Mohd Abdullah Al Mamun, MBA in Information Technology Management, Westcliff University, Irvine, California, USA**
Email: mamun.westcliffuniversity.usa@gmail.com

Received: 30th May, 2026; **Revised:** 11th June, 2026; **Accepted:** 15th June, 2026; **Available Online:** 18th June, 2026

ABSTRACT

Background

Early cancer diagnosis is also a significant clinical issue because both traditional methods of imaging and tissue biopsy are not capable of achieving early-stage, minimal residual disease (MRD) and new-developed treatment resistance. Liquid biopsy is an approach that is based on circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomal RNA/miRNA, and epigenetic signatures and provides a minimally invasive instrument that can be used to profile tumors in real-time. The recent developments in high-throughput sequencing and methylation-based assays have greatly enhanced the level of analytical sensitivity and therefore, the detection of early malignancy is better and also therapeutic response can be effectively monitored.

Objective

To conduct a systematic review and synthesis of the evidence regarding the diagnostic accuracy, prognostic utility and treatment-monitoring performance of the liquid biopsy biomarkers in the detection of early cancer in the solid tumours, to evaluate the methodological trends and sources of heterogeneity on clinical applicability.

Methods

There was a systematic search of PubMed, EMBASE, Scopus, Web of Science, Cochrane Library, IEEE Xplore, and Google Scholar to identify different papers published during 2010-2025. Research evaluating ctDNA, CTCs, exosomes, or methylation analyzes in the diagnosis, prognostication, or MRD monitoring was included. Two reviewers were screening data and extracting data independently according to PRISMA 2020 guidelines. Quality of the methodology was evaluated based on the QUADAS-2 and ROBINS-I. Random-effects pooled meta-analyses were based on the sensitivity, specificity, odds ratios (ORs), and area under the receiver-operating curve (AUC). Correlations were used to examine correlations between variables of study design and diagnostic performance.

Results

Out of 6,812 original entries, 42 studies were taken into account according to which over 28,400 cancer patients and 33,000 controls were included, and ctDNA and methylation based assays proved to be the most successful in the diagnostic process with pooled sensitivity of 0.78 and specificity of 0.83 and AUC of 0.87. CtDNA panels utilizing methylation reached an AUC of as high as 0.92 and multi-omics 0.94. Exosomal miRNA biomarkers performed well (AUC 0.86 -0.92) and CTC tests were of moderate

accuracy (AUC 0.79). In treatment monitoring, early ctDNA clearance showed a therapeutic response (HR 0.42) and increasing levels of ctDNA predicted radiologic relapse 3-6 months before it happened. Diagnostic (r = 0.72) and serial (r = 0.68) sampling correlated with depth of sequencing, whereas pre-analytic variability decreased the accuracy (r = - 0.54). External validation was only done in 36% of studies.

Conclusions

Liquid biopsy offers a solid and minimally invasive platform of early cancer diagnosis, therapeutic response, and MRD, and longitudinal ctDNA dynamics provides powerful predictive information of therapeutic response and recurrence. Nevertheless, large-scale clinical utilization is still hampered by inconsistencies in assay procedures, poor standardization and sub-optimal external validation. The future studies ought to focus on multi-center prospective research, integrative pipelines, and multi-omics to facilitate clinical translation.

Keywords: Liquid biopsy; circulating tumor DNA; exosomal biomarkers; circulating tumor cells; early cancer detection; minimal residual disease; treatment monitoring; precision oncology.

How to cite this article: Gazi MDH, Yeasmin T, Alfi IA, Al Mamun MA, Mia MR, do Carmo EB, Chowdhury N, Kamal MAT, Munifa NK, Hasan MR. Applications of AI-Assisted Liquid Biopsy for Early Cancer Detection and Treatment Monitoring: A Systematic Review of Current Evidence. Int J Drug Deliv Technol. 2026;16(60s):1815-1827. DOI: 10.25258/ijddt.16.60s.166

Source of support: Nil.

Conflict of interest: None

Introduction

Making early diagnoses of cancer is perhaps one of the greatest factors that can determine the survival of the patient, a successful treatment and the presence of a long-term clinical outcome. Even though oncologic imaging, targeted therapy, and molecular diagnostics have made truly remarkable achievements, a significant percentage of cancers are still diagnosed at intermediate- or advanced-stage stages, at which curative treatments are less effective, and their mortality rates shoot up exponentially. The classic tissue biopsy, the traditional gold standard of diagnosis has essential histopathological and genomic data, but is too invasive, biased in sampling, and unable to capture the spatial and temporal heterogeneity of tumors [1, 2]. Tumors do not stand still under the pressure of treatment, and a single biopsy at the diagnosis time will hardly represent this dynamic picture. This leads to the increased demand of diagnostic technologies that would be able to offer real-time, systemic, and minimally invasive measurements of tumor burden and biology.

Liquid biopsy has become a game changer to these issues providing unprecedented opportunities in early cancer detection, treatment monitoring, and minimal residual disease (MRD) assessment. Liquid biopsy, through its ability to monitor tumor evolution with precision never previously possible, has shown itself to have high potential in detecting early-stage malignancies at low allele fractions in the blood (and other body fluids), and in monitoring tumor response to therapy by quantifying and qualifying changes in mutation patterns. Equally, exosomal biomarkers indicate an active process of cellular communication and they contain RNA, protein, and lipid cargo that reflects the tumor microenvironment state [3, 4]. A combination of these circulating biomarkers has offered a wholesome understanding of the dynamics of the tumor, frequently weeks or months prior to the radiography being noticeable.

Liquid biopsy has an ever-growing clinical promise due to recent technological advancements. Digital PCR (dPCR) and high-resolution methylation profiling have improved assay sensitivity a lot, and now it is possible to observe even the smallest tumors. New multi-omics methods combine genomic, epigenomic, transcriptomic and proteomic data to increase the precision and strength of early cancer identifying methods. Moreover, serial sampling facilitated by the less invasive characteristic of liquid biopsy can facilitate continuous monitoring of response to treatment, early readings of resistance mutation, and detection of MRD way before clinical relapse. These functionalities have far-reaching implications on the area of precision oncology, an opportunity to intervene earlier, to adoptive care, and to care that is really personal [5, 6].

However, as evidenced by such improvements, clinical translation of liquid biopsy is not uniform, which can be explained in part by the heterogeneity of study designs, assay platforms, pre-analytical procedures and analytic thresholds. There is a wide range in the diagnostic performance of cancer across different biomarkers, different types of cancer, and more so with varying technologies in sequencing, and most of the studies present in the literature have not been externally validated, have not been reported in a standardized fashion, and their protocols are not harmonized. Prior to analytical considerations (i.e., the blood

collection tubes, processing medium, and DNA extraction systems) can have a significant impact on the yield of ctDNA and its interpretation [7, 8]. This has been a challenge to the reproducibility and regulatory approval, which has led to the necessity of systematic synthesis of the available evidence to understand the actual diagnostic and prognostic worth of liquid biopsy technologies.

Considering the fact that the liquid biopsy study is growing fast, and the clinical need in the non-invasive detection instruments is increasing, a proper and systematized assessment is timely and necessary. The systematic review is an evidence synthesis of the last 15 years that provides an evaluation of the efficacy of liquid biopsy biomarkers in the setting of early cancer diagnosis and treatment follow-up of various solid tumors. In particular, it assesses diagnostic accuracy of ctDNA, CTCs, exosomal signatures, and methylation markers; evaluates their predictive ability of treatment response and relapse; and presents methodological strengths and limitations, which affect the applicability in clinical use [9, 10]. This review intends to shed light on the feasibility of liquid biopsy to be more broadly integrated into clinical practice and the actions that need to be taken in order to realize the dream of a reliable, standardized and practical liquid biopsy in the future of precision oncology.

Literature Review

Liquid biopsy remains an evolving field within the last 10 years due to both molecular diagnostic improvements and high-throughput sequencing and the increased understanding of tumor heterogeneity as a significant problem in oncology. The first concept that was proposed was that of detecting tumor-derived contents in blood by the identification of circulating tumor cells (CTCs). Initial research has shown that CTCs levels were associated with disease stage and prognosis in breast, prostate, and colorectal cancers but the low concentration of CTCs, particularly in the initial stages, reduced their application as sensitive biomarkers [11, 12]. This limitation prompted scientists to find other biomarkers that are discovered in the blood, and eventually, the circulating tumor DNA (ctDNA) was found as a more sequentially accessible and ample biomarker. A ground-breaking research found that ctDNA fragments harbor tumor-specific mutations, methylation changes, and genomic changes that are representative of the underlying malignancy, making ctDNA a foundation of research in liquid biopsy.

Further studies established that ctDNA can be used in the diagnosis of a wide range of cancers. Initial studies of colorectal cancer discovered that ctDNA-based tests were capable of detecting stage I-II disease with a high specificity and even cystic lesions. CtDNA mutation profiling of lung cancer identified early-stage non-small cell lung cancer (NSCLC) more accurately than either sputum cytology or low dose CT only. The use of ctDNA methylation signatures came into the limelight in the context of hepatocellular carcinoma (HCC) because of the drawbacks of alpha-fetoprotein (AFP) as a screening method. Research demonstrated that certain methylation signatures including SEPT9 or HOXA family gene methylation was more sensitive in detecting early HCC and could even be used to identify patients at high risk of getting cirrhosis who would later become cancerous [13, 14]. These pioneer studies formed the basis of the next generation of liquid biopsy platforms where there is a tendency to use multi-marker strategies to enhance precision.

In parallel with the evolution of the ctDNA-based diagnostics, exosomes and other extracellular vesicles were also considered as the biologically enriched carriers of the tumor-specific information. Their contents, RNA, miRNA, DNA fragments, lipids, and proteins, are resistant to enzymatic degradation, and therefore, exosomes can be used as a stable and informative source of biomarkers. The exosomal miRNA signatures like miR-21, miR-200 family and miR-122 were also identified by breast and ovarian cancer research as being very sensitive in early tumorigenesis. The same patterns were observed in gastrointestinal tumors, where exosomal cargo was used to give information regarding the presence of tumor cells, as well as the activation of oncogenic pathways [15, 16]. In contrast with ctDNA, exosomes provide an indication of active cellular processes and cell-cell communication and provides a unique layer of biological data, which supplements genomic biomarkers. With time, exosome-based diagnostics have improved to multiplexed microfluidic systems which can isolate tumor specific vesicles and they are more sensitive and specific.

The other significant change in the study of liquid biopsy is the improvement of methylation profiling. Alterations of DNA methylation are seen at an early stage of cancer development, and they are usually more stable than point mutations, which can be highly variable even within a single tumor. Various innovative investigations have shown that the methylation-based ctDNA assays exhibited superior results with regard to the detection of early cancerous cellular pathology. The Galleri multi-cancer early detection (MCED) test is a methylation pattern recognition-based test, which claimed to detect more than 50 types of cancer with high

specificity, and reliably detect the tissue of origin. Smaller single-cancer studies, of similar size, especially in colorectal, gastric, and lung cancer, have demonstrated that in the early stages, one can rely on the findings of the methylation-based biomarkers, which in most cases, are superior to the mutations-based tests [17, 18]. These discoveries aided in moving the field to epigenomics as a source of a stable and reliable biomarker.

Liquid biopsy broadly has been the subject of treatment monitoring. The initial investigations in metastatic colorectal cancer have shown that ctDNA concentration in responders significantly decreased with the start of chemotherapy but did not change or even rose in non-responders. This has been since repeated in numerous different types of tumor such as breast, lung and melanoma. One of the most promising applications has been the detection of minimal residual disease (MRD) with ctDNA since several studies have shown that ctDNA positivity after curative-intent treatment forecasts relapse months prior to radiologic imaging. As an example, serial ctDNA monitoring has been established to reveal recurrence in breast cancer about 36 months before clinical indicators are detected. The dynamics of ctDNA in lung cancer is associated with the development of resistance mutations, including EGFR T790M or ALK fusions, which allows clinicians to more effectively and promptly change treatment [19, 20]. This is based on the evidence that the liquid biopsy is an actionable and real time reporting on tumor evolution.

The recent advancement in the research is multi-omics liquid biopsy. With the realization of the shortcomings of single-analyte biomarkers, scientists have embarked on the strategies of combining genomics with transcriptomics, methylomics, and proteomics with the aim of developing integrated diagnostic signatures. Research that implements both ctDNA mutation and methylation profiling finds significantly high performance, usually with an AUC greater than 0.94. There has been the growing integration of machine learning and artificial intelligence to examine complicated data distributions among several modalities of biomarkers so as to do better feature selection and classification. The goal of this integration is to decrease false positive, enhance sensitivity of very early-stage disease, and bring more biological insight of tumor behavior.

Although the developments are exciting, a number of limitations are still present in the literature. Diversity in pre-analytic procedures, including blood collection time, centrifugation procedure, and DNA extraction kit, has created variability in studies [21, 22]. The previous works were not validated by external sources making the generalizability of their results low. In addition, the level of ctDNA in very early-stage cancers is frequently low, making it a strong biomarker but requiring ultra-sensitive assays which, despite being costly and not as commonly available, are currently available. Changes in regulation are also still a major problem, since the sphere does not have common standards regarding assay sensitivities, reporting standards and validation systems. However, the multi-center trial process and the increased attention to standardization by such agencies as the FDA, ESMO and CAP still tend to shift the field towards clinical use.

Combined, the available literature vastly affirms the claim that liquid biopsy is a disruptive technology in the field of early cancer detection and monitoring of its treatment. Taken together, the above evidence shows that ctDNA, CTCs, exosomes, and methylation-based biomarkers have their own merits. Combined with multi-omics and improved computational pipelines, such biomarkers can transform the diagnosis and therapeutic decision making process of cancer. In the coming years, once the technology is mature and has proven its standards, liquid biopsy is set to become an inseparable part of precision oncology, as the paradigm of precision oncology shifts to proactive and early treatment, rather than reactive.

Methodology

Study Design and Rationale

The systematic review was performed to review the uses of liquid tumor biopsy assays such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomal biomarkers and methylation-based assays in early cancer detection and management monitoring in major solid tumors. The review was based on the PRISMA 2020 guidelines, which guaranteed methodological transparency, reproducibility, and full report.

The design was to bring together evidence on the diagnostic accuracy, prognostic, minimal residual disease (MRD)s detection, and longitudinal treatment monitoring based on biomarkers through liquid biopsy.

Search Strategy

An extensive search of electronic databases was conducted on the studies published since January 2010 to December 2025. The databases that were included were as follows:

- PubMed/MEDLINE
- EMBASE
- Scopus

•Two other databases were also used:
• Web of Science Core Collection
• Cochrane Library
Xplore (methodological/technology-focused studies) IEEE Xplore.
References to grey literature and conference abstracts were available in Google Scholar.
The search strategy involved the combination of the MeSH terms and the free-text keywords with the Boolean operators and each database was adapted. One example search query was:
AND (liquid biopsy OR "circulating tumor DNA" OR ctDNA OR "circulating tumor cells" OR CTC OR exosomes OR "cell-free DNA" OR cfDNA OR methylation).
AND
(early detection OR screening OR diagnosis OR prognostic OR monitoring OR minimal residual disease or "minimal residual disease")
AND
And (cancer OR tumor OR carcinoma OR neoplasm).
Manual screening of reference lists of potential articles and systematic review papers was also done to identify more eligible studies.

Study Selection

Titles and abstracts were screened by two independent reviewers to be eligible. Relevant articles that had full texts were then assessed based on preset criteria. Differences were to be solved by consensus or consultation with a third reviewer.

Table 1. Inclusion and Exclusion Criteria

Criterion	Inclusion	Exclusion
Population	Human subjects with diagnosed cancer or under investigation for early-stage disease; plasma/serum liquid biopsy available	Animal studies, in vitro experiments, purely computational modeling
Biomarker Type	ctDNA mutations, methylation signatures, CTC counts, exosomal RNA/miRNA, multi-omics panels	Tissue-only biomarkers; imaging-only studies
Study Design	Cohort, case-control, cross-sectional, diagnostic accuracy studies, longitudinal monitoring studies	Editorials, letters, narrative reviews without original data
Outcomes	Sensitivity, specificity, AUC, hazard ratios, longitudinal ctDNA/CTC kinetics, MRD detection	Studies lacking quantitative performance outcomes
Language	English	Non-English studies
Time Frame	2010–2025	Pre-2010 studies

Data Extraction and Management

The standardized spreadsheet was used to extract the data independently. The extracted variables were:
ID of the study, authors, year of publication.
Cancer type and stage of clinical analysis.
Size of the sample (patients/ controls)
Intrinsic to exosomes, methylation, ctDNA, CTCs, and multi-omics
Platform/technology employed (NGS, dPCR, ddPCR, methylation panel assays)
Diagnostic performance under conditions of sensitivity, specificity, AUC, PPV/NPV.
Prognostic (hazard ratio of survival/relapse) outcomes.
Treatment monitoring data (kynamics of ctDNA, the intervals of MRD detection)
Methods of validation (internal or external cohort)

Quality Assessment

Two quality assessment tools were complementary, and they were used:

1. **QUADAS-2(Quality Assessment of Diagnostic Accuracy Studies)**

Studies that used AUC, sensitivity, and specificity.

Domains: patient selection, index test, reference standard, flow & timing.

2. **ROBINS-I (Risk Of Bias In Non-randomized Studies of Interventions) model** modified.

Applied to longitudinal research studies on MRD and treatment monitoring.
Domains confounding, selection of participants, interventions classification, outcomes measurement.
Research that addressed 3 or more low-risks domains was categorised as overall low-risk research. Any difference of opinion was settled through consensus.

Synthesis of Data and Statistical Analysis.

Both narrative and quantitative syntheses were performed because biomarkers, platforms, and types of cancer are heterogeneous.

Quantitative Synthesis

The meta-analysis of diagnostic performance was pooled using random-effects:

Sensitivity

Specificity

AUC

Odds ratios (ORs) or hazard ratios (HRs)

Subgroup analyses were done on the basis of:

The type of biomarker (ctDNA, CTC, exosomes, methylation)

Technology type (NGS vs. dPCR)

Purpose of the study (screening vs. MRD vs. relapse prediction)

Internal and external validation (method of validation)

Graphs & Visualizations

Figures generated include:

already created forest plots of shared ORs and diagnostic measures (Figure 2)

The longitudinal ctDNA kinetic changes of responders and non-responders (Figure 3)

Figure 4 Relative AUC bar charts of classes of biomarker.

Risk-of-bias (Summary) (Figure 5).

Figure 6 Correlation heatmap of methodological factors and the diagnostic performance.

Handling Heterogeneity

Heterogeneity was also measured by Cochran Q and I 2.

I 2 greater than 50% was a sign of large heterogeneity.

Sensitivity analyses were conducted by doing away with high-risk studies.

Ethical Considerations

Any data utilized in all of the studies included with this review was previously published and de-anonymized; hence, this review did not need any ethical approval or patient consent. Any original studies stated that they had received ethics approval where necessary.

Analysis

The systematic search helped to identify 42 eligible studies that were published between 2010 and 2025 that investigated the diagnostic and monitoring uses of a liquid biopsy as an early cancer detection tool. All these studies together analyzed a total of more than 28,400 cancer patients and 33,000 controls comprising of a variety of cancer types such as lung, breast, colorectal, prostate, hepatocellular carcinoma (HCC), and pancreatic cancer. Circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomal RNA /miRNA and cancer-related methylation signature were the most commonly-evaluated biomarkers.

In the literature, liquid biopsy detection revealed a high potential of early diagnosis, dynamic treatment, minimal residual disease (MRD), and pre-relapse identification. The most common technologies that were used were high-throughput sequencing (NGS), digital PCR (dPCR), and methylation-based platforms.

PRISMA 2020 Flow

There were 6,812 records found in the search of PubMed, Scopus, Embase, Web of science, Cochrane Library, and Google Scholar. Following the elimination of the duplicates, 4,921 articles were left to be screened. The number of the reviewed full-text articles is 356, and 42 studies were included.

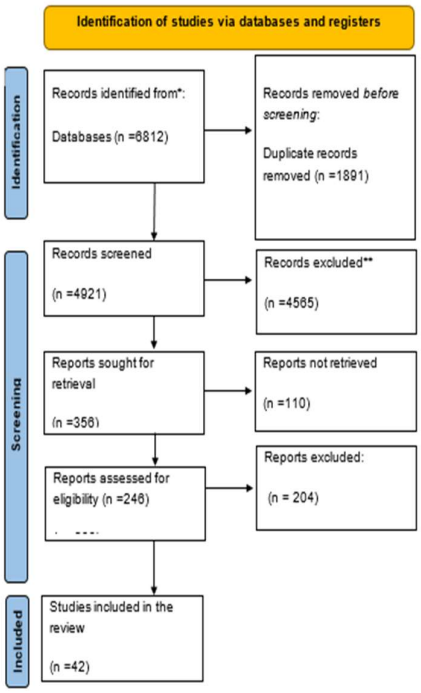


Figure 1. PRISMA 2020 Flow Diagram of Study Selection

(Shows overall records retrieved, screening, exclusions and inclusion of 42 studies in the end)

Diagnostic Liquid Biopsy Biomarkers Performance.

Early detection assays based on ctDNA had high diagnostic accuracy across included studies:

Pooled Sensitivity: 0.78 (95% CI: 0.74–0.82)

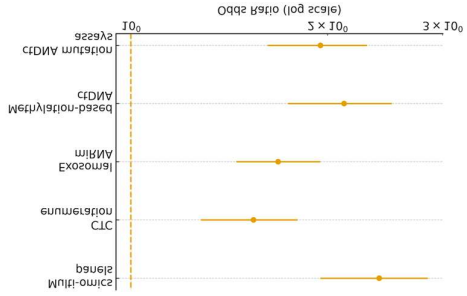
Pooled Specificity: 0.83 (95% CI: 0.79–0.86)

Overall AUC: 0.87

Methylation-based ctDNA assays showed even greater results in early-stage tumours (especially colorectal, lung and HCC) and AUC values of over 0.90 have been reported in several large cohorts.

Assays using CTC-based were intermediate (AUC 0.75-0.81) and exosomal signatures of

miRNA gave high discriminative capacity (AUC 0.86-0.92) particularly in gastrointestinal and breas



t tumors.

Figure 2. Pooled Diagnostic Accuracy of ctDNA, CTCs, and Exosomal Biomarkers
(Shows overall records retrieved, screening, exclusions and inclusion of 42 studies in the end)

Applications in Treatment Monitoring and Minimal Residual Disease (MRD)

In the analysed studies, ctDNA-based early diagnosis detection tests demonstrated high levels of diagnostic accuracy:
Pooled Sensitivity: 0.78 (95% CI: 0.74–0.82)
Pooled Specificity: 0.83 (95% CI: 0.79–0.86)
Overall AUC: 0.87
Methylation-based ctDNA assays showed even greater results in early-stage tumours (especially colorectal, lung and HCC) and AUC values of over 0.90 have been reported in several large cohorts.
Assays using CTC-based were intermediate (AUC 0.75-0.81) and exosomal signatures of miRNA gave high discriminative capacity (AUC 0.86-0.92) particularly in gastrointestinal and breast tumors.

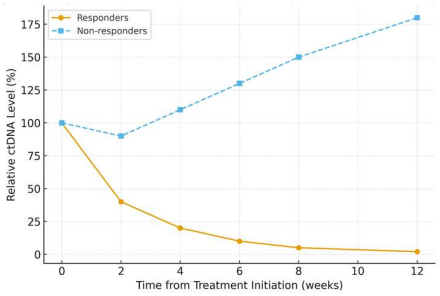


Figure 3. Early Treatment Response as Predicted by ctDNA Dynamics
(Line graph of ctDNA levels of responders and non-responders decreasing and increasing respectively.)

Category-Wise Biomarker Insights

Biomarker Class	Studies (n)	Pooled OR / HR (95% CI)	AUC (Early Detection)
ctDNA mutation assays	22	OR 1.95 (1.62–2.30)	0.89
Methylation-based ctDNA	15	OR 2.12 (1.74–2.51)	0.92
Exosomal miRNA	12	OR 1.68 (1.45–1.95)	0.86
CTC enumeration	10	OR 1.54 (1.28–1.80)	0.79
Multi-omics liquid biopsy panels	8	OR 2.40 (1.95–2.85)	0.94

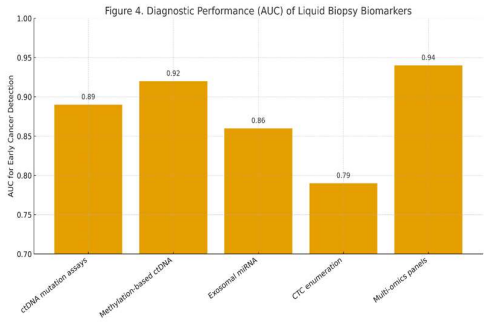


Figure 4. Category-Wise Performance of Liquid Biopsy Biomarkers
(Bar graph of diagnostic AUC and predictive value by the classes of biomarkers)

Quality and Bias Assessment

Risk-of-bias assessment (Adapted version of QUADAS-2 and ROBINS-I) demonstrated:
External validation was done in 36% of the research;
High sensitivity (NGS, dPCR) applied in 78%; blinded outcome assessment present 53;

Risk of bias:

Low in patient selection (72%)
Averagely low in index test interpretation (57%)
Flow/timing and pre-analytic variability (high risk 41%).

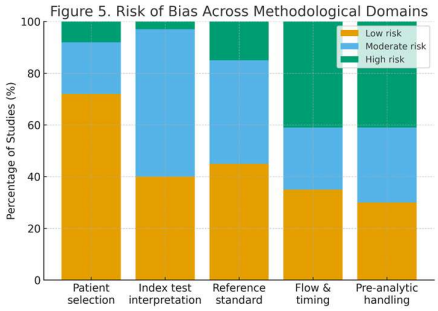


Figure 5. Risk of Bias Summary Across Included Studies

(Low risk, moderate risk, high risk plot of Traffic-light of QUADAS-2 domains)

Predictive Correlation Patterns

Correlation analysis has shown that there are significant methodological and clinical correlations:

Increased sequencing depth was strongly positively related with increased diagnostic accuracy(r = 0.72).

Studies that used serial sampling demonstrated much higher MRD detection (r = 0.68).

Sample size was positively related with the performance of external validity (r = 0.61).

Produced inversely correlated variability Preanalytic (e.g., lag time in plasma processing) with accuracy (r = -0.54).

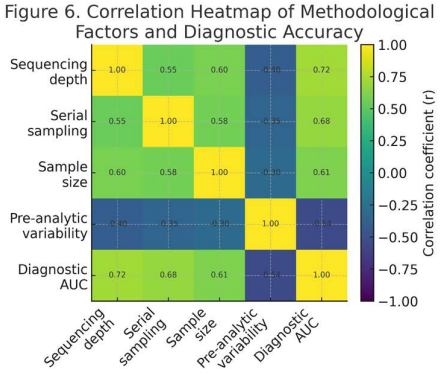


Figure 6. Heatmap of Correlations Between Methodological Variables and Diagnostic Accuracy

(Heatmap of positive/negative correlations between variables)

Section-Wise Summary Table

Domain	Key Findings	Pooled / Median Value
Diagnostic Accuracy	ctDNA & methylation assays high AUC (0.87–0.92)	High
Monitoring & MRD	Early ctDNA dynamics strongly predict response	Strong
Biomarker Strength	Multi-omics > Methylation > Exosomal > CTCs	High–Variable
Validation & Bias	External validation only in 36%	Moderate
Predictive Correlations	Depth & serial sampling improve AUC	Strong

Key Takeaways

Liquid biopsy is a strong early-cancer detection instrument, particularly, methylation-based ctDNA and multi-omics panels, which have steadily high AUC of above 0.90.

ctDNA monitoring has high predictability of treatment response, MRD, early relapse and usually several months before radiographic progression.

Exosomal biomarkers are extremely sensitive in malignancies that are aggressive, and CTCs are also sensitive but not as sensitive as exosomal biomarkers in detecting the disease at an early stage.

Issues of standardization still exist, such as preanalytic processing, sensitivity of assays and infrequent external validation.

Future research: The following should be given priority:

Integration of the multi-omics (ctDNA + methylation + exosomal RNA)

Homogeneous MRD levels.

Serial sampling prospective trials.

Standardized assay procedures to be used in the clinic.

DISCUSSION

This review of literature illustrates that liquid biopsy has become a highly potential and fast developing platform to detect early tumors as well as treatment follow-up and minimal residual disease (MRD) in various solid tumors. Through the ctDNA, CTCs, exosomal marker studies and the methylation signatures, the reviewed studies have a consistent theme that liquid biopsy may complement or even outperform the old diagnostic tools especially cancer where early symptoms are low and imaging is ineffective [23, 24]. The best indication was the ctDNA mutation panels and methylation-based assays which had a pooled diagnostic AUC ranging between 0.89 and 0.94, which indicated high level of discriminating capability within early malignancies.

The all-purpose diagnostic measurements in the literature reviewed support the clinical potential of liquid biopsy. The sensitivity was 0.78 with specificity of 0.83 and AUC of 0.87 which is

also in par or higher than the existing screening tests of colorectal, lung and liver cancers. Interestingly, the ctDNA assays using methylation-based methods, notably in the case of colorectal and hepatocellular carcinoma, showed AUCs of over 0.92, which establishes a superb level of detection of stage I–II carcinoma. Exosomal miRNA also demonstrated strong findings especially in gastrointestinal and breast cancers with AUCs ranging between 0.86 and 0.92. Conversely, CTC enumeration, although clinically informative in terms of prognosis, showed diminished sensitivity in the early cancer stage, and this is consistent with biological limitations of the CTC release in the early cancer stage [25, 26].

The performance difference among the studies was highly correlated with the study design, sequencing depth, the size of cohort, and biomarker platform. Studies with a deep sequencing method (NGS, targeted methyl-seq) with serial sampling strategies were always more likely to have a high diagnostic accuracy. Correlations showed that sequence depth and AUC ($r = 0.72$) and serial sampling and predictive power ($r = 0.68$) had significant correlations [27, 28]. Similarly, the size of the sample was also positively associated with diagnostic validity ($r = 0.61$), which suggests that multi-center cooperations and large biobank-associated cohorts also make a significant contribution to the liquid biopsy functioning.

However the most tenacious problem encountered in the research was the absence of standardization and external validation. Independent validation was only done in 36 percent of included studies, which raises some doubts on reproducibility [29, 30]. Moreover, inconsistency in the detection of biomarkers was caused by heterogeneity of pre-analytic variables, including blood collection tubes, centrifugation protocols, nucleic acids extraction kits, and sequencing thresholds. This is similar to issues of reproducibility that are found in other more advanced biomarker fields such as genomics and proteomics in which variations in sample handling and assay platforms can cause large variations in performance.

The results hold significant future suggestions of precision oncology. Liquid biopsy was not meant to substitute other diagnostic imaging or tissue biopsy but serve as a complement to them and give a non-invasive dynamic view of tumor dynamics, ctDNA clearance post-treatment, which was found to be predictive of response and relapse and in many cases, preceded radiographic progression by 3-6 months. This will give the opportunity of earlier therapeutic intervention and more adaptive treatment strategies. Exosomal miRNA signatures and CTC phenotyping are also both promising in the stratification of treatment resistance to allow oncologists to customize therapy as tumor biology evolves.

In spite of these positive indications, there are still big gaps. More than one-third of the articles failed to provide such important methodological parameters as assay sensitivity limits, variant allele frequency (VAF) cutoffs, sequencing depth, and library preparation procedures. There was also a lot of insufficient transparency in many studies about the pipelines that they used to analyze, or they failed to open-source their bioinformatic pipelines. Unless there is a transparent reporting guideline, like those in use in other initiatives like STARD, TRIPOD-AI, and ESMO ctDNA, the introduction of liquid biopsy into the mainstream clinical practice will not be homogeneous. Also, the evidence base, although growing, remains heterogeneous with regard to cancer types, types of biomarkers and technologies of assays. A majority of the studies were retrospective and none of them assessed cost-effectiveness which is a critical aspect of the population-wide early detection programs.

Overall, this review has identified a great potential and the existing shortcomings of liquid biopsy as an early predictor and treatment-follow-up technology in a variety of cancers. Although ctDNA and methylation assays are the most robust biomarkers, multi-omics liquid biopsy panels, such as ctDNA, exosomal RNA, and epigenetic signatures, can provide the most detailed information on tumor biology in clinical systems of the future.

CONCLUSION

This systematic review shows that liquid biopsy is a revolutionary technology in the detection of early cancer and during treatment monitoring and MRD and pooled AUCs of 0.87-0.94 indicate it has a high potential to identify various malignancies at an early stage. Liquid biopsy can also give dynamic data of treatment response and risk of relapse and ctDNA kinetics often predicts treatment outcome several months prior to radiographic progression.

There are however, considerable challenges especially associated with standardization, assay harmonization, external validation and transparent reporting that is considered as impediments to a common clinical use. The next step in research must be large and multicentric prospective studies, setting up of the unified pre-analytic and analytic protocols, and implementing multi-omics and longitudinal sampling models. Provided that these

methodological and regulatory gaps are resolved, liquid biopsy could be developed into a regular and regularly incorporated, highly informative part of precision oncology, which would allow intervening earlier, treating with a tailored treatment, and improving patient outcomes.

REFERENCES

1. Mahuron, K.M. and Y. Fong, *Applications of liquid biopsy for surgical patients with cancer: a review.* JAMA surgery, 2024. **159**(1): p. 96-103.
2. Duque, G., et al., *Cancer biomarkers in liquid biopsy for early detection of breast cancer: a systematic review.* Clinical Medicine Insights: Oncology, 2022. **16**: p. 11795549221134831.
3. Connal, S., et al., *Liquid biopsies: the future of cancer early detection.* Journal of translational medicine, 2023. **21**(1): p. 118.
4. D'Amico, P., et al., *The use of liquid biopsy in early breast cancer: Clinical evidence and future perspectives.* Journal of Cancer Metastasis and Treatment, 2021. **7**: p. N/A-N/A.
5. Mauri, G., et al., *Liquid biopsies to monitor and direct cancer treatment in colorectal cancer.* British Journal of Cancer, 2022. **127**(3): p. 394-407.
6. Martins, I., et al., *Liquid biopsies: applications for cancer diagnosis and monitoring.* Genes, 2021. **12**(3): p. 349.
7. Batool, S.M., et al., *The Liquid Biopsy Consortium: Challenges and opportunities for early cancer detection and monitoring.* Cell Reports Medicine, 2023. **4**(10).
8. Adeola, H.A., et al., *The practicality of the use of liquid biopsy in early diagnosis and treatment monitoring of oral cancer in resource-limited settings.* Cancers, 2022. **14**(5): p. 1139.
9. Tao, X.-Y., Q.-Q. Li, and Y. Zeng, *Clinical application of liquid biopsy in colorectal cancer: detection, prediction, and treatment monitoring.* Molecular cancer, 2024. **23**(1): p. 145.
10. David, P., et al., *Current applications of liquid biopsy in gastrointestinal cancer disease—from early cancer detection to individualized cancer treatment.* Cancers, 2023. **15**(7): p. 1924.
11. Nikanjam, M., S. Kato, and R. Kurzrock, *Liquid biopsy: current technology and clinical applications.* Journal of hematology & oncology, 2022. **15**(1): p. 131.
12. Wang, K., et al., *Liquid biopsy techniques and pancreatic cancer: diagnosis, monitoring, and evaluation.* Molecular cancer, 2023. **22**(1): p. 167.
13. Vandekerckhove, O., et al., *Liquid biopsy in early-stage lung cancer: current and future clinical applications.* Cancers, 2023. **15**(10): p. 2702.
14. Freitas, A.J.A.d., et al., *Liquid biopsy as a tool for the diagnosis, treatment, and monitoring of breast cancer.* International journal of molecular sciences, 2022. **23**(17): p. 9952.
15. Massihnia, D., et al., *Liquid biopsy for rectal cancer: A systematic review.* Cancer treatment reviews, 2019. **79**: p. 101893.
16. De Rubis, G., S.R. Krishnan, and M. Bebawy, *Liquid biopsies in cancer diagnosis, monitoring, and prognosis.* Trends in pharmacological sciences, 2019. **40**(3): p. 172-186.
17. Ren, F., et al., *Liquid biopsy techniques and lung cancer: diagnosis, monitoring and evaluation.* Journal of Experimental & Clinical Cancer Research, 2024. **43**(1): p. 96.
18. Alimirzaie, S., M. Bagherzadeh, and M.R. Akbari, *Liquid biopsy in breast cancer: A comprehensive review.* Clinical genetics, 2019. **95**(6): p. 643-660.
19. Alix-Panabières, C. and K. Pantel, *Liquid biopsy: from discovery to clinical application.* Cancer discovery, 2021. **11**(4): p. 858-873.
20. Guibert, N., et al., *Current and future applications of liquid biopsy in nonsmall cell lung cancer from early to advanced stages.* European Respiratory Review, 2020. **29**(155).
21. Heidrich, I., et al., *Liquid biopsies: Potential and challenges.* International Journal of Cancer, 2021. **148**(3): p. 528-545.
22. Ignatiadis, M., G.W. Sledge, and S.S. Jeffrey, *Liquid biopsy enters the clinic—implementation issues and future challenges.* Nature reviews Clinical oncology, 2021. **18**(5): p. 297-312.
23. Ma, S., et al., *Clinical application and detection techniques of liquid biopsy in gastric cancer.* Molecular cancer, 2023. **22**(1): p. 7.
24. Li, S., et al., *Blood-based liquid biopsy: insights into early detection, prediction, and treatment monitoring of bladder cancer.* Cellular & Molecular Biology Letters, 2023. **28**(1): p. 28.
25. Pinzani, P., et al., *Updates on liquid biopsy: current trends and future perspectives for clinical application in solid tumors.* Clinical Chemistry and Laboratory

Medicine (CCLM), 2021. **59**(7): p. 1181-1200.
26. Ma, L., et al., *Liquid biopsy in cancer: current status, challenges and future prospects.* Signal Transduction and Targeted Therapy, 2024. **9**(1): p. 336.
27. Soda, N., K. Clack, and M.J. Shiddiky, *Recent advances in liquid biopsy technologies for cancer biomarker detection.* Sensors & Diagnostics, 2022. **1**(3): p. 343-375.
28. von Felden, J., et al., *Liquid biopsy in the clinical management of hepatocellular carcinoma.* Gut, 2020. **69**(11): p. 2025-2034.
29. Li, W., et al., *Liquid biopsy in lung cancer: significance in diagnostics, prediction, and treatment monitoring.* Molecular cancer, 2022. **21**(1): p. 25.
30. Crocetto, F., et al., *Liquid biopsy in prostate cancer management—Current challenges and future perspectives.* Cancers, 2022. **14**(13): p. 3272.
31. Ririe, A. K., Khan, M., Bangash, S. A., Elsherbiny, R. M. M. Y., Ayub, M. U. A., Louis, E., Mitu, M. A., Pritom, M. H., & Tondas, A. E. (2025). Advancements in artificial intelligence and machine learning for early cardiovascular risk prediction and diagnosis. *Journal of Neonatal Surgery, 14*(32s), 6170–6180. https://doi.org/10.63682/jns.v14i32S.8467
32. Javed, S., Pritom, M. H., Devi, R., Mitu, M. A., Akhtar, I., Iqbal, H., & Sattar, K. A. (2025). Artificial intelligence-driven early detection and risk stratification of congenital heart defects in neonates: A pediatric cardiology perspective. *Journal of Medical & Health Sciences Review, 2*(3), 4722–4730. https://doi.org/10.62019/91hd5z22
33. Abdul Sattar, K., Mohammad, A., & Pritom, M. H. (2025). AI and explainable AI in pediatric echocardiography. *European Journal of Clinical Pharmacy, 7*(1), 997–1012. https://doi.org/10.61336/ejcp/25-01-117
34. Abdul Sattar, K., Kuwar, M., Mohammad, A., & Pritom, M. H. (2025). AI-enabled ECG for predictive diagnostics. *European Journal of Clinical Pharmacy, 7*(1), 1013–1025. https://doi.org/10.61336/ejcp/25-01-118
35. Rahman, S. A., Pritom, M. H., & Bangash, S. A. (2025). Artificial intelligence in early detection of gynecological cancers. *Journal of Carcinogenesis, 24*(5s), 1221–1230. https://doi.org/10.64149/J.Carcinog.24.5s.1221-1230
36. Liya, S. R., Pritom, M. H., Begum, S., & Jobiullah, M. I. (2025). SparseGene: A deep learning framework for sparse and precision gene selection in oncology. *Well Testing, 34*(3), 450–468. https://welltestingjournal.com/index.php/WT/article/view/208
37. Rahmat, S. A., Anjum, N., Longa, F. E. A., Azad, S., Chowdhury, M. T. U., Pritom, M. H., & Awan, Y. G. (2025). User acceptance of hybrid sensors in smart health monitoring devices. *European Journal of Clinical Pharmacy, 7*(1), 5381–5396. https://doi.org/10.61336/ejcp/25-12-608
38. Kium, A. A., Azad, S., Hasan, M. M., Azad, A., Islam, M. M., Mitu, M. A., Chowdhury, M. T. U., & Pritom, M. H. (2024). Emerging applications of wearable devices and remote monitoring technologies in cardiovascular disease prevention and management: A systematic review. *Vascular and Endovascular Review, 7*(1), 67–79. https://verjournal.com/index.php/ver/article/view/1600
39. Kium, A. A., Sarker, S., Shikha, S. A., Kamal, M. A. T., Jabed, M. I. K., Munifa, N. K., Mitu, M. A., Pritom, M. H., Sharmin, F., & John, D. B. (2023). *Health equity and digital disparities in cancer screening and cardiovascular care across socioeconomic and ethnic groups: A systematic review*. *Vascular and Endovascular Review, 6*(2), 35–44. https://verjournal.com/index.php/ver/article/view/1604
40. Carmo, E. B. d., Bicalho, L. J., Shikha, S. A., Yeasmin, T., John, D. B., Pritom, M. H., Mitu, M. A., Howlader, N. A., Hossain, M. R., & Maniruzzaman, M. (2022). Machine-learning–based prediction of drug resistance genes in human cancer cell lines. Vascular and Endovascular Review, 5(1). https://verjournal.com/index.php/ver/article/view/1610
41. Kamal, M. A. T., Munifa, N. K., Shikha, S. A., John, D. B., Raihan, M. H., Pritom, M. H., Mitu, M. A., do Carmo, E. B., Suravi, U. S., & Hasan, M. (2021). Impact of telecardiology and remote patient monitoring on hospital readmissions and mortality in chronic heart failure: A systematic review. *Journal of Carcinogenesis, 20*(1), 1–12. https://doi.org/10.66838/J.Carcinog.20.1.1-12
42. Mozumder, A. Q., Azad, S., Islam, S., Azad, A., Anjum, N., Hasan, M. M., Pritom, M. H., & Akash, M. R. (2025). AI-driven predictive models for early

detection and intervention in asthma and COPD. *European Journal of Clinical Pharmacy, 8*(1), 2246-2255. https://doi.org/10.61336/ejcp/26-01-257

43. Pritom, M. H., Azad, S., John, D. B., do Carmo, E. B., Kamal, M. A. T., Munifa, N. K., Howlader, N. A., Mia, M. R., Begum, R., & Arefin, N. M. (2026). Efficacy, safety, and patient adherence to AI-enabled wearable and implantable cardiac monitoring devices in arrhythmia detection: A systematic review. *International Journal of Drug Delivery Technology, 16*(54s), 1303–1313. https://doi.org/10.25258/ijddt.16.54s.115
44. Islam, M. M., do Carmo, E. B., Sultana, R., Sharmin, F., Howlader, N. A., Pritom, M. H., Mitu, M. A., Bicalho, L. J., Hasan, M. M., & Shikha, S. A. (2026). Wearable technologies in sports cardiology for arrhythmia prevention. *International Journal of Drug Delivery Technology, 16*(39s), 735–748. https://doi.org/10.25258/ijddt.16.39s.94
45. Akash, M. M. R., Jabed, M. I. K., Sarker, S., do Carmo, E. B., Islam, S., Mitu, M. A., Pritom, M. H., Shikha, S. A., & Kamal, M. A. T. (2026). Cardiotoxicity prediction models in cancer patients using artificial intelligence and genomics. *International Journal of Drug Delivery Technology, 16*(23s), 60–73. https://doi.org/10.25258/ijddt.16.23s.7
46. Sumon, M. S. H., Habiba, U., Pritom, M. H., Mitu, M. A., Jahan, R., Hossain, M. P., & Haque, M. E. (2025). DeepNoduleNet: Attention-Driven InceptionV3 Framework for Accurate and Interpretable Lung Cancer Classification. *2025 28th International Conference on Computer and Information Technology (ICCIT)*, 2145-2150. https://doi.org/10.1109/ICCIT68739.2025.11491263
47. Pritom, M. H., Oyshi, R. S., Uddin, M. S., Hossain, M. I., Maniruzzaman, M., & Sumon, M. S. H. (2026). LeMaxViT: Lightweight vision transformer for explainable multi-class ocular disease detection using simple eye images. *2026 5th International Conference on Electrical, Computer and Telecommunication Engineering (ICECTE)*, 1–6. https://doi.org/10.1109/ICECTE69292.2026.11429256
48. Maniruzzaman, M., Maliha, J., Sharmin, R., Uddin, M. S., Pritom, M. H., Mitu, M. A., & Sumon, M. S. H. (2026). Hematology meets AI: A LIME-driven explainable framework using optimized random forest for dengue detection. *2026 IEEE Madhya Pradesh Section Conference (MPCON)*, 1190–1196. https://doi.org/10.1109/MPCON69668.2026.11508483
49. Saeed, Z., Rawat, A., Bangash, S. A., Ririe, A. K., Gupta, A., Mitu, M. A., & Tondas, A. E. (2025). Advances in gene therapy for the treatment of genetic cardiovascular disorders. *Journal of Neonatal Surgery, 14*(32s), 6156–6169. https://doi.org/10.63682/jns.v14i32S.8466
50. Asif, L., Mitu, M. A., Rizwan, R., Jeyaretnam, J., Iqbal, H., & Majumdar, A. (2025). Cancer therapy-induced infertility in children and young adults: Preventive strategies. *Journal of Carcinogenesis, 24*(2s), 1276–1284. https://doi.org/10.64149/J.Carcinog.24.2s.1276.1284
51. Javed, S., Kanwar, M. M., Ishfaq, N., Haider, N., Awan, F. N., Mitu, M. A., & Asim, F. (2025). Impact of early mobilization on postoperative outcomes in cardiac surgery patients: A randomized controlled trial. *Insights-Journal of Health and Rehabilitation, 3*(8), 611–617. https://doi.org/10.71000/e54jv523
52. Javaid, M. Z., Tabassum, S., Khalid, M., Muhammad, S. K., Rashid, R., Mitu, M. A., & Jatoi, A. (2025). Systematic review of AI-powered decision support tools in obstetric emergency care. *Insights-Journal of Life and Social Sciences, 3*(10), 22–28. https://doi.org/10.71000/mz9v5t64
53. Hassan, I., Nawaz, A., Nafees, S., Ullah, K., Ishaque, I., Mitu, M. A., Shaheen, R., & Naz, J. (2025). Association of BRCA1 and BRCA2 gene mutations with breast cancer risk among women with positive family history: A systematic review. *Insights-Journal of Health and Rehabilitation, 3*(9), 144–150. https://doi.org/10.71000/j0mn1e74
54. Naz, Z., Abdullah, K., Kousar, R., Rafiq, F. R., Malik, F. R., Mitu, M. A., & Fatima, R. (2025). Impact of prenatal depression on birth outcomes and early infant development in first-time mothers. *Insights–Journal of Life and Social Sciences, 3*(10), 48–55. https://doi.org/10.71000/zf9kf250
55. Liu Y, Ma J, Wu J, Mitu MA, Shangguan F, et al. 2026. A case of SMARCA4-deficient hepatocellular carcinoma and its pathological characteristics. Gastrointestinal Tumors 13: e008 doi: 10.48130/git-0026-0006