

Review

Not peer-reviewed version

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Posted Date: 1 April 2026

doi: 10.20944/preprints202603.2516.v1

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Review

Beyond Mutation Detection: Cell-Free DNA for Functional Inference and Adaptive Oncology

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Abstract

Liquid biopsy has evolved beyond its original role as a minimally invasive approach for mutation detection and is now being developed as a broader analytical framework for cancer detection, stratification, and longitudinal monitoring. Improvements in next-generation sequencing, assay chemistry, and computational analysis have increased analytical sensitivity, including in settings with low tumor fraction and very low variant allele abundance. These advances have expanded the utility of cfDNA analysis in measurable residual disease assessment and in the detection of low-abundance tumor-derived signals across multiple clinical contexts. **At** the same time, the field has shifted toward interpreting cfDNA as a carrier of higher-order biological information rather than solely a substrate for mutation calling. Fragmentation profiles, nucleosome positioning, and chromatin accessibility patterns derived from plasma DNA have been used to infer transcriptional and regulatory states, raising the possibility that cfDNA may capture functional tumor states not readily accessible through genotype-focused assays alone. **These** developments have prompted growing interest in chromatin-informed cfDNA analysis as a means of identifying pathway activity, enhancer usage, transcription factor occupancy, and potentially actionable biological dependencies. However, the translational relevance of many such inferences remains incompletely established. In this review, we examine the analytical advances underlying these approaches, assess the current evidence supporting their biological and clinical utility, and consider the extent to which cfDNA-derived regulatory inference may contribute to adaptive oncology and therapeutic decision-making.

Keywords: cfDNA; ctDNA; liquid biopsy; fragmentomics; epigenomics; DNA methylation; adaptive oncology; target discovery

1. Introduction

Liquid biopsy has substantially expanded the analytical toolkit available for cancer monitoring by enabling minimally invasive access to tumor-derived material in blood and other biofluids. Tumors release multiple analytes into circulation, including cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), circulating tumor cells, and extracellular vesicles. Collectively, these analytes can provide a dynamic view of disease that partly mitigates the sampling bias and temporal limitations inherent to tissue biopsy [1–4]. Among them, cfDNA has emerged as a central component of precision oncology because it can be repeatedly sampled and profiled for genomic, epigenomic, and structural features associated with tumor evolution and treatment response [2,3]. In this review, cfDNA refers to extracellular DNA recovered from plasma using standard isolation workflows, which predominantly capture nucleosome-associated fragments; extracellular vesicle-associated DNA is not specifically resolved in most current sequencing protocols and is therefore not considered separately.

The field was initially driven by the demonstration that circulating DNA could reveal tumor-derived genomic alterations, enabling minimal residual disease (MRD) assessment. With the introduction of ultra-deep sequencing, molecular barcoding, and duplex error-correction approaches, ctDNA assays achieved sensitivity sufficient for clinically relevant detection at very low allele fractions. These technical advances enabled practical applications in advanced disease,

recurrence monitoring, and increasingly, early detection [5–8]. Nevertheless, mutation-centered approaches provide only a partial view of tumor biology and are less informative in tumors shaped by epigenetic dysregulation, transcriptional plasticity, or adaptive resistance programs that arise without recurrent genomic alterations [9,10].

This limitation has motivated a broader interpretation of cfDNA. Plasma DNA retains structural and epigenetic features related to its chromatin origin, including information linked to nucleosome positioning, transcription factor occupancy, chromatin accessibility, and fragmentation topology [11–15]. cfDNA should therefore not be regarded solely as sequence material. Under appropriate analytical conditions, it may also function as a readout of chromatin organization and regulatory state.

Accordingly, fragmentomics has expanded from descriptive analysis of fragment size into a broader framework encompassing fragment-length distributions, fragment-end motifs, nucleosome phasing, regional fragmentation patterns, and methylation-associated cleavage signatures [13,14,21,22]. These features may provide access to functional aspects of tumor biology that are not readily captured by mutation profiling alone [9–12,14]. Artificial intelligence and machine learning have further accelerated this area by enabling multimodal integration of fragmentation, methylation, copy-number, and sequence-derived features for cancer detection, tissue-of-origin prediction, and biological interpretation [15–18,24]. Some recent models aim to move beyond classification by inferring regulatory activity, pathway dysregulation, and aspects of transcriptional state from cfDNA data [12,14,27–29].

A related and more speculative extension is the use of cfDNA-derived chromatin features to nominate candidate therapeutic vulnerabilities, particularly when integrated with CRISPR dependency maps, regulatory atlases, and orthogonal tumor profiling data [12,14,27,28]. At present, this does not justify treating cfDNA as a stand-alone target discovery platform. It does, however, support the view that cfDNA may serve as a useful discovery layer for generating and prioritizing mechanistically grounded therapeutic hypotheses.

In this review, we evaluate how cfDNA is being positioned for this broader role in oncology. We first consider the biological basis of cfDNA as an informational substrate, then examine multimodal and AI-enabled analytical strategies, and finally assess how genomic and epigenomic cfDNA features may be applied to resistance monitoring, patient stratification, target nomination, and treatment optimization. We also address the methodological and translational limitations that currently constrain the field.

2. Biological Foundations and Translational Utility of cfDNA in Adaptive Oncology

2.1. Biological Foundations of cfDNA as an Informational Substrate

2.1.1. Origins and Release Mechanisms

Most circulating cfDNA is thought to arise from apoptotic cells, in which endonuclease-mediated chromatin cleavage generates nucleosome-protected double-stranded DNA fragments associated with histones and other chromatin proteins [11,21,23]. As a result, a substantial fraction of plasma cfDNA exists as cell-free nucleosomes. This is a central observation because it provides the biological basis for interpreting cfDNA as a structured analyte rather than merely a degradation product.

Additional release mechanisms contribute further heterogeneity. Necrosis typically generates longer and more heterogeneous fragments because chromatin degradation is less orderly [2,30]. NETosis contributes extracellular chromatin derived from neutrophils and links cfDNA biology to inflammation, immune signaling, and thrombosis [33,34]. Active secretion through extracellular vesicles may also contribute DNA cargo, including nucleosomal and supercoiled forms, particularly in settings of inflammation or treatment-induced stress [2,23]. The plasma cfDNA pool should

therefore be understood as a composite output of multiple cell death and secretion processes rather than a uniform analyte.

2.1.2. Fragmentation Biology, Nucleosome Footprints, and Regulatory Information

cfDNA fragmentation is non-random and is shaped by chromatin architecture, nuclease activity, local DNA accessibility, and methylation state [11–13,21,22]. Genome-wide analyses have shown that plasma DNA preserves an in vivo nucleosome footprint: nucleosome-protected regions are relatively shielded from cleavage, whereas open chromatin regions are more accessible and generate shorter fragments with characteristic endpoint distributions [11–13]. Nuclease activity contributes an additional layer of specificity. DNASE1L3, for example, has been associated with C-rich fragment ends, while altered nuclease activity in cancer influences end-motif composition and motif diversity [21,22]. Preferential cleavage at methylated CpG sites further links fragmentation patterns to local epigenetic states.

At actively transcribed loci, nucleosome depletion near transcription start sites and phased fragmentation patterns in adjacent regions have been used to infer transcriptional activity directly from plasma DNA [12,14]. Collectively, these observations underpin fragmentomics and nucleosomics as analytical strategies that use fragment length, endpoints, end motifs, and nucleosome phasing to reconstruct regulatory landscapes from cfDNA [11–14,21–23]. Although the biological rationale is strong, the degree to which such inferences are robust across platforms, cohorts, and clinical settings remains an active question.

2.1.3. Distinctive Features of ctDNA and the Relevance of Epigenetic Signals

Tumor-derived cfDNA differs from background plasma DNA across several feature classes, including fragment size, end-motif composition, methylation state, chromatin accessibility signatures, and genomic content [13–16,21–23]. These differences may offer a richer representation of tumor biology than mutation profiling alone, particularly in cancers driven by epigenetic instability, lineage plasticity, or adaptive resistance mechanisms that do not depend on recurrent coding mutations [9,10,14–18].

However, biological richness should not be conflated with clinical utility. Only a subset of circulating molecules is tumor derived, and many high-dimensional cfDNA features are strongly influenced by tumor fraction, tissue admixture, preanalytical handling, library preparation, sequencing depth, and computational modeling choices [2,7,20,24,25]. Consequently, the field has produced increasingly detailed characterizations of fragment length, end motifs, nucleosome spacing, chromatin accessibility, and vesicle-associated DNA without yet achieving comparable gains in routine treatment decision-making. At present, the strongest clinical use cases remain mutation detection, methylation-based classification, and residual disease assessment. Many higher-order structural and epigenomic features are better regarded as biologically informative research characteristics than as validated clinical endpoints [15–20].

The critical issue is therefore not whether cfDNA can generate additional descriptors, but whether these descriptors can be converted into reproducible functional inferences—such as pathway activation, lineage transition, resistance state, or therapeutic vulnerability—that improve patient stratification or treatment selection. Until such relationships are prospectively demonstrated and analytically standardized, much of the apparent richness of cfDNA remains descriptive rather than decisional.

2.2. Multimodal cfDNA Analysis and AI/ML-Enabled Translation

2.2.1. Rationale for Multimodal Integration

Multimodal cfDNA analysis arises from a fundamental constraint: no single feature class adequately captures the complexity of tumor-derived signals in plasma, particularly in low-tumor-fraction settings. Current frameworks therefore integrate partially independent signals—including

DNA methylation, fragment size, fragment-end motifs, copy-number alterations, and somatic variants—within unified computational models [15–18]. In principle, this approach can improve analytical sensitivity when individual features are too weak or noisy to support stable inference on their own.

However, multimodal integration should not be assumed to confer greater biological insight or clinical utility. Combining analytes introduces additional technical variability, increases model complexity, and complicates attribution of model outputs to specific biological features. In practice, reported gains are often modest and highly dependent on preprocessing, cohort composition, and model training, raising the possibility that performance improvements reflect technical rather than biological integration [24].

The central translational question is therefore not whether additional cfDNA features can be combined, but whether multimodal models yield outputs that are reproducible, interpretable, and clinically actionable. At present, such advantages have not been consistently demonstrated in prospective or interventional settings [16–20,24].

2.2.2. Representative Multimodal Research Platforms

Several recent studies illustrate the analytical potential of multimodal cfDNA modeling. THEMIS demonstrated that enzyme-mediated methylome sequencing can preserve fragmentation information while simultaneously capturing whole-genome methylation, fragment size, fragment-end motifs, and copy-number alterations from a single cfDNA sample [15]. MESA extended this approach by incorporating chromatin-derived features, including nucleosome occupancy and window protection scores, supporting the view that plasma DNA retains structurally informative signatures of tumor chromatin organization [16]. Additional platforms, such as AlphaLiquid and SPOT-MAS, further suggest that integrated models combining methylation, copy number, and fragmentation may outperform single-feature approaches for cancer detection and tissue-of-origin prediction [17,18].

A conceptual extension of this work is that certain fragmentation-derived features may support functional interpretation rather than classification alone. For example, promoter fragmentation entropy and nucleosome-depleted region signals have been linked to inferred gene-expression states, raising the possibility that cfDNA may encode aspects of regulatory architecture in addition to tumor presence [14]. These observations are biologically informative, but most mechanistic applications remain investigational and require validation across independent datasets and clinical contexts.

2.2.3. Clinical Translation and Commercial Assays

Clinical translation of AI- and ML-based cfDNA analysis has advanced most clearly in cancer detection and tissue-of-origin classification. Its role in treatment selection or target nomination remains substantially less developed. Galleri, developed through the CCGA and PATHFINDER programs, is a prominent example of a methylation-based multi-cancer early detection assay that relies on machine-learning classification [19,20]. In colorectal cancer screening, Shield and the PREEMPT CRC validation study further demonstrate that blood-based classification frameworks can be deployed in large prospective settings [31,32].

By contrast, multimodal platforms such as THEMIS and MESA remain primarily research-oriented. Their principal contribution has been to show that a single cfDNA dataset can encode multiple layers of biologically relevant information, including methylation, fragmentation, copy-number changes, and chromatin-related structure [15,16]. However, this analytical breadth has not yet translated into treatment-directing applications supported by comparable prospective validation.

Accordingly, the more meaningful distinction in the field is not simply between unimodal and multimodal assays, but between platforms optimized for clinically validated detection and those designed to extract richer biological information that may eventually support adaptive oncology. The latter remain conceptually important but largely investigational [15,16,19,20].

2.3. Mining Genomic and Epigenomic cfDNA Features for Adaptive Oncology

2.3.1. Resistance Monitoring Through Serial cfDNA Profiling

A core objective of adaptive oncology is to modify treatment according to the evolving molecular state of the tumor rather than relying solely on baseline tissue profiling. cfDNA is particularly well suited to this application because it enables repeated, minimally invasive monitoring of tumor-derived genomic and epigenomic signals over time [2,5,20]. This practical advantage has been a major driver of its clinical adoption.

Importantly, plasma cfDNA mutation analysis is no longer confined to exploratory use. In selected settings, professional recommendations and disease-specific clinical literature support its use when tumor tissue is unavailable, insufficient, or impractical to re-biopsy [42,43]. This establishes a defined role for cfDNA in real-world molecular genotyping for certain cancers, albeit in a context-dependent manner.

Its clearest clinical utility lies in the serial detection of resistance-associated alterations. Such changes may emerge before or near the time of overt progression and can therefore inform earlier therapeutic intervention than conventional imaging or repeat tissue biopsy alone [2,5]. Representative examples include ESR1 mutation monitoring in hormone receptor-positive metastatic breast cancer, prospectively demonstrated in PADA-1 [38]; plasma detection of EGFR T790M to guide osimertinib therapy in EGFR-mutant non-small cell lung cancer [39]; emergent KRAS-mediated resistance during anti-EGFR therapy in metastatic colorectal cancer [40]; and BRCA1/2 reversion mutations associated with resistance to platinum therapy or PARP inhibition [41].

These examples support the use of cfDNA as a practical readout of molecular escape mechanisms and, in selected contexts, as a tool for therapy switching or rational combination strategies. Nonetheless, the strength of evidence remains uneven across tumor types and therapeutic settings, and plasma findings do not uniformly obviate the need for tissue confirmation.

2.3.2. Copy-Number, Structural Variant, and Synthetic Lethality Inference

The contribution of cfDNA to adaptive oncology extends beyond single-nucleotide variants. Plasma sequencing can identify clinically relevant copy-number alterations, amplifications, structural rearrangements, gene fusions, and subclonal drivers that emerge during metastatic progression or treatment [2,44,45].

Examples include ERBB2 or MET amplification and oncogenic kinase rearrangements, which may refine patient stratification and uncover treatment opportunities not captured by limited genotyping panels [44,45]. cfDNA profiling may also contribute to synthetic-lethal inference. Detection of BRCA1/2 alterations remains relevant to PARP inhibitor selection, whereas BRCA1/2 reversion mutations can signal acquired resistance and loss of therapeutic vulnerability [41].

However, the broader translational value of structural and copy-number inference still depends on sufficient tumor fraction, assay sensitivity, analytical standardization, and the limited availability of prospective evidence that these findings improve treatment assignment beyond established mutation-focused workflows [2,41,44,45].

2.3.3. Epigenomic Inference

Epigenomic cfDNA profiling extends adaptive oncology beyond mutation tracking by attempting to capture tumor states not adequately explained by genotype alone [14–18]. Methylome- and fragmentome-derived features may, in principle, reflect pathway activation, tumor-suppressor silencing, lineage plasticity, epithelial-to-mesenchymal transition programs, and dependence on chromatin regulators. This is particularly relevant when resistance is driven by transcriptional reprogramming or lineage switching rather than newly acquired coding mutations [10,46,47].

Although the biological rationale is compelling, the translational significance of such inferences remains uncertain. Combined analyses of methylation, nucleosome organization, and fragmentation

can reveal meaningful tumor states, but the extent to which these features can be converted into robust treatment-directing biomarkers has not yet been established [15–20].

A more plausible near-term role for epigenomic cfDNA lies in risk stratification and therapeutic hypothesis generation. In this context, cfDNA-derived patterns may help identify lineage switching, therapy-associated dedifferentiation, or chromatin-regulator dependence, which can then be integrated with orthogonal data to refine patient selection and guide follow-up investigation [14–16,46,47].

2.3.4. cfDNA as a Real-Time Therapeutic Control System

This treatment-directed perspective reframes liquid biopsy as more than a diagnostic assay and positions cfDNA as a potential real-time therapeutic monitoring system. Within this framework, methylation programs may suggest sensitivity to targeted or epigenetic therapies, chromatin accessibility signatures may inform immunotherapy responsiveness, and serial cfDNA measurements may reveal emerging resistant subclones or epigenetic state transitions before they become radiographically apparent [14–18,20].

The near-term translational objective is therefore not limited to disease detection, but extends to dynamic treatment optimization through repeated cfDNA-guided assessment. Whether this concept can be implemented reliably in routine clinical practice remains uncertain. Even so, the shift in perspective is important because it recasts cfDNA from a passive marker of disease burden into a potentially actionable tool for therapeutic control.

The principal cfDNA signal classes discussed in this review, together with the biology they may capture, their potential translational relevance, and their current evidentiary maturity, are summarized in Table 1.

Table 1. cfDNA feature classes, inferred biology, and translational relevance for adaptive oncology and therapeutic hypothesis generation.

cfDNA feature class	Inferred biological information	Potential translational applications	Current evidence maturity	Major limitations	References
Somatic mutations	Tumor genotype, emerging resistance alterations, clonal evolution	Therapy selection, serial resistance monitoring, measurable residual disease assessment	Clinically established in selected settings	Limited by low tumor fraction, assay sensitivity, clonal hematopoiesis, and inability to capture non-mutational resistance states	[2,5,7,20,38–43]
Copy-number alterations and amplifications	Structural instability, focal amplification events, subclonal genomic evolution	Patient stratification, resistance profiling, context-dependent inference of pathway activation	Clinically relevant but less standardized than mutation testing	Requires sufficient tumor fraction; plasma CNA calls may be noisy; prospective treatment-directing evidence remains limited	[2,41,44]
Structural variants and gene fusions	Rearrangements, oncogenic fusions, and other structural drivers	Detection of actionable rearrangements, resistance profiling, complementary genotyping when tissue is limited	Useful in selected contexts	Analytical sensitivity varies by assay design; structural resolution may remain incomplete in plasma; broader	[2,39,45]

Fragment size, fragment ends, and fragmentation topology	Nuclease activity, chromatin accessibility, tumor-associated fragmentation patterns	Cancer detection, tissue-of-origin modeling, exploratory biological inference	Strong analytical evidence, but limited routine clinical decision utility	standardization is needed Highly sensitive to preanalytical variables, sequencing workflow, and computational modeling; biological interpretation is often indirect Signals are indirect and require computational inference; prospective validation for treatment direction remains limited	[13,21–25]
Nucleosome positioning and promoter fragmentation features	Chromatin organization, nucleosome depletion, inferred transcriptional activity	Functional-state inference, pathway nomination, exploratory therapeutic hypothesis generation	Research-stage	Rich biological information does not yet consistently translate into actionable therapeutic decisions	[11,12,14,27,28]
DNA methylation patterns	Lineage identity, tissue of origin, tumor class, silencing states, epigenomic reprogramming	Multi-cancer early detection, classification, risk stratification, exploratory therapeutic redirection	Clinically advanced for detection and classification; investigational for treatment direction	Vulnerable to overfitting, data leakage, confounding, batch effects, and weak cross-cohort generalization	[15–20,31,32]
Multimodal integrated cfDNA models	Composite tumor state derived from combined methylation, fragmentation, CNA, and mutational signals	Improved classification, adaptive-oncology modeling, exploratory target nomination frameworks	Promising, but not yet treatment-directing in routine practice		[15–18,24,46–48]

This table summarizes the major categories of cfDNA-derived signals discussed in this review, the biology they may capture, their potential clinical or drug-discovery relevance, and their current evidentiary maturity. Not all signal classes carry equivalent translational weight. Somatic mutation profiling and selected serial ctDNA applications are already clinically useful in defined settings, whereas many fragmentation-, chromatin-, methylation-, and multimodal AI-derived features remain primarily research-stage and are more appropriately viewed as tools for biological inference, target nomination, or therapeutic hypothesis generation than as stand-alone treatment-directing biomarkers.

2.3.5. Target Nomination

A more ambitious extension of the field is the proposition that cfDNA may contribute to target discovery. Framed cautiously, this is plausible. cfDNA can reveal tumor states associated with actionable dependencies, including pathway activation, lineage switching, enhancer engagement, and transcription factor programs not evident from mutation analysis alone [12,14,27,28].

However, cfDNA is better regarded as a prioritization tool rather than a stand-alone discovery platform. Its value lies in narrowing the search space by identifying candidate vulnerabilities that can then be evaluated through integration with CRISPR dependency maps, tumor profiling, and functional validation.

2.4. Future Directions: From Multimodal Biomarkers to Therapeutic Decision Engines

The translational landscape of cfDNA currently spans a spectrum from clinically established mutation-based applications to research-stage efforts aimed at functional inference and therapeutic guidance. Most target-related uses remain provisional and continue to require orthogonal validation, as summarized in Table 1.

2.4.1. Foundation-Style Multimodal Models

The next generation of cfDNA models are increasingly being developed to be multimodal by design, integrating methylation, fragmentomics, copy number, mutations, and clinical variables within unified analytical frameworks [15–18]. The objective is not simply to improve binary classification, but to align complementary biological signals with clinically relevant outputs spanning diagnosis, prognosis, resistance emergence, and likely treatment sensitivity [15,16].

A logical extension of this trend is the development of foundation-style models trained on large cfDNA datasets. In principle, such systems could generate integrated predictions across several clinically relevant dimensions rather than a single narrow endpoint. However, their value will depend not only on predictive performance, but also on interpretability, biological robustness, and prospective validation.

If such frameworks mature, they may support patient stratification, improve pharmacodynamic monitoring, enrich biomarker-driven trials, and help prioritize rational combination strategies [17–20]. At present, however, these applications remain aspirational rather than established.

2.4.2. Integration into Biomarker-Driven Clinical Trials

An important next step is the integration of genomic and epigenomic cfDNA features into biomarker-driven clinical trials [17–20,31,32]. These readouts could improve patient selection, enable earlier identification of likely responders or non-responders, and support the use of molecular residual disease or early plasma ctDNA response as intermediate endpoints.

If validated prospectively, this approach could make trials smaller, faster, and more biologically focused while improving alignment between therapy mechanism and enrolled population [19,20,31,32]. However, its utility will depend on assay reproducibility, interpretability, and evidence that cfDNA-based readouts improve trial decisions rather than merely add correlates.

2.5. Pitfalls

Confounding and batch effects further complicate interpretation. Cases and controls may differ not only in cancer status, but also in collection site, preanalytical handling, sequencing chemistry, and clinical characteristics. As a result, classifiers may capture technical or demographic artifacts rather than tumor biology. These issues are particularly pronounced in retrospective and multicenter datasets lacking harmonization and independent validation [24,46–48].

More broadly, prospective evidence demonstrating that AI-guided cfDNA strategies improve clinical outcomes remains limited. Many studies are retrospective or case-control in design, and the gap between analytical performance and treatment-directed utility remains substantial [19,20,24].

2.6. Conclusions

At present, the strongest clinical validation remains in detection and classification. The more consequential long-term opportunity lies in adaptive oncology, where genomic and epigenomic cfDNA features may eventually support resistance monitoring, patient stratification, and treatment redirection [16,24].

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