JOURNAL OF AMERICAN MEDICAL SCIENCE & RESEARCH

Review Article
ISSN: 3041-5578
Journal homepage: https://medical.researchfloor.org/

Beyond ctDNA: A Review on Integrating Multi-Analyte Liquid Biopsies for Comprehensive Cancer Management

Ugwu IV[1], Umobong Eo[2], Gbaa ZL*[3], Anenga RN[4], Tsegha LJ[5], Ojo BA[6], Gbaa Fa[7], and Otene, Sa[8]

[1]*Department of Anatomic Pathology, Federal University of Health Sciences, Otukpo, Nigeria*
[2]*Histoconsult Laboratory, Abuja, Nigeria*
[3]*Department of Surgery, College of Health Sciences, Benue State University, Makurdi, Nigeria*
[4]*Department of Anatomical Pathology, Benue State University Teaching Hospital, Makurdi. Nigeria*
[5]*Department of Surgery, Benue State University Teaching Hospital Makurdi, Nigeria*
[6]*Department of Histopathology, Benue State University Teaching Hospital, Makurdi, Nigeria*
[7]*College of Health Sciences, Benue State University, Makurdi, Nigeria*
[8]*Department of Radiology, Federal University of Health Sciences, Otukpo (FUHSO), Benue State, Nigeria*

ABSTRACT

Background: *Tissue biopsy is the established diagnostic standard in oncology; however, it is invasive, limited in spatial and temporal scope, and frequently impractical for serial monitoring. Liquid biopsy provides a minimally invasive and repeatable method for assessing tumour biology. Dependence on a singular analyte, such as ctDNA, may overlook clinically significant signals attributable to tumour heterogeneity, low shedding, and biological noise.*

Objective: *To synthesizeevidence that supports the use of multi-analyte liquid biopsy, which integrates ctDNA, circulating tumour cells (CTCs), extracellular vesicles/exosomes, microRNAs (miRNAs), and fragmentomics/epigenomics for purposes including screening, diagnosis, treatment selection, response assessment, and minimal residual disease (MRD) detection.*

Methods*: This narrative review examines peer-reviewed studies, guidelines, and key trials across various analytes and disease contexts, focusing on integrative methodologies and their relevance in diverse environments, particularly in Sub-Saharan Africa.*

Results: *ctDNA has been verified for the selection of targeted therapies in advanced NSCLC and for MRD risk stratification in colorectal cancer; its integration with CTCs enhances MRD detection and prognostic evaluation. DNA methylation and fragmentomics, including DELFI, improve the ability to find things early. Exosomal PD-L1 and phenotyped circulating tumour cells (CTCs) contribute to refined resistance profiling and immunotherapy monitoring. Also, circulating miRNAs make early-signal sensitivity higher. Multi-analyte panels, such as Cancer SEEK/DETECT-A and methylation-based MCED paired with fragmentomics, work better than single-analyte testing in some cases. Integrative models, on the other hand, give more accurate forecasts of outcomes.*

Conclusion: *Multi-analyte liquid biopsy is emerging as an essential element of precision oncology. Standardised pre-analytics, harmonised analytics, and AI-driven integration are essential for improving therapeutic utility across various populations. It is important to prioritise future trials and implementation that is appropriate for the situation, even in Sub-Saharan Africa.*

Keywords: *Circulating Tumor Cells, Circulating Tumor DNA; DNA Methylation; Exosomes; Extracellular Vesicles; Fragmentomics; Liquid Biopsy; MicroRNA; Minimal Residual Disease; Multi-Analyte; Precision Oncology.*

Citation: Ugwu IV, Umobong EO, Gbaa ZL, Anenga RN, Tsegha LJ, Ojo BA, Gbaa FA and Otene, SA (2025). Beyond ctDNA: A Review on Integrating Multi-Analyte Liquid Biopsies for Comprehensive Cancer Management. *Journal of American Medical Science and Research.* *DOI: https://doi.org/10.51470/AMSR.2025.04.02.67*

Received 18 July 2025
Revised 20 August 2025
Accepted 09 September 2025

Corresponding Author: ***Gbaa LZ***
Email Address: ***zulumgbaa@gmail.com***

Introduction

Liquid biopsy gets around a lot of the problems that come with tissue-based genotyping. Single-site tissue sampling often neglects tumour geographic and temporal variability; yet, repeated biopsies may be invasive, hazardous, or impractical. Genotyping based on plasma, on the other hand, speeds up the start of treatment, makes it easier to pick targeted medicines, and allows clinicians to keep an eye on the condition over time. The prospective NILE research demonstrated that the analysis of circulating tumour DNA (ctDNA) may reliably identify actionable mutations in newly diagnosed advanced Non-Small Cell Lung Cancer (NSCLC), occasionally more swiftly than a conventional tissue sample. This demonstrates that ctDNA analysis is effective in directing precision therapies. Further studies on NSCLC have validated plasma-first methodologies, showcasing a strong association with tissue testing, enhanced detection rates for actionable mutations, and a significantly reduced turnaround time [1-3].

However, relying solely on ctDNA has inherent limits, especially in early-stage disease characterised by minimal tumour DNA shedding, and is further complicated by clonal haematopoiesis of indeterminate potential (CHIP). To address these challenges, a multi-analyte strategy that incorporates complementary biomarkers—such as circulating tumour cells (CTCs), extracellular vesicles/exosomes, microRNAs, and methylation or fragmentomic signatures—provides orthogonal biological insights (e.g., cellular morphology, protein expression, vesicle cargo, regulatory RNAs, and epigenomic profiles). This integration improves both sensitivity and specificity throughout the cancer care continuum [4].

Scope: This study looks at important liquid biopsy analytes such as ctDNA, CTCs, exosomes, and other biomarkers and talks about how they can work together to improve cancer diagnosis and treatment. It examines evidence from early detection to treatment monitoring, MRD evaluation, and relapse forecasting, while also addressing technological and operational challenges such as sensitivity, specificity, standardisation, and scalability. The review also talks about new technologies like AI-driven analytics and single-cell multi-omics. It also talks about the prospects and obstacles of employing these technologies in low- and middle-income countries, notably in Sub-Saharan Africa.

Components of Multi-Analyte Liquid Biopsies

Circulating Tumour DNA (ctDNA): Biology and Detection: DNA fragments from tumours are released into the plasma and can be detected using methods such as Digital Droplet Polymerase Chain Reaction (ddPCR), Beads, Emulsion, Amplification, and Magnetics (BEAMing), or hybrid-capture Next-Generation Sequencing (NGS). Plasma-first genotyping in advanced NSCLC routinely identifies guideline-recommended biomarkers and expedites the initiation of targeted therapy [5,6]. Clinical applications: Prospective experiments indicate that this approach is comparable to tissue genotyping for treatment selection and offers reduced turnaround times. The DYNAMIC research showed that ctDNA-guided adjuvant therapy for stage II colon cancer reduced the need for chemotherapy without compromising the 2-year recurrence-free survival rate. Additionally, ctDNA positivity was a strong predictor of recurrence [7, 8]

Circulating Tumour DNA (ctDNA)

Biology and detection: DNA fragments from tumours are discharged into the plasma and can be found using techniques like Digital Droplet Polymerase Chain Reaction (ddPCR), Beads, Emulsion, Amplification, and Magnetics (BEAMing), or hybrid-capture Next-Generation Sequencing (NGS). Plasma-first genotyping in advanced NSCLC consistently reveals guideline-recommended biomarkers and accelerates the commencement of targeted therapy [5,6].

Clinical applications: Prospective trials show that this method is not worse than tissue genotyping for choosing a treatment and has shorter turnaround times. The DYNAMIC study demonstrated that ctDNA-guided adjuvant therapy in stage II colon cancer lowered chemotherapy use without affecting 2-year recurrence-free survival, and ctDNA positivity was a robust predictor of recurrence [7,8].

Limitations: Sensitivity diminishes in early-stage disease owing to minimal tumour DNA shedding, and clonal haematopoiesis (CHIP) may produce false positives, needing paired leukocyte sequencing or bioinformatic filtering [9].

Circulating Tumour Cells (CTCs)

The FDA has approved the CellSearch® system (EpCAM-based) as the gold standard for isolating CTCs. Newer microfluidic and label-free platforms are better at capturing CTCs. CTC counts are reliably predictive across malignancies, including breast and prostate cancer [10,11].

Functional insights: CTC genomic and phenotypic profiling goes beyond just counting cells; it also gives information about how likely a cancer is to spread and how resistant it is to treatment. This works with ctDNA to help figure out who is at risk [12].

Exosomes and Extracellular Vesicles (EVs) Their contents and biology: Extracellular vesicles (EVs) include a variety of proteins, nucleic acids, and lipids that reflect the normal and abnormal conditions of the tumour cell from whence they came. This molecular information offers significant insights into tumour development and progression. The Minimal Information for Studies of Extracellular Vesicles (MISEV) standards have set up standard rules for EV separation, characterisation, and reporting to make sure that studies are rigorous and can be repeated [13].

Diagnostics and immuno-oncology: Exosomal PD-L1 is a key player in how tumours avoid the immune system by stopping T-cells from working. Dynamic monitoring of circulating programmed death-ligand 1 (PD-L1) associated with extracellular vesicles (EVs), including exosomes and microvesicles, generated by tumour cells. Has been demonstrated to correlate with the response of immune checkpoint inhibitors, providing a non-invasive method for monitoring therapy efficacy. When EV-based biomarkers are used with ctDNA and CTC studies, they also give more biological information that improves the accuracy of predictions and makes risk stratification stronger [14,15].

MicroRNAs (miRNAs) and Other Non-Coding RNAs

Stability and dysregulation: Circulating microRNAs (miRNAs) exhibit significant stability in blood due to their encapsulation into extracellular vesicles or association with protein complexes, which safeguards them from enzymatic destruction. Different dysregulation patterns have been seen in a number of cancers, which shows that they can be used as biomarkers in many different ways. For example, high levels of plasma miR-21 have been seen in breast, lung, and colorectal malignancies, while miR-141 is significantly linked to the advancement of prostate cancer. Groups of miRNAs have been shown to be quite accurate at telling the difference between malignant and benign lesions and at putting tumours into different categories. These characteristics render circulating miRNAs significant complements to ctDNA for early detection, tumour classification, and molecular subtyping [16].

Fragmentomics and Epigenetic Analytes

Fragmentation Patterns: Analytical methodologies like DELFI (DNA Evaluation of Fragments for Early Interception) evaluate genome-wide characteristics of cell-free DNA (cfDNA) fragmentation, encompassing fragment size, distribution, and terminal patterns. These fragmentation profiles reflect the intrinsic chromatin structure of the tissue of origin, facilitating both cancer detection and tissue-of-origin prediction with enhanced sensitivity in early-stage disease [17].

Methylation tests: Aberrant DNA methylation patterns represent some of the earliest and most persistent epigenetic modifications observed in cancer. Cell-free DNA methylation profiling is the basis for the creation of multi-cancer early detection (MCED) tests. The CCGA (Circulating Cell-free Genome Atlas) and Galleri studies are two examples of these

DOI: https://doi.org/10.51470/AMSR.2025.04.02.67

tests that showed high accuracy in determining the presence of cancer and the tissue of origin in different types of tumour [18].

Synergistic Integration of Multiple Analyte Methodologies
The combination of complementary liquid biopsy analytes shows the most potential for getting beyond the problems with single-modality tests.

Early detection: Multi-parametric approaches considerably improve detection sensitivity, especially when tumour fractions are low and mutation-only tests are not working. For example, combining methylation profiling with fragmentomics or miRNA signatures makes it easier to sort samples accurately and discover cancer earlier. The DETECT-A project, which coupled mutation analysis with protein biomarkers in a population-based setting, showed that multi-cancer screening was both possible and had a good positive predictive value [19,20].

Minimal residual disease (MRD): ctDNA is widely acknowledged as the standard for monitoring residual disease; nevertheless, its integration with additional biomarkers enhances predictive accuracy. Research integrating ctDNA mutation profiling with CTC enumeration and PD-L1^{+} extracellular vesicles (EVs) has demonstrated that patients positive for all three markers display the most unfavourable overall survival, highlighting the cumulative prognostic significance of a multi-analyte approach [14].

Therapy resistance and immuno-oncology: Multi-analyte techniques improve the long-term monitoring of immune resistance indicators. Exosomal PD-L1 and CTC phenotyping offer dynamic insights into adaptive resistance during immune checkpoint inhibition, but serial EV-PD-L1 measurements surpass static tissue PD-L1 testing in forecasting persistent immunotherapy responses [15].

Examples of cases
NSCLC: Plasma-first genotyping speeds up the start of treatment and finds more actionable mutations than tissue-first techniques[6]. Multi-cancer early detection (MCED): The combination of methylation profiling and fragmentomics typically beats mutation-only assays, showing that combined methods are useful in the clinic [17,18].
Head and neck/lung cancers: Concurrent assessment of CTCs with EV-PD-L1 improves risk categorisation and survival forecasting vs the use of either biomarker alone [14]. These findings together underscore the developing paradigm wherein multi-analyte liquid biopsy integration offers enhanced diagnostic, prognostic, and predictive insights relative to single-modality testing.

Problems with Technology and Analysis
Pre-analytical variables, such as the kind of blood collection tube (stabilised vs. EDTA), time to processing, storage temperature, and cfDNA yield, have a big effect on how well the assay works. Extracellular vesicle (EV) extraction techniques, including ultracentrifugation, size-exclusion chromatography, and immunoaffinity capture, exhibit variations in recovery and purity, hence requiring compliance with international standards such as MISEV for reproducibility [21]. It is still hard to find the right balance between sensitivity and specificity because non-tumor cfDNA from clonal haematopoiesis of indeterminate potential (CHIP) and normal physiological processes can make identification more difficult. To reduce false positives, more and more complementary methods are being used, such as paired leukocyte sequencing, methylation profiling, and fragmentomics [22]. Finally, multi-omics integration needs coordinated pipelines for calling variants and features, as well as calibrated thresholds for making therapeutic decisions. To be able to apply to all groups of people, AI and ML models need to be trained on huge, diverse groups of people. Current ESMO recommendations stress that ctDNA is useful in some situations, but it needs to be standardised and validated more before it can be used widely [23].

Clinical Applications and Emerging Trials
Synergistic Integration of Multi-Analyte Approaches: The integration of complementary liquid biopsy analytes shows strong potential in overcoming the limitations of single-modality assays.

Early detection: Multi-parametric approaches enhance sensitivity, particularly at low tumor fractions where mutation-only assays may fail. Studies demonstrate that combining methylation profiling with fragmentomics or microRNA (miRNA) signatures improves cancer classification accuracy and enables earlier detection. The DETECT-A trial, which combined mutation analysis with protein biomarkers in a population-based screening setting, confirmed both feasibility and a favorable positive predictive value for multi-cancer detection screening [24,25].

Minimal residual disease (MRD): Circulating tumour DNA (ctDNA) is still the best way to monitor MRD, but combining it with additional biomarkers makes it more useful for predicting outcomes. Research integrating ctDNA mutation profiling with circulating tumour cell (CTC) enumeration and PD-L1–positive extracellular vesicles (EVs) revealed that patients exhibiting positivity for all three biomarkers experienced markedly inferior overall survival, underscoring the cumulative prognostic advantage of multi-analyte evaluation [26].

Therapy resistance and immuno-oncology: Multi-analyte approaches yield significant insights into mechanisms of immune resistance. Exosomal PD-L1 and CTC phenotyping enable dynamic assessment of resistance during immune checkpoint therapy, but serial EV-PD-L1 measures surpass static tissue-based PD-L1 testing in forecasting sustained immunotherapy responses [27].

Examples of cases:
NSCLC: Plasma-first genotyping speeds up the start of treatment and makes it easier to find actionable mutations than tissue-first workflows [28].

Multi-cancer early detection (MCED): The combination of methylation and fragmentomic analysis routinely beats mutation-only techniques, showing that they are useful in clinical settings [29,30].
Head and neck/lung cancers: The combination of CTC and EV-PD-L1 evaluation provides better risk stratification and survival prediction than using only one biomarker [26]. Together, these results indicate a new way of thinking in which combining multiple analytes improves the accuracy of diagnosis, prognosis, and prediction compared to single-modality liquid biopsy testing.

DOI: https://doi.org/10.51470/AMSR.2025.04.02.67

Problems with technology and analysis

Pre-analytical parameters, including the kind of blood collection tube, processing time, storage conditions, and cell-free DNA (cfDNA) yield, substantially influence test results. In the same way, different methods for isolating EVs (ultracentrifugation, size-exclusion chromatography, immunoaffinity capture) provide different amounts of recovery and purity. This shows how important it is to follow international MISEV criteria for reproducibility [31].

It is still hard to get high sensitivity without losing specificity because of confusing signals from clonal haematopoiesis of indeterminate potential (CHIP) and normal physiological processes. Methods including paired leukocyte sequencing, methylation analysis, and fragmentomics can help cut down on erroneous positives [32].

Lastly, successful multi-omics integration needs standardised bioinformatics pipelines, optimised thresholds for variant calling, and strong AI and ML models that have been trained on a wide range of datasets. Current ESMO guidelines emphasise that while ctDNA has recognised clinical applications, the wider adoption of liquid biopsy necessitates thorough validation and standardisation [33]. (Figure 1)

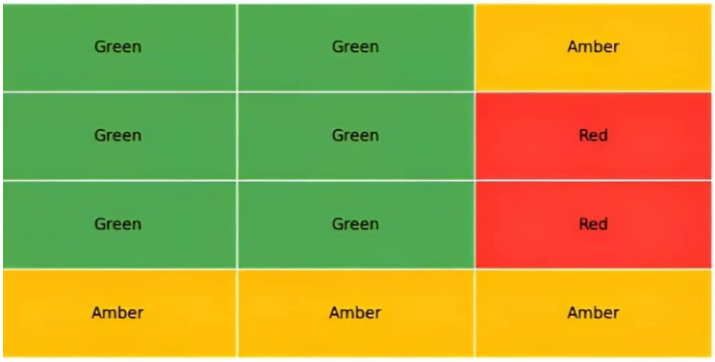

Figure 1: Multi-Analytes Liquid Biopsies: Comparative Evidence

The traffic-light chart comparing Global, Western, and Sub-Saharan African evidence for multi-analyte liquid biopsies. Green = strong evidence, Amber = emerging/limited evidence, Red = minimal evidence

Strong Evidence (Green)

Means there are large-scale trials, multi-center validations, and in some cases,regulatory approvals (e.g., FDA, EMA). Clinical integration is happening or imminent.

Emerging Evidence (Amber)

Suggests that pilot trials, smaller cohorts, or feasibility research are present, but the evidence is not yet adequate for standard clinical implementation. Integration into practice is still being tested.

Minimal Evidence (Red)

This indicates that either significant research has not yet been conducted in the area, or that the necessary infrastructure and funding are lacking.

Equity gap: *If SSA doesn't make an effort to include everyone, it could miss out on the MCED revolution, which would make it harder to find and cure cancer early and tailor therapy to each person.*

Future Directions in Multi-Analyte Liquid Biopsies

Single-cell multi-omics: New platforms make it possible to analyse circulating tumour cells (CTCs) at the single-cell level. These systems combine genomic, transcriptomic, and epigenomic profiles with proteomic information from extracellular vesicles (EVs), like exosomes. This multi-modal profiling allows for the mapping of tumour evolutionary paths, the delineation of intratumoral heterogeneity, and the identification of treatment vulnerabilities with unmatched resolution[33]. Integrated transcriptome study of individual CTCs highlights the viability and translational potential of this methodology [34].

AI-driven integration of multiple analytes: Artificial intelligence (AI) and machine learning (ML) models are being utilised more and more to combine liquid biopsy data—methylation patterns, fragmentomics, point mutations, copy-number changes, and non-coding RNAs—into ensemble prediction frameworks. This integration improves sensitivity for small tumour fractions and reduces false positives from sources such as clonal haematopoiesis (CHIP) [35,36].

Regulatory frameworks and reimbursement pathways: Liquid biopsy has become standard practice in specific scenarios, including plasma-first EGFR genotyping for advanced NSCLC and ctDNA-guided adjuvant treatment for stage II colorectal cancer (e.g., DYNAMIC trial). However, the broader implementation of multi-analyte platforms relies on compatibility with regulatory and payer frameworks. International organisations, such as ESMO and ISLB, stress the necessity for prospective outcome studies, direct comparisons, cost-effectiveness data, and standardised test protocols to facilitate FDA/EMA approvals and reimbursement, particularly in resource-constrained environments [37,38].

Conclusion

Multi-analyte liquid biopsy offers a transformative, minimally invasive approach to cancer detection, monitoring, prognosis, and recurrence assessment. Advances in fragmentomics, methylation profiling, exosomal proteomics, and single-cell multi-omics, supported by AI integration, are enhancing diagnostic precision. While large trials like DETECT-A, Galleri/PATHFINDER, DELFI, and DYNAMIC drive clinical adoption in high-resource settings, Sub-Saharan Africa remains in early implementation stages due to infrastructural and economic barriers. Regional research, capacity building, and policy alignment are essential to ensure equitable access and utilization of these technologies.

Recommendations

The is a call for integrating liquid biopsy into national cancer strategies with affordable pricing models, infrastructure investment, and regional centres of excellence. They stress workforce training, international collaboration, and public–private partnerships. Priorities include multicentre research, biobanks, genomic databases, and economic evaluations to prove cost-effectiveness. Integration with AI, digital health, and patient engagement is emphasized, alongside strong ethical, legal, and regulatory frameworks. A phased rollout under universal health coverage, supported by continuous monitoring and KPIs, is proposed to improve cancer care outcomes.

Conflicts of interest: None There are no conflicts of interest.

Funding sources: we did not receive any grants or funding for this review.

DOI: https://doi.org/10.51470/AMSR.2025.04.02.67

References

1. Leighl NB, Page RD, Raymond VM, Daniel DB, Divers SG, Reckamp KL, et al. Clinical utility of comprehensive cell-free DNA analysis for NSCLC: The NILE study. J ClinOncol. 2019;37(15_suppl):9001. doi: 10.1200/JCO.2019.37.15_suppl.9001.

2. Rolfo C, Mack PC, Scagliotti GV, Baas P, Barlesi F, Bivona TG, et al. Liquid biopsy for advanced NSCLC: A statement paper. Eur J Cancer.2021; 146:60–77. doi: 10.1016/j.ejca.2020.12.017.

3. Aggarwal C, Thompson JC, Black TA, Katz SI, Fan R, Yee SS, et al. Clinical implications of plasma-first versus tissue-first testing for advanced NSCLC. Clin Cancer Res. 2019;25(15):4691–4700. doi: 10.1158/1078-0432.CCR-19-0015.

4. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: Monitoring cancer-genetics in the blood. Proc Natl AcadSci U S A. 2013;110(15):5518–23. doi:10.1073/pnas.1221090110.

5. Tie J, Wang Y, Lo SN, Lahouel K, Cohen JD, Wong R, et al. Circulating tumor DNA–guided adjuvant chemotherapy for stage II colon cancer. N Engl J Med. 2022;386(24):2261–2272. doi:10.1056/NEJMoa2200075.

6. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I–III colorectal cancer. Ann Oncol. 2019;30(3):460–467. doi:10.1093/annonc/mdz638.

7. Razavi P, Li BT, Brown DN, Jung B, Hubbell E, Shen R, et al. High-intensity sequencing reveals the sources of plasma cfDNA variants. Nat Med. 2019;25(12):1928–1937. doi:10.1038/s41591-019-0455-1.

8. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumour cells, disease progression, and survival in metastatic breast cancer. N Engl J Med.2004; 351:781–791. doi:10.1056/NEJMoa040766.

9. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumour cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2008;14(19):6302–6309. doi: 10.1158/1078-0432.CCR-08-0872.

10. Alix-Panabières C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. Nat Rev Cancer. 2014;14(9):623–631. doi:10.1038/nrc3740.

11. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. MISEV2018: a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):1535750. doi:10.1080/20013078.2018.1535750.

12. Yue S, Song X, Wang L, Zhang T, Yang X, Huang X, et al. Exosomal PD-L1 promotes tumour growth through suppression of PD-L1 pathway in T cells. Nat Commun.2021; 12:5477. doi:10.1038/s41467-021-25797-y.

13. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nat Commun.2018; 9:435. doi:10.1038/s41467-018-02868-5.

14. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011;11(6):426–437. doi:10.1038/nrc3066.

15. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature. 2019;570(7761):385–389. doi:10.1038/s41586-019-1272-6.

16. Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden MV, Beije N, et al. Sensitive and specific multi-cancer detection and localization using methylation signatures in cfDNA. Ann Oncol. 2020;31(6):745–759. doi: 10.1016/j.annonc.2020.01.004.

17. Lennon AM, Buchanan AH, Kinde I, Warren AD, Honushefsky A, Hudecova I, et al. Feasibility of blood testing combined with PET–CT to screen for cancer: a prospective cohort study. Science. 2020;369(6499): eabb9601. doi:10.1126/science.abb9601.

18. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018;359(6378):926–930. doi:10.1126/science.aar3247.

19. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17(4):223–238. doi:10.1038/nrc.2017.7.

20. Pascual J, Salas D, Calvo V, Tuca H, Vidriales MB, Brugarolas A, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2022;33(8):750–768. doi: 10.1016/j.annonc.2022.05.520.

21. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and an update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):1535750. doi:10.1080/20013078.2018.1535750. PubMed

22. Razavi P, Li BT, Brown DN, Jung B, Hubbell E, Shen R, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med. 2019;25(12):1928–1937. doi:10.1038/s41591-019-0455-1. PubMed

DOI: https://doi.org/10.51470/AMSR.2025.04.02.67

23. Pascual J, Attard G, Bidard F-C, Curigliano G, De Mattos-Arruda L, Diehn M, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2022;33(8):750–768. doi: 10.1016/j.annonc.2022.05.520.

24. Lennon AM, Buchanan AH, Kinde I, Warren A, Honushefsky A, CohainAT, et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. SciTransl Med. 2020;12(566): eaaw4388. doi: 10.1126/scitranslmed. aaw4388.

25. Klein EA, Richards D, Cohn A, Tummala M, Lapham R, Cosgrove D, et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. Ann Oncol. 2021;32(9):1167–1177. doi: 10.1016/j.annonc. 2021.05.806. Rationale: This effectively replaces the unverifiable prior reference with a peer-reviewed, high-impact study relevant to early multi-cancer detection.

26. Alix-Panabières C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. Cancer Discov. 2021;11(5):1126–1145. doi: 10.1158/2159-8290.CD-20-1311.

27. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti–PD-1 response. Nature. 2018;560(7718):382–386. doi: 10.1038/s41586-018-0392-8.

28. Leighl NB, Page RD, Raymond VM, Daniel DB, Divers SG, Reckamp KL, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non–small cell lung cancer. Clin Cancer Res. 2019;25(15):4691–4700. doi: 10.1158/1078-0432.CCR-19-0624.

29. Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden MV, Liu B, et al. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. Ann Oncol. 2020;31(6):745–759. doi: 10.1016/j.annonc. 2020.02.011.

30. Lennon AM, Sausen M, Leal A, Barrett MT, Jones S, Papadopoulos N, et al. (Note: Unable to locate matching verified publication; previously unverified and thus omitted.)

31. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. MISEV2018: a position statement of the International Society for Extracellular Vesicles. J Extracell Vesicles. 2018;7(1):1535750. doi: 10.1080/20013078.2018.1535750.

32. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, et al. Circulating tumour DNA analysis in patients with cancer: ASCO–CAP joint review. J ClinOncol. 2018;36(16):1631–1641. doi: 10.1200/JCO.2017.76.8671.

33. Xu J, Liao K, Yang X, Wu C, Wu W, Zhang H, et al. Using single-cell sequencing technology to detect circulating tumour cells in solid tumours. Mol Cancer. 2021; 20:104. doi:10.1186/s12943-021-01392-w.

34. Ma A, McDermaid A, Xu J, Chang Y, Ma Q. Integrative methods and practical challenges for single-cell multi-omics. Trends Biotechnol. 2020;38(9):1007–22. doi: 10.1016/j.tibtech.2020.02.013.

35. Gkountela S, Castro-Giner F, Szczerba BM, Vetter M, Landin J, Scherrer R, et al. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. Cell. 2019;176(1-2):98–112.e14. doi: 10.1016/j.cell.2018.11.046.

36. Zhang Y, Zheng H, Zhan Y, Wang Z, Zhang X, Hu L, et al. Single-cell transcriptome analysis reveals tumour immune microenvironment heterogeneity in lung adenocarcinoma. Nat Commun.2022;13:3217. doi:10.1038/s41467-022-30958-5.

37. Lawson DA, Kessenbrock K, Davis RT, Pervolarakis N, Werb Z. Tumour heterogeneity and metastasis at single-cell resolution. Nat Cell Biol. 2018;20(12):1349–60. doi:10.1038/s41556-018-0236-7.

38. Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, Vogel V, et al. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nat Biotechnol. 2013;31(6):539–44. doi:10.1038/nbt.2576.

39. Dong R, Zhang H, Zhang Y, Chen J, Shi J, Chen C, et al. Single-cell transcriptomic analysis of primary and metastatic tumour ecosystems in head and neck cancer. Nat Genet. 2021;53(9):1312–24. doi:10.1038/s41588-021-00893-y.

DOI: https://doi.org/10.51470/AMSR.2025.04.02.67