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REVIEW

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Exploring the potential of multiomics liquid biopsy testing in the clinical setting of lung cancer

Andrea Gottardo[1] | Tancredi Didier Bazan Russo[1] | Alessandro Perez[1] | Marco Bono[1] | Emilia Di Giovanni[1] | Enrico Di Marco[1] | Rita Siino[1] | Carla Ferrante Bannera[1] | Clarissa Mujacic[1] | Maria Concetta Vitale[1] | Silvia Contino[1] | Giuliana Ianni[1] | Giulia Busuito[1] | Federica Iacono[2] | Lorena Incorvaia[1] | Giuseppe Badalamenti[1] | Antonio Galvano[1] | Antonio Russo[1] | Viviana Bazan[3] | Valerio Gristina[1]

[1]Department of Precision Medicine in Medical, Surgical and Critical Care (Me. Pre.C.C.), University of Palermo, Palermo, Italy

[2]A.R.N.A.S. Hospital Di Cristina, Palermo, Italy

[3]Department of Biomedicine, Neuroscience and Advanced Diagnostic (Bi.N.D.), University of Palermo, Palermo, Italy

Correspondence
Antonio Russo, Department of Precision Medicine in Medical, Surgical and Critical Care (Me.Pre.C.C.), "P. Giaccone" University Hospital (A.O.U.P.) of Palermo, Via del Vespro 129, 90127 Palermo, Italy.
Email: antonio.russo@usa.net

Abstract

The transformative role of artificial intelligence (AI) and multiomics could enhance the diagnostic and prognostic capabilities of liquid biopsy (LB) for lung cancer (LC). Despite advances, the transition from tissue biopsies to more sophisticated, non-invasive methods like LB has been impeded by challenges such as the heterogeneity of biomarkers and the low concentration of tumour-related analytes. The advent of multiomics – enabled by deep learning algorithms – offers a solution by allowing the simultaneous analysis of various analytes across multiple biological fluids, presenting a paradigm shift in cancer diagnostics. Through multi-marker, multi-analyte and multi-source approaches, this review showcases how AI and multiomics are identifying clinically valuable biomarker combinations that correlate with patients' health statuses. However, the path towards clinical implementation is fraught with challenges, including study reproducibility and lack of methodological standardization, thus necessitating urgent solutions to solve these common issues.

KEYWORDS
artificial intelligence, biomarkers, deep learning, liquid biopsy, lung neoplasm, multiomics

1 | INTRODUCTION

Lung cancer (LC) remains a formidable health challenge, ranking as the second most common cancer by incidence and the leading cause of cancer-related mortality according to recent statistics.[1] Despite significant technological advancements over the past decades, tissue biopsy has largely remained the diagnostic gold standard since the late 1990s.[2] However, the National Lung Screening Trial (NLST) of 2011[3] illuminated a path forward, demonstrating a 20% reduction in LC mortality with the adoption of low-dose computed tomography (LDCT) over chest radiography for population screening. Although LDCT has become a widely accepted method for early-stage LC detection in numerous countries, its high false-positive rate has imposed substantial time and resource burdens on national healthcare systems (NHS).

In response, institutions such as the International Society of Liquid Biopsy (ISLB)[4] have begun exploring liquid biopsy (LB) for its potential to revolutionize LC detection. LB offers a promising alternative to classical diagnostic methods, boasting reduced invasiveness, cost and turn-around times (TAT) times,[5] while maintaining

Andrea Gottardo and Tancredi Didier Bazan Russo contributed equally to this work.

Viviana Bazan and Valerio Gristina Co-last authors.

high diagnostic and prognostic accuracy.[6] This method has become particularly relevant for detecting new-onset diseases and assessing recurrence risks.[7] The past decade has seen a surge in the identification of biomarkers like circulating tumour cells (CTCs), circulating free DNA/RNA (cfDNA/RNA) and extracellular vesicles (EVs), among others.[8,9] These developments have given rise to 'liquidomics' – a term encapsulating the vast potential of LB biomarkers.[6]

The advent of a new 'omic', namely 'multiomics',[10] marks a significant leap forward in this field. Enabled by sophisticated *deep learning* algorithms, multiomics approaches allow for the simultaneous analysis of various analytes, offering insights previously unattainable. This shift towards integrating genomic, epigenomic, transcriptomic, proteomic, metabolomic and lipidomic data, alongside clinical parameters, and imaging, heralds a new era in LC diagnosis and treatment.[11–13] The capability to analyse multiple biomarkers across various biological fluids (e.g. blood, saliva, urine, faeces, pleural fluid and cerebrospinal fluid) underscores the versatility of LB, presenting a paradigm shift in cancer diagnostics.[6,14,15]

Yet, the transition from experimental success to clinical implementation has been tempered by challenges, including the interpretation of complex datasets, the heterogeneity of information obtainable from various biosources, and, above all, the low concentration of tumour-related analytes present in the various biosources, which often borders on the lower limit of sensitivity of current laboratory methods, although even here there are improvements given by new ultra-sensitive methods such as ddPCR, NSG and BEAMing.[14] The potential of multiomics lies in its ability to aggregate a broad spectrum of analytes, promising to overcome these hurdles through algorithm optimization and method standardization.

This review seeks to consolidate the burgeoning evidence supporting the multiomics approach in LB for LC. By synthesizing studies that test various markers simultaneously, we aim to provide a comprehensive overview of the early successes and ongoing challenges in this field. Our goal is not only to highlight the promise of multiomics LB but also to outline the steps necessary for its integration into clinical practice, thereby contributing to the ongoing evolution of lung cancer diagnostics and treatment.

2 | METHODS

On 18 March 2024 and 19 March 2024, various computer searches were conducted on *PubMed*, *ScienceDirect* and *CochraneLibrary*; the strings used were as follows:

- ("Lung Neoplasms"[Mesh] OR "Carcinoma, Non-Small-Cell Lung"[Mesh] OR "NSCLC"[tiab] OR "Small Cell Lung Carcinoma"[Mesh] OR "SCLC"[tiab]) AND Liquid Biopsy AND (multianalyte OR multi-analyte OR multimarker OR multi-marker OR multi-source OR multi-target)
- "Lung cancer" AND "Liquid Biopsy" AND (multi-omics OR multi-analyte OR multi-analyte OR multi-marker OR multi-source OR multi-target)
- "Lung Cancer" AND "Liquid Biopsy" AND (("Extracellular Vesicles" OR EV OR Exosome OR TDX) AND (CTC OR "Circulating Tumor Cell"))
- "Lung Cancer" AND "Liquid Biopsy" AND (("Extracellular Vesicles" OR EV OR Exosome OR TDX) AND ("circulating tumor DNA" OR ctDNA))
- "Lung Cancer" AND "Liquid Biopsy" AND (("Extracellular Vesicles" OR EV OR Exosome OR TDX) AND ("circulating free DNA" OR cfDNA))
- "Lung Cancer" AND "Liquid Biopsy" AND (("Extracellular Vesicles" OR EV OR Exosome OR TDX) AND ("Tumor Educated Platelets" OR TEP))
- "Lung Cancer" AND "Liquid Biopsy" AND ((CTC OR "Circulating Tumor Cell") AND ("circulating tumor DNA" OR ctDNA))
- "Lung Cancer" AND "Liquid Biopsy" AND ((CTC OR "Circulating Tumor Cell") AND ("circulating free DNA" OR cfDNA))
- "Lung Cancer" AND "Liquid Biopsy" AND ((CTC OR "Circulating Tumor Cell") AND ("Tumor Educated Platelets" OR TEP))
- "Lung Cancer" AND "Liquid Biopsy" AND (("circulating tumor DNA" OR ctDNA) AND ("circulating free DNA" OR cfDNA))
- "Lung Cancer" AND "Liquid Biopsy" AND (("circulating tumor DNA" OR ctDNA) AND ("Tumor Educated Platelets" OR TEP))
- "Lung Cancer" AND "Liquid Biopsy" AND (("circulating free DNA" OR cfDNA) AND ("Tumor Educated Platelets" OR TEP))

The inclusion criteria subsequently used for screening were the presence of free full text, English or Italian language, and the presence of research on LB (either clinical studies or reviews) that dealt with the simultaneous use of different molecules, analytes and/or sources. Beyond these, additional articles in the possession of the authors or found in the bibliographies of the included articles are added.

3 | RESULTS

3.1 | 'Multi-marker' approach

In our exploration of the multiomics landscape, we first investigate the 'multi-marker' approach. This strategy enables the simultaneous analysis of virtually all molecules within our analyte, offering a comprehensive snapshot of its molecular composition. An illustrative example of this approach's potential is seen in the study of extracellular vesicles (EVs). EVs are notable for their diverse cargo, including proteins, metabolites and various forms of RNA, both coding and non-coding. These entities play crucial roles in tumour progression and the emergence of treatment resistance, as demonstrated in numerous studies.[11,16] However, not all components of a given analyte correlate with disease states to the same extent. For instance, research led by Purcell et al.[17] highlighted that in a cohort of 10 EGFR-positive NSCLC patients, protein content in EVs (EV-Prot) showed a 100% correlation with the samples analysed, whereas EV-derived RNAs displayed variable positivity

rates, ranging from 60% to 78%, depending on the specific mutation. This variability underscores the heterogeneity in composition, function and dynamics of EVs, making them prime candidates for multiomics investigations.

This principle extends beyond EVs. Platelets, for example, actively transform the cancer milieu, after a process known as 'tumour education', which lead to the acquisition of the 'tumour-educated platelets (TEPs) phenotype'.[8] This change is predominantly due to the absorption of tumour-derived molecules, encompassing both proteins and nucleotides, over their lifespan. Consequently, TEPs emerge as another valuable analyte for the multi-marker approach, given their dynamic interaction with the tumour environment and their molecular complexity.

Among the numerous studies investigating the molecular constituents of platelets for correlations with LC, one particularly stands out. It analysed both circular RNAs and mRNAs within TEPs,[18] finding that utilizing 28 mRNAs yielded an Area Under the Curve (AUC) of 0.81, which increased to 0.88 with the use of 21 circular RNAs. Remarkably, a tailored panel combining 6 mRNAs and 2 circular RNAs – derived through sophisticated bioinformatic algorithms – further enhanced the AUC to 0.92 for the cohort, and an impressive 0.96 for patients with early-stage disease. This finding illustrates the power of a multi-marker approach, leveraging the intricate biochemistry of circulating biomarkers to achieve unparalleled diagnostic precision.

3.2 | 'Multi-analyte' approach

Beyond merely combining the different molecules that constitute each analyte, multiomics introduces a pivotal strategy – the 'multi-analyte approach'. This method, often regarded as the quintessence of multiomics due to its extensive clinical research footprint, involves assessing various combinations of biomarkers such as EVs, CTCs, ctDNA and others. The goal is to identify the most diagnostically significant combination of these biomarkers. For a comprehensive view, [Table 1] presents a summary of the key studies that have been explored under this approach.

3.3 | 'Multi-source' approach

The final facet of our exploration into multiomics approaches focuses on the 'multi-source' methodology, characterized by the integration of data derived from diverse tissues or distinct analytical natures. Indeed, LB is able to obtain valuable clinical information starting from different biological fluids, for example, blood, saliva, urine, faeces, pleural fluid, cerebrospinal fluid[6,14,15]; thus, thanks to AI, we can now merge all the information collected separately from all of the aforementioned biosources, to combine them together or with other clinical and/or molecular data. This strategy is emblematic of the transformative potential heralded by advancements in AI and machine learning within biomedical research. As previously highlighted,[12] these technologies are refining the analysis of conventional LB experiments, yielding results that are not only more precise but also faster. The true innovation, however, lies in the algorithms' capacity to interrelate data of wholly different types,[29] enabling a comprehensive evaluation of various parameters simultaneously. This approach facilitates clinical decisions that are more accurately aligned with a patient's overall health status, showcasing several emblematic cases identified in our research.

One pioneering instance of the multi-source approach involves the use of saliva for LB,[13] a medium chosen for its accessibility despite traditionally exhibiting low sensitivity for cytological analyses. Yet, molecular investigations of non-coding RNAs (ncRNAs) in saliva are showing promising outcomes, advocating for its inclusion in larger research cohorts.[30–32] Notably, studies have successfully combined saliva-derived miRNAs with CT scans[33] and plasma miRNAs,[34] exceeding the diagnostic efficacy achievable through single biosource analyses. However, these innovative methods currently face challenges related to standardization and the representativeness of study cohorts, delaying their integration into clinical practice.

Furthermore, attention has shifted towards analysing malignant pleural effusions (MPE) through LB.[35] MPEs, often linked to lung cancer, especially NSCLC,[36] are relatively easier to collect than neoplastic tissue samples and are rich in tumour markers[37] that correlate well with solid biopsy results.[38] The literature underscores the abundance of crucial analytes like EVs, ncRNAs and notably cf/ctDNA within MPE samples, with cf/ctDNA appearing in higher concentrations due to its release from tumour lesions.[39] Despite the promising correlation of these markers with biopsy results in EGFR+ NSCLC cases,[39–44] the approach's applicability is tempered by the variable presence of MPE in early-stage lung cancers[38] and a slight increase in false positives in certain conditions (in particular, with chronic pulmonary diseases[35,45]).

For all these reasons, a multi-source approach application of MPE was hypothesized: indeed, in the study by Kim and colleagues,[46] the presence of EGFR mutations was evaluated in 54 plasma samples and 13 pleural fluid samples from patients with confirmed EGFR+ NSCLC diagnosis. The results showed that combining the two biosources always yielded the best results, both via ddPCR and via NGS: in fact, although the specificity was always 100%, the sensitivity in detecting the two mutations 'exon 19 deletion' and 'L858R' went from 93% of the plasma cfDNA alone to 93.8% of the combined test via NGS, and 95.3% of the combined test via ddPCR; while for the 'T790M' mutation the sensitivity with the plasma cfDNA alone was 64.7%, with the combined test via ddPCR 88.2% and with the combined test via NGS 93.3%.

Lastly, the analysis of cerebrospinal fluid (CSF) in LB presents a still experimental frontier. One study utilized CSF alongside ctDNA and plasma EV-RNA to characterize brain metastases in LC patients,[47] illuminating the clonal heterogeneity and identifying potential molecular targets for therapy. Such approaches promise to revolutionize diagnosis and monitoring, potentially obviating the need for invasive brain biopsies and heralding a new era of personalized and minimally invasive cancer care.

TABLE 1 - Included clinical studies about the 'multi-analyte' approach.

Article	Setup	Result
19	*Single tube liquid biopsy* with CTCs, EVs & ctDNA (Cohort: 97 stage IIIB-IV NSCLC)	Survival plots for single versus grouped biomarkers in NSCLC patients show lower *p*-values for the latter graph (0.001) than for the former one (0.009).
20	CTCs, ctDNA & classical circulating markers (Cohort: 99 LCs + 12 benign lesions)	Diagnostic Accuracy: • CEA + CYFRA-21-1 + NSE = 67.6% • CTCs = 63.1% • ctDNA = 69.4% • CEA + CYFRA-21-1 + NSE + CTCs = 82.9% • CEA + CYFRA-21-1 + NSE + ctDNA = 82.9% • CTCs + ctDNA = 86.5% • CEA + CYFRA-21-1 + NSE + CTCs + ctDNA = 89.2%
21	CTCs, cf/ctDNAs, *CTECs* (*Circulating Tumour-derived Endothelial Cells*) & classical circulating markers (CTCs + CTECs cohort: 24 HC + 31 benign lesions +29 LCs; cf/ctDNA cohort = 8 benign lesions +20 LCs)	AUC values: • CTCs = 0.815 • CTECs = 0.793 • Total CTCs + total CTECs = 0.826 • Small CTCs + small CTECs = 0.898 • Triploid CTCs + triploid CTECs = 0.872 No correlations between ct/cfDNA and other biomarkers, except for some clinical parameters (tumour size and maximum tumour diameter) and with CYFRA-21-1 concentration.
22	CTCs & cfDNA (Final cohort: 6 HC + 23 NSCLC EGFR+)	Concordance between tissue biopsy and combination of CTCs and cfDNA • True negatives = 6/6 • Concordant mutation status = 13/23 • Discordant mutation status = 10/23 N.B.: very old tissue specimens, collected from 1 to 7 years before blood sampling!
23	CTCs & cfDNA (Cohort: 50 advanced NSCLC)	Variables with *p*-values ≤0.05 in multivariate analysis: PFS • Baseline CTC count, CellSearch (≥1 vs. 0, *n* = 30) = 0.006 • Sex (male vs. female, *n* = 50) = 0.04 • Number of metastasis (>2 vs. ≤ 2, *n* = 50) = 0.006 • Smoking (yes vs. no, *n* = 50) = 0.03 • Combined changes in CTC and cfDNA levels, Group B (CTCs <1 and a high cfDNA level or CTCs ≥1 and a low cfDNA level or CTCs ≥1 and a high cfDNA level, *n* = 18) = 0.009 OS • Baseline CTC count, CellSearch (≥1 vs. 0, *n* = 30) = 0.01 • Number of metastasis (>2 vs. ≤2, *n* = 42) = 0.001 • Smoking (yes vs. no, *n* = 42) = 0.02 • Combined changes in CTC and cfDNA levels, Group 3 (CTCs ≥1 and a high cfDNA level, *n* = 8) = 0.01 • Combined changes in CTC and cfDNA levels, Group B (CTCs <1 and a high cfDNA level or CTCs ≥1 and a low cfDNA level or CTCs ≥1 and a high cfDNA level, *n* = 18) = 0.05
24	CTCs & cfDNA (Cohort: 25 stage III-IV NSCLC)	PFS prediction: • CTCs count at T0, *p*-value = 0.1872 • CTCs cluster count at T0, *p*-value = 0.0711 • cfDNA levels at T0, *p*-value = 0.1662 • cfDNA levels at T1, *p*-value = 0.0684 • cfDNA T0 + cfDNA T1 + CTCs cluster T0, *p*-value = 0.0022
25	CTC & cfDNA (Cohort: 49 stage I-IIIA NSCLC +22 HC)	In multivariate analysis for *Recurrence-Free Survival* (*RFS*), the only variable with a *p*-value ≤0.05 was *'At least one mutation in plasma-ct DNA (yes vs. no)'*, with *p*-value = 0.015
26	EVs & cfDNA (Cohort: 20 stage III-IV NSCLC)	Concordance between tissue biopsy and combination of EVs & cfDNA • Sensitivity = 83.3% (5/6) • Specificity = 100% (14/14)

TABLE 1 (Continued)

Article	Setup	Result

27	EVs + CTC
	(Cohort: 54 stage III-IV NSCLC)
		Multivariate analysis for OS
		• PD-L1+ sEV concentration, *p*-value = 0.008
		• Presence of CTCs, *p*-value <0.001
		• Number of previous treatment lines, *p*-value = 0.026
		• Squamous cell/basaloid carcinoma, *p*-value = 0.003
		Multivariate analysis for PFS
		• PD-L1+ sEV concentration (*p*-value = 0.044):
		  • Patients without CTCs, *p*-value = 0.007
		  • Patients with CTCs, *p*-value = 0.935
		• Number of previous treatment lines, *p*-value = 0.047
		• Squamous cell/basaloid carcinoma, *p*-value = 0.019

28	EV ncRNA + plasmatic ncRNA + classical circulating markers (Validation cohort: 75 HC + 47 LUAD +62 LUSC)	AUC in validation cohort = 0.973 Notably, plasmatic ncRNA *CTA-384D8.35* was the greatest contributor, followed by exosome ncRNA *CTA-384D8.35* and Exosome ncRNA *SFTA1P*, and finally, NSE, SCC and $\log_{10}$CEA contributed equally. All those markers were used to build a predictive nomogram.

4 | CONCLUSIONS

The integration of AI and multiomics is revolutionizing LB, enhancing diagnostic and prognostic capabilities beyond what was previously achievable. By employing multi-marker, multi-analyte and multi-source approaches, we are on the cusp of identifying biomarker combinations that truly resonate with clinical utility, reflecting a comprehensive correlation with patients' health statuses. This leap forward promises not only to justify the developmental costs of such technologies through improved analytical precision and reduced TATs, but also to elevate treatment outcome metrics, as pursued with (now gladly widespread) molecular tumour boards (MTBs),[48] or as (yet commonly) evaluated in cost–benefit analyses (CBAs).[49,50]

What is more, next generation technical advancements are state-of-the-art laboratory technologies that aim to overcome the limitations of current single-gene testing techniques: these, such as Bias-Corrected Targeted NGS or eTAm-Seq,[51,52] indeed aim to obtain 'ultra-deep' sequencing, a type of molecular sequencing capable of multiplexing and capturing up to the least represented of the genomic variants present within our starting sample, thus achieving sensitivity rates hitherto unattainable with current single-gene testing methods. Being able to unite multiple such technologies through AI (as done, e.g. by de Wit et al.[18] with their single tube LB assay) would thus enable the capture of every molecular variation in the patient's circuloma, allowing this to be correlated immediately with the clinical outcome of treatment, or with a recurrence of the disease, or with early diagnosis, and so on.

Yet, declaring victory prematurely would be unwise. The path forward is tempered by the ongoing need for rigorous, large-scale studies. A glaring challenge highlighted by recent literature on novel LB methodologies, particularly those leveraging AI, is the inconsistency in study reproducibility and a pervasive lack of methodological standardization, both in the laboratory and computationally.

Therefore, it is the earnest hope of this review's authors that the initiation of numerous multi-centre studies will address these challenges. Such studies should not only explore diverse combinations of variables (spanning software, hardware, sample types and pathologies) but also insist on the standardization of laboratory and computational protocols across all participating centres. Achieving this level of uniformity is crucial for producing results that are not only informative, but critically, reliable and comparable. If we succeed in this endeavour, AI and multiomics are poised to secure their rightful place in the panorama of contemporary clinical practice.

AUTHOR CONTRIBUTIONS

A.Go., T.D.B.R, Conceptualization; Study design; Data acquisition, analysis & interpretation; Draft writing & review. A.P., Study design; Data analysis & interpretation; Draft writing & review. M.B., Data analysis & interpretation; Draft writing. E.D.G., E.D.M., R.S., C.F.B., C.M., M.C.V., Data acquisition & analysis. S.C., G.I., G.Bu., Data acquisition & analysis; Draft writing. F.I., Data interpretation; Draft writing. L.I., G.Ba., Data interpretation; Draft review. A.Ga., Conceptualization; Study design; Data interpretation; Draft review. A.R., Conceptualization; Data interpretation; Draft review. V.B., Study design; Data interpretation; Draft review. V.G., Conceptualization; Study design; Data acquisition & interpretation; Draft writing & review. All authors had full access to all study data and take responsibility for their integrity and for the accuracy of the data analysis. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Andrea Gottardo https://orcid.org/0009-0004-3321-2218
Clarissa Mujacic https://orcid.org/0009-0002-1748-6337
Antonio Russo https://orcid.org/0000-0002-4370-2008

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