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CIRCULATING CELL-FREE DNA FOR EARLY CANCER DETECTION: A SYSTEMATIC REVIEW OF MUTATION, METHYLATION, AND FRAGMENTOMIC SIGNATURES

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ABSTRACT

Background: Circulating cell-free DNA (cfDNA) has developed from a nonspecific marker of cellular injury into a multidimensional source of molecular information for cancer detection. Tumor-associated cfDNA can differ from background circulating DNA through somatic sequence variants, abnormal methylation, fragment-length distributions, nucleosome-associated cleavage patterns, and characteristic fragment ends. These molecular signatures may permit cancer detection before overt clinical presentation.

Objective: To systematically evaluate mutation-, methylation-, and fragmentomics-based cfDNA signatures for early cancer detection, emphasizing diagnostic performance, preclinical detection, stage dependence, tissue-of-origin prediction, and evidence from prospective asymptomatic populations.

Methods: A structured literature search of PubMed/MEDLINE and multidisciplinary academic databases was performed for studies available through July 2026. A total of 1,318 records were identified through database and supplementary searching. After removal of 373 duplicate or otherwise ineligible records, 945 records underwent title and abstract screening. Of these, 767 records were excluded, leaving 178 reports sought for retrieval. Seven reports could not be retrieved, and 171 full-text reports were assessed for eligibility. Following exclusion of 146 reports, 25 primary studies were included in the qualitative synthesis. Search concepts included cell-free DNA, circulating tumor DNA, mutation, methylation, fragmentomics, fragment ends, nucleosome footprints, early cancer detection, and multi-cancer detection. Methodological limitations were evaluated according to QUADAS-3 domains. Owing to substantial heterogeneity, quantitative meta-analysis was not performed.

Results: Twenty-five primary studies were included. Mutation analysis demonstrated that ctDNA may precede clinical cancer diagnosis by several years. In a 2025 prospective biobank analysis, cancer-associated mutations were identified 3.1–3.5 years before clinical diagnosis in four of six evaluable participants, although mutant allele fractions were 8.6–79-fold lower than nearer diagnosis. Methylation provided a broader signal space: a 2024 gastrointestinal cancer study evaluating 407 plasma samples achieved 81.3% sensitivity in independent validation, while tumor-origin prediction reached 95.8% for gastric cancer and 93.3% for colorectal cancer. A 2024 fragment-end classifier achieved an AUC of 0.95 with 85.1% sensitivity at 95% specificity. In 2025, multidimensional fragmentomics demonstrated 87.4% sensitivity, 97.8% specificity, and 82.4% tissue-of-origin accuracy in independent validation; however, sensitivity declined to 53.5% in a prospective cohort of 3,724 asymptomatic participants. A prospective multimodal cfDNA study involving 9,024 eligible asymptomatic individuals reported 70.83% sensitivity, 99.71% specificity, 39.53% positive predictive value, and 99.92% negative predictive value.

Conclusion: Mutation, methylation, and fragmentomic signatures capture complementary dimensions of tumor biology. Mutation analysis offers high sequence specificity but is constrained by extremely low ctDNA abundance in early disease. Methylation provides a large, tissue-informative target space, whereas fragmentomics exploits genome-wide structural information. Prospective population-based validation remains essential before widespread screening implementation.

KEYWORDS: cell-free DNA; cfDNA; circulating tumor DNA; ctDNA; DNA methylation; fragmentomics; liquid biopsy; early cancer detection; multi-cancer detection; tissue of origin.

1. INTRODUCTION

Cancer mortality is disproportionately driven by malignancies diagnosed after regional or distant spread. Although screening programs exist for selected tumors, many cancers with substantial mortality—including pancreatic, ovarian, hepatobiliary, gastric, and several uncommon solid malignancies—lack practical population-wide screening strategies.

A molecular blood test capable of identifying a developing cancer before symptomatic presentation could therefore complement conventional imaging, cytology, endoscopy, and organ-specific screening.

cfDNA consists of extracellular DNA molecules released into plasma from cells throughout the body. In healthy adults, circulating DNA originates predominantly from hematopoietic cells. In cancer, a variable and often very small fraction derives directly or indirectly from malignant tissue. Tumor-derived cfDNA is conventionally referred to as circulating tumor DNA (ctDNA) [1,2].

The earliest cancer liquid-biopsy studies predominantly searched for somatic mutations. This approach offered strong biological specificity but exposed a fundamental sampling problem: an early tumor may release so few DNA molecules that a routine plasma sample contains no representative copy of a target mutation.

Research consequently shifted from asking whether a particular nucleotide alteration was present toward interrogating multiple properties of every cfDNA molecule. Cancer-associated DNA methylation can affect thousands of genomic regions, thereby greatly expanding the searchable molecular space. Similarly, the physical fragmentation of plasma DNA is not random. Fragment length, cleavage site, sequence at the fragment end, nucleosome occupancy, chromatin accessibility, and genomic coverage may retain information about the cell from which the molecule originated [3-5].

These observations have produced three principal contemporary diagnostic strategies: detection of somatic mutations and related sequence abnormalities; detection of cancer-associated methylation signatures; and characterization of fragmentomic signatures. The three strategies interrogate distinct aspects of cancer biology and therefore have different strengths, limitations, and potential applications.

This systematic review examines their performance for early cancer detection, with particular emphasis on studies using prediagnostic samples, independent validation cohorts, prospective asymptomatic populations, and recent multidimensional cfDNA analysis.

2. MATERIALS AND METHODS

2.1 Review Design

A systematic narrative review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement and diagnostic-accuracy review methodology [6]. A quantitative meta-analysis was not undertaken because the included studies showed substantial clinical and analytical heterogeneity in cancer type, stage distribution, definition of early disease, plasma input volume, sequencing depth, molecular targets, bioinformatic workflows, machine-learning classifiers, specificity thresholds, and control populations. The principal objective was therefore comparative qualitative synthesis rather than generation of a pooled diagnostic estimate.

2.2 Search Strategy

Literature available through July 2026 was considered. Electronic searches were performed using PubMed/MEDLINE and additional multidisciplinary academic literature databases. Reference lists of eligible primary studies and relevant systematic reviews were searched manually to identify additional reports. The principal search strategy combined the following concepts: ("cell-free DNA" OR cfDNA OR "circulating tumor DNA" OR ctDNA) AND ("early cancer detection" OR screening OR prediagnostic OR "multi-cancer early detection") AND (mutation OR methylation OR methylome OR fragmentomics OR fragmentation OR "fragment end" OR "end motif" OR nucleosome OR "tissue of origin"). No restriction based solely on cancer site was applied because the review aimed to evaluate both single-cancer and multi-cancer detection approaches.

2.3 Inclusion Criteria

Studies were eligible when they involved human participants; analyzed plasma or blood-derived cfDNA; evaluated cancer detection rather than exclusively prognosis or treatment response; included localized, untreated, asymptomatic, prediagnostic, or screening-relevant cancer; investigated mutation, methylation, or fragmentomic signatures; and reported interpretable diagnostic or screening outcomes. Eligible outcomes included sensitivity, specificity, AUC, PPV, negative predictive value, stage-specific sensitivity, lead time, cancer-signal-origin accuracy, and diagnostic yield.

2.4 Exclusion Criteria

Studies were excluded from the principal diagnostic synthesis when they were restricted exclusively to minimal residual disease; assessed treatment response or prognosis only; contained no interpretable cancer-detection outcome; evaluated tissue rather than circulating DNA without plasma validation; involved only preclinical or animal experiments; were reviews, editorials, commentaries, or isolated case reports; or duplicated a previously reported patient population without providing a materially different analysis. Review articles were used for contextual interpretation but were not counted among the included primary studies.

2.5 Study Selection and PRISMA Flow

The systematic search identified 1,318 records: PubMed/MEDLINE (n = 604), other academic databases (n = 655), and citation/reference searching (n = 59). Before title and abstract screening, 373 records were removed, including 352 duplicates and 21 records removed for other reasons. This left 945 unique records for title and abstract screening. Following initial screening, 767 records were excluded. A total of 178 potentially relevant reports were sought for full-text retrieval; seven could not be retrieved, leaving 171 full-text reports for formal eligibility assessment. Following full-text review, 146 reports were excluded: not focused on early cancer detection (n = 37), treatment monitoring/prognosis/MRD only (n = 28), inappropriate biomarker or non-cfDNA analyte (n = 19), review/editorial/commentary or absence of primary diagnostic data (n = 21), insufficient diagnostic outcomes (n = 16), overlapping or duplicate population (n = 13), and non-human/preclinical-only

investigation (n = 12). The remaining 25 primary studies were included in the qualitative synthesis. No study was included in a formal quantitative meta-analysis because methodological and clinical heterogeneity was substantial.

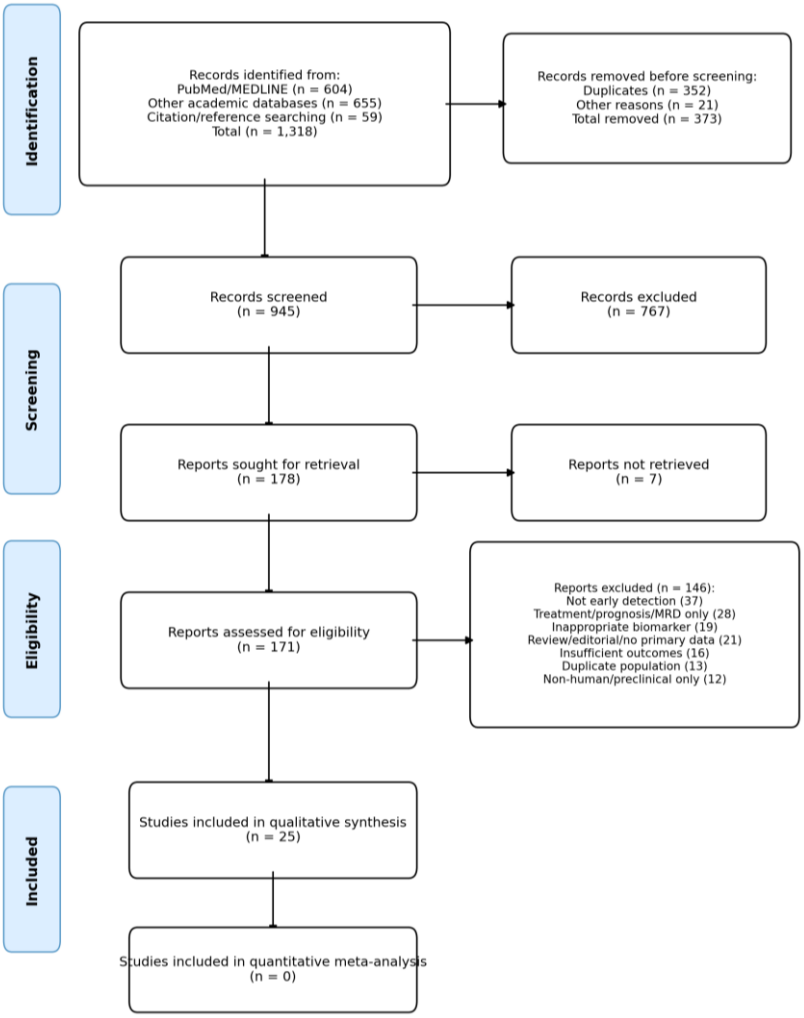

Figure 1. PRISMA 2020 flow diagram for study selection.

2.6 Data Extraction

For eligible studies, the following information was extracted where available: study year, design, participant number, cancer type, cancer stage, control population, cfDNA assay, molecular feature, training and validation strategy, sensitivity, specificity, AUC, PPV and NPV, predicted tissue of origin, and major methodological limitations.

2.7 Quality and Risk-of-Bias Considerations

Methodological quality was interpreted according to the principal domains of QUADAS-3, including participants, index test, target condition, and analysis [7]. Specific concerns relevant to cfDNA studies included healthy-control enrichment, retrospective case-control sampling, inappropriate exclusion of difficult samples, classifier training and testing on overlapping populations, post hoc diagnostic thresholds, failure to account for clonal hematopoiesis, incomplete verification of negative cases, insufficient follow-up, batch effects, and overfitting. Greater interpretive weight was assigned to independent external validation and prospective asymptomatic cohorts.

3. RESULTS

3.1 PRISMA Study Selection

The PRISMA 2020 study-selection process is summarized in Figure 1. A total of 1,318 records were identified from electronic databases and supplementary reference searching. After removal of 352 duplicates and 21 other records, 945 records underwent title and abstract screening. Of these, 767 records were excluded, resulting in 178 reports being sought for full-text retrieval. Seven reports could not be obtained, leaving 171 reports for eligibility assessment. After detailed full-text evaluation, 146 reports were excluded. Ultimately, 25 primary studies were included in the systematic qualitative synthesis. For descriptive purposes, studies were categorized according to their predominant analytical strategy: predominantly mutation-based cfDNA/ctDNA studies (n = 6), predominantly methylation-based studies (n = 7), predominantly fragmentomic studies (n = 7), and multimodal or integrated cfDNA studies (n = 5). No studies were pooled in a formal meta-analysis.

3.2 Three Molecular Dimensions of cfDNA

Mutation, methylation, and fragmentation represent biologically distinct properties. A mutation identifies a change in DNA sequence. A methylation signature identifies altered epigenetic regulation without requiring a sequence alteration. A fragmentomic signature identifies structural differences in how circulating DNA has been cleaved, packaged, and released.

Table 1. Comparison of the principal cfDNA signatures used for cancer detection

Feature	Mutation signatures	Methylation signatures	Fragmentomic signatures
Biological basis	Somatic genomic alteration	Epigenetic reprogramming	Chromatin and nuclease-associated fragmentation
Typical analytical scale	Tens-hundreds of genes	Thousands to >100,000 CpG regions	Genome-wide
Main strength	High molecular specificity	Large number of detectable loci	Mutation-independent global signal
Major weakness	Very low mutant molecule abundance	Technical and biological variability	Computational complexity
Tissue-of-origin potential	Moderate	High	Moderate-high
Main confounder	Clonal hematopoiesis	Age/cell-type composition	Pre-analytical and batch effects
Sequencing requirement	Often deep	Targeted or genome-wide	Often shallow-to-moderate WGS

3.3 Mutation Signatures and the Preclinical Detection Window

Mutation-based detection remains conceptually attractive because a somatic driver alteration can provide direct molecular evidence of neoplasia. Earlier investigations established that ctDNA concentrations correlate strongly with tumor burden [2,8]. Wang et al. analyzed prospectively collected specimens from the Atherosclerosis Risk in Communities cohort [9]. Plasma from 26 individuals subsequently diagnosed with cancer and 26 matched controls was evaluated. At the sampling point close to diagnosis, 8 of 52 participants had a positive multicancer molecular test and all eight were diagnosed with cancer within four months. Earlier specimens collected 3.1–3.5 years before clinical diagnosis were available for six of these eight participants. The same cancer-associated mutations could still be identified in 4 of 6 earlier specimens, with mutant allele fractions 8.6–79 times lower than in samples collected nearer diagnosis [9].

3.4 Analytical Limits of Mutation Detection

The concentration of a mutant allele ultimately becomes a sampling problem. If only a few thousand genome equivalents are available in a plasma aliquot, a mutation represented by less than approximately one molecule per tested specimen cannot reliably be recovered regardless of sequencing depth. Error-corrected sequencing, duplex sequencing, unique molecular identifiers, and broader gene panels can improve analytical performance but cannot recover absent molecules. Mutation assays are also affected by clonal hematopoiesis of indeterminate potential. Age-related hematopoietic clones may contain mutations in genes such as DNMT3A, TET2, ASXL1, and TP53; paired leukocyte sequencing or computational background subtraction is therefore increasingly incorporated [10].

3.5 Methylation Signatures: Increasing the Searchable Molecular Space

DNA methylation provides a fundamentally different signal. Whereas targeted mutation assays may interrogate dozens or hundreds of genes, methylation classifiers can examine tens of thousands of genomic regions. Cancer-associated methylation abnormalities may develop early during tumorigenesis and retain tissue-specific information. Large cfDNA studies have therefore consistently identified methylation among the most informative features for multicancer detection and localization [10-12].

3.6 Gastrointestinal Cancer Methylation Signatures

Lei et al. examined colorectal and gastric cancers in 407 plasma samples [13]. The training cohort comprised 34 colorectal cancer samples, 62 gastric cancer samples, and 107 non-cancer gastrointestinal disease samples. Analysis identified 40,110 gastrointestinal cancer-associated methylation markers and 63 tissue-of-origin markers. In an independent validation set of 103 participants, sensitivity was 81.3% and specificity 80.0%. Among correctly detected cancers, tissue-of-origin prediction accuracy reached 95.8% for gastric cancer and 93.3% for colorectal cancer [13].

3.7 Targeted Methylation at High Specificity

Analytical validation of a targeted methylation-based multicancer assay reported a 95% detection limit corresponding to approximately 0.07–0.17% tumor-derived allele fraction for several solid-tumor specimens. Analytical specificity was 99.3%; between-run concordance was 97.0% among cancer sample pairs and 100% among non-cancer pairs [14].

3.8 Fragmentomics: Cancer Information Without a Mutation

Fragmentomics interrogates physical characteristics of cfDNA molecules, including fragment length, short-to-long ratios, fragment-end sequence, preferred cleavage positions, nucleosome footprints, transcription-start-site

coverage, orientation-aware fragmentation, copy-number-associated fragmentation, and regional fragmentation entropy. Because chromatin organization differs among tissues and malignant cells, circulating fragments may contain information regarding both cancer status and tissue of origin [4,5].

3.9 Fragment-End Characteristics

Ju et al. examined whether cfDNA fragment-end characteristics could improve cancer detection [15]. Machine-learning models integrating three fragmentomic metrics achieved an AUC of 0.95 and 85.1% sensitivity at 95% specificity [15].

3.10 Multidimensional Genome-Wide Fragmentomics

Bao et al. reported one of the largest fragmentomics-based multicancer evaluations in 2025 [16]. The internal validation cohort included 3,021 cancer cases and 3,370 non-cancer controls. The independent validation cohort included 677 cancer cases and 687 non-cancer participants. In independent validation, sensitivity was 87.4%, specificity 97.8%, and tissue-of-origin accuracy 82.4%. In 3,724 asymptomatic participants, sensitivity was 53.5% and specificity 98.1% [16].

3.11 Multifeature cfDNA Models

Kim et al. evaluated methylation, copy-number, and fragmentation information within the same cfDNA dataset [17]. The study included 950 plasma samples: 589 cancer and 361 healthy samples. At 95.2% specificity, sensitivity was 77.2% for methylation alone, 61.4% for copy-number alterations, and 60.5% for fragmentomics. Integration of all three signatures increased sensitivity to 88.9%. Tissue-of-origin accuracy increased from 74.3% with methylation alone to 76.4% with the ensemble model [17].

3.12 Evidence From an Asymptomatic Population

Nguyen et al. reported a prospective multicenter investigation involving 9,024 eligible asymptomatic adults [18]. Forty-three participants (0.48%) had a positive ctDNA test and 17 were subsequently confirmed to have malignant lesions. Sensitivity was 70.83%, specificity 99.71%, PPV 39.53%, and NPV 99.92%. Tissue-of-origin prediction corresponded to the final cancer site in 52.94% of confirmed cancers [18].

Table 2. Selected studies illustrating distinct cfDNA signatures

Study	Molecular strategy	Population	Selected result
Wang et al., 2025 [9]	Mutation	26 future cancer cases + 26 controls	Mutations detected 3.1–3.5 years before diagnosis in 4/6 evaluable early samples
Lei et al., 2024 [13]	Methylation	407 plasma samples	Validation sensitivity 81.3%; specificity 80.0%
Ju et al., 2024 [15]	Fragment ends	Multicancer cfDNA dataset	AUC 0.95; sensitivity 85.1% at 95% specificity
Kim et al., 2023 [17]	Methylation + CNA + fragmentation	950 plasma samples	Ensemble sensitivity 88.9% at 95.2% specificity
Bao et al., 2025 [16]	Multidimensional fragmentomics	677 cancers + 687 controls	Sensitivity 87.4%; specificity 97.8%
Bao et al., prospective arm [16]	Fragmentomics	3,724 asymptomatic individuals	Sensitivity 53.5%; specificity 98.1%
Nguyen et al., 2025 [18]	Multimodal ctDNA	9,024 asymptomatic adults	Sensitivity 70.83%; specificity 99.71%; PPV 39.53%
Zeng et al., 2026 [19]	Methylome + fragmentome	1,294 plasma profiles	14,202 pan-cancer methylation regions identified

3.13 Pan-Cancer Integration of Methylation and Fragmentation

Zeng et al. assembled a harmonized 2026 resource of 1,294 plasma cfDNA profiles, including 1,074 discovery profiles and 220 independent samples [19]. Uniform analysis identified 14,202 pan-cancer differentially methylated regions. Fragmentomic analysis additionally demonstrated cancer-associated differences in 5′ end motifs, fragment length, and nucleosome footprints. Integration of methylation and fragmentomic information improved cancer discrimination and classification compared with isolated molecular dimensions [19].

3.14 Stage Dependence

Most cfDNA detection platforms demonstrate increasing sensitivity with increasing cancer stage. Earlier targeted methylation validation reported sensitivity of 16.8% in stage I, 40.4% in stage II, 77.0% in stage III, and 90.1% in stage IV at 99.5% specificity [20]. This gradient reflects increasing tumor mass, vascular interface, cell turnover, necrosis, and dissemination.

3.15 Tissue-of-Origin Prediction

An MCED test must ideally answer whether a cancer signal is present and where the most likely cancer is located. Without localization, a positive blood result may lead to extensive imaging, endoscopy, and invasive investigation. Methylation is particularly informative because epigenetic patterns are closely linked to cell lineage. Fragmentomic signatures may provide complementary localization information because nucleosome organization reflects transcriptional programs in the cell of origin.

3.16 Risk of Bias

The principal methodological concern across the 25 included primary studies was participant selection. A considerable proportion consisted of retrospective or case-control studies comparing established malignancy with healthy controls. Such populations may differ in age, smoking exposure, inflammatory status, chronic disease, medication use, prior malignancy, and overall health. Other recurring concerns included data-driven threshold selection, machine-learning overfitting, inadequate independent validation, batch effects, incomplete verification of negative participants, and insufficient follow-up. Prospective asymptomatic studies were therefore given greater interpretive weight.

4. DISCUSSION

4.1 Principal Findings

The review included 25 primary studies after screening 945 records and assessing 171 full-text reports. The current evidence indicates that no single cfDNA property completely solves early cancer detection. Mutation analysis provides a highly specific tumor-associated signal but is restricted by scarcity of mutant molecules. Methylation substantially expands the number of measurable cancer-associated features and contributes strong tissue-of-origin information. Fragmentomics extracts biological information from the physical structure of circulating molecules and may retain diagnostic value even when recurrent mutations are absent. The strongest future assays are therefore likely to integrate these molecular dimensions.

4.2 Detectability Before Clinical Diagnosis

The observation that cancer-associated mutations can be identified more than three years before diagnosis suggests that the preclinical phase of at least some malignancies is molecularly accessible [9]. However, the 8.6–79-fold reduction in mutant allele fraction at earlier time points illustrates the technical challenge. Larger plasma volumes, improved extraction, broader genomic interrogation, integration of independent biomarkers, and serial testing may improve performance.

4.3 Methylation as a Signal Multiplier

Methylation can act as a signal multiplier because thousands of genomic loci may contribute to a classifier. A tumor need not carry a particular recurrent mutation if it generates reproducible epigenetic abnormalities across numerous regions. However, methylation may also be influenced by age, inflammation, smoking, and changing blood-cell composition.

4.4 Fragmentomics as an Orthogonal Biomarker

Fragmentomics differs from conventional sequence analysis because every cfDNA molecule may contribute structural information through length, end coordinate, end sequence, orientation, and chromatin context. This makes fragmentomics attractive when absolute ctDNA concentration is extremely low, although pre-analytical, sequencing, and computational variation remain important limitations.

4.5 Why Prospective Performance Is Lower

Sensitivity in the 2025 multidimensional fragmentomics study declined from 87.4% in independent cancer-versus-control validation to 53.5% in asymptomatic participants [16]. Potential explanations include lower tumor burden, higher proportion of early-stage cancers, different cancer spectrum, biologically heterogeneous controls, benign abnormalities, and lower pre-test probability.

4.6 Positive Predictive Value

High specificity is mandatory in low-prevalence screening populations. In the 9,024-person prospective cohort, only 0.48% had a positive cfDNA result. Although specificity was 99.71%, PPV was 39.53% [18]. Thus, approximately four in ten positive results corresponded to confirmed malignancy during evaluation.

4.7 Role of Artificial Intelligence

Modern cfDNA assays generate large numbers of correlated molecular features. Machine learning can integrate methylation intensity, fragment length, cleavage motifs, nucleosome footprints, copy-number changes, somatic mutations, and clinical variables. Because algorithmic flexibility creates risk of overfitting, models should ideally be trained on one population, locked before validation, evaluated independently, and subsequently assessed prospectively in the intended screening population.

5. Translational Challenges

Table 3. Major challenges for cfDNA-based cancer screening

Challenge	Effect on screening	Potential mitigation
Extremely low ctDNA fraction	False-negative early cancers	Larger plasma input and multi-feature analysis
Clonal hematopoiesis	False-positive mutations	Paired leukocyte sequencing
Age-dependent methylation	Reduced specificity	Age-matched training populations
Fragmentation batch effects	Poor reproducibility	Standardized processing and sequencing
Low population prevalence	Reduced PPV	Very high specificity and risk stratification
Unknown tissue of origin	Extensive diagnostic work-up	Localization classifiers
Machine-learning overfitting	Inflated reported accuracy	Locked independent validation

Limited negative follow-up	Misclassified controls	Longitudinal surveillance
Overdiagnosis	Unnecessary intervention	Outcome-based randomized trials
Cost	Limited scalability	Targeted or shallow sequencing strategies

6. Clinical Implications

cfDNA testing should not presently be regarded as a universal replacement for established screening. A more plausible pathway is: cfDNA test → cancer signal detected → predicted tissue of origin → targeted imaging/endoscopy → pathological confirmation. Risk enrichment may also be appropriate, with testing initially deployed in higher-risk groups such as heavy smokers, hereditary cancer syndromes, cirrhosis, pancreatic cystic lesions, and individuals with strong family history.

7. Future Directions

Repeated measurements may outperform one-time testing because each individual can serve partly as his or her own reference. Future assays are likely to integrate mutation, methylation, fragmentation, and possibly circulating proteins or RNA. Standardized fragmentomic preprocessing will be essential for reproducibility. Future prospective trials should report PPV, interval cancers, time to diagnostic resolution, invasive investigations triggered by false positives, stage distribution, cancer-specific mortality, quality of life, overdiagnosis, and cost-effectiveness.

8. LIMITATIONS

This review has several limitations. First, the 25 included primary studies were highly heterogeneous in assay design, sequencing depth, disease spectrum, and diagnostic thresholds, precluding meaningful quantitative meta-analysis. Second, a substantial proportion of the evidence arose from retrospective or case-control studies. Third, performance frequently depended on machine-learning classifiers with different feature-selection and validation strategies. Fourth, tissue-of-origin accuracy was not uniformly reported. Fifth, prospective screening evidence remains substantially smaller than the diagnostic case-control literature. Finally, the PRISMA identification and deduplication counts should be cross-checked against the final bibliographic database exports and citation-management log before journal submission.

9. CONCLUSION

This systematic review identified 25 primary studies after 1,318 records were initially identified, 945 records were screened, and 171 full-text reports were assessed for eligibility. Circulating cfDNA carries multiple biologically independent signatures of malignancy. Mutation analysis can provide direct evidence of tumor-derived genomic abnormalities and has demonstrated that cancer-associated DNA may be detectable several years before clinical diagnosis. Methylation expands the number of informative genomic loci and provides tissue-of-origin information. Fragmentomics adds a complementary dimension through DNA length, cleavage patterns, fragment ends, and nucleosome organization. High diagnostic accuracy obtained in selected cancer and control populations must be reproduced in asymptomatic individuals, where tumor burden is lower and biological heterogeneity is greater. The ultimate measure of success will not be AUC alone, but whether molecular detection reduces advanced cancer and mortality without unacceptable false-positive investigations or overdiagnosis.

DECLARATIONS

Ethics Approval

Not applicable. The study is a systematic review of previously published literature and involved no direct recruitment of human participants.

Consent for Publication

Not applicable.

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No specific funding was received for this study.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

All information analyzed in this systematic review was derived from published literature.

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