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Mini Review

Ultrasensitive Detection of Biomarkers for Guiding Immunotherapy in Lung Cancer: A Liquid Biopsy Approach

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Abstract

Liquid biopsy (LB) represents a promising strategy for the early diagnosis and treatment of lung cancer. However, relying solely on single-biomarker immunohistochemistry for predictive purposes has shown limited efficacy, often leading to suboptimal responses in certain patients. LB provides a complementary or alternative approach to immunohistochemistry by aiding in the identification of patients better suited for immunotherapy, thereby improving treatment precision. This review highlights key LB targets, including circulating tumor cells, exosomes, and small protein molecules, and explores the predictive and prognostic value of LB in immunotherapy for lung cancer and other tumors. These biomarkers play complex and multifaceted roles in liquid biopsies. Consequently, researchers have developed numerous targeted detection methods to study and identify key factors among multiple biomarkers in lung cancer and other tumor diseases. In addition, the limitations and future directions of LB are examined, aiming to advance its clinical application and support the development of personalized and precise immunotherapy. The integration of LB with artificial intelligence holds significant clinical potential for guiding immunotherapy and advancing precision medicine in lung cancer and other tumors.

Introduction

Lung cancer is a significant malignant disease that poses a serious threat to human health. The development of effective strategies for prognosis assessment, diagnosis, and treatment of lung cancer is hindered by a limited understanding of tumor cell heterogeneity and the key regulatory factors involved in the onset and progression of the disease. Over the past decade, cancer immunotherapy has emerged as a pivotal antitumor strategy, complementing conventional treatments such as chemotherapy, radiotherapy, and surgical intervention.[1] Specifically, in studies evaluating the efficacy of immunotherapy for lung cancer, immune checkpoint inhibitors (ICIs) targeting the programmed death ligand 1 (PD-L1), programmed death 1 (PD-1), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) pathways have demonstrated the ability to restore T cell function and enhance antitumor immune responses.[2–5]

Currently, several ICIs have received approval for the treatment of various cancer types from the U.S. Food and Drug Administration and the European Medicines Agency, including anti-PD-L1 antibody atezolizumab, anti-PD-1 antibody pembrolizumab, and anti-CTLA-4 antibody ipilimumab.[6,7] These highlight the growing importance of immunotherapy in clinical cancer treatment.[2] Determining the suitability of first-line immunotherapy and predicting individual responses in lung cancer patients currently rely primarily on assessing PD-L1 expression through immunohistochemistry (IHC).[8] However, the use of PD-L1 as a biomarker and predictive factor presents several limitations and challenges. One major issue is the inherent variability in IHC outcomes due to the use of different PD-L1 antibodies, such as the 28-8 rabbit monoclonal antibody, 22C3 mouse monoclonal antibody, Roche Ventana SP142 rabbit monoclonal antibody, and Roche Ventana SP263 rabbit monoclonal antibody, across studies and clinical settings.[9,10] Additionally, variability in defining PD-L1 positivity thresholds across different detection platforms used in clinical trials further complicates the comparability of results.[11] Another challenge is the intratumoral heterogeneity of PD-L1 expression, which can lead to underesti-

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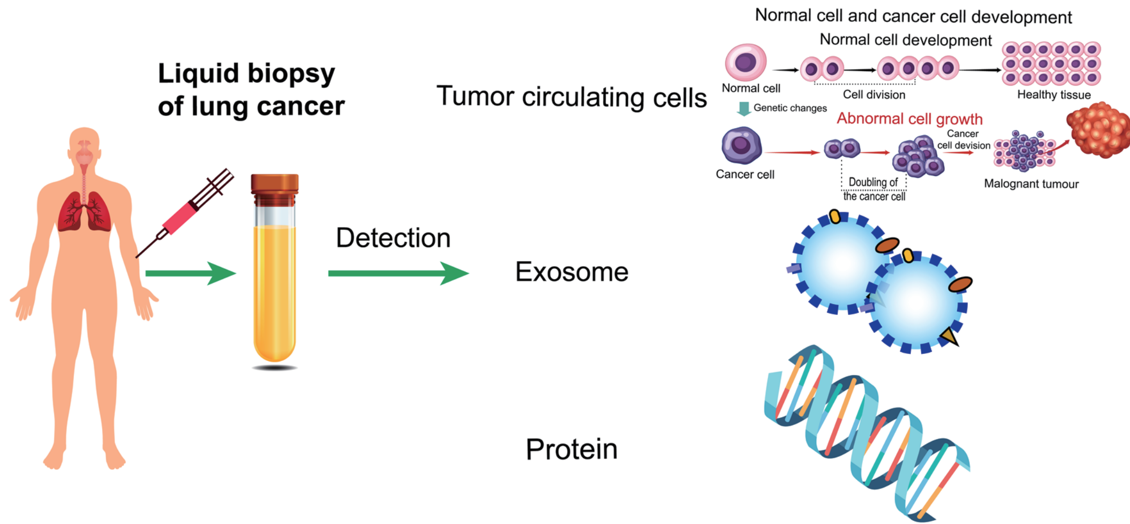

Fig. 1. Distribution of key targets for liquid biopsy in lung cancer.

mation of the true expression status within the entire tumor when assessments are based on small biopsy samples. This limitation prevents the capture of the full distribution of PD-L1 within the tumor.[3] Furthermore, the predictive value of IHC-based PD-L1 expression for immunotherapy response remains uncertain.[12] This uncertainty stems partly from practical challenges, such as obtaining sufficiently large, high-quality tumor tissue samples and isolating an adequate number of viable tumor cells from limited biopsy specimens.[13] Given these challenges, there is a pressing need to develop and validate more robust and reliable predictive biomarkers to improve patient selection and enhance personalized treatment approaches.

Liquid biopsy (LB) has recently garnered attention as a promising, minimally invasive approach for guiding immunotherapy decisions in lung cancer.[14–16] LB enables the evaluation of various tumor-associated components in peripheral blood, including circulating tumor cells (CTCs), tumor-associated exosomes, and protein molecules closely related to tumors (Fig. 1). Compared to traditional tissue biopsy, LB offers advantages such as minimal invasiveness, high repeatability, and rapid result turnaround.[16,17] This review summarizes recent advancements in LB research for ICI therapy in lung cancer, with a focus on current findings and future directions. We provide a comprehensive discussion of the advantages and limitations of LB as a biomarker for lung cancer immunotherapy, including its potential to identify responders, detect resistance mechanisms, and predict clinical outcomes. Ongoing research aimed to further validate the clinical utility of LB-based biomarkers, with the goal of optimizing patient selection and management in ICI therapy. Moreover, with the development of ultrasensitive detection technologies, LB holds promise for identifying tumor biomarkers at extremely low concentrations during the early stages of cancer. Early detection enables patients to undergo immunotherapy before disease progression, significantly improving cure rates and therapeutic outcomes.

Application of LB technology in the diagnosis and prognosis of lung cancer

Combining potentially valuable biomarkers such as CTCs, circulating tumor DNA, extracellular vesicles (EVs), and tumor-associated antigens can guide the application and development of LB in the early diagnosis and prognosis of tumor diseases, including lung cancer (Table 1).[18–22] Although LB offers advantages comparable to traditional lung cancer detection methods, there are still limitations and challenges in large-scale clinical applications. (1) The high heterogeneity of CTC means that the sensitivity, accuracy, and quality of detection need to be improved. For example, fluorescence flow cytometry requires 5–7 mL of peripheral blood to detect a single circulating tumor cell cluster (CTCC), and its sensitivity is only 35.3%.[18] (2) There are also unavoidable false positives and false negatives in LB.[23] For instance, the low abundance of CTCs in peripheral blood can lead to false positives.[24,25] (3) LB lacks industry standards and has a high cost associated with detection technology.[23,26] There is no unified standardization in clinical practice. For example, in the clinical setting, the early stage of lung cancer requires high sensitivity in detection methods, while the prognosis and treatment of the disease require good specificity in the detection method. Here, we comprehensively outline the recent technical updates on LB and propose a feasible method for early diagnosis and prognosis management of lung cancer, supported by new LB technologies.

The role of circulating tumor cells in lung cancer diagnosis and prognosis

This section primarily discusses how to quickly collect, identify, and correlate CTCs as one of the tumor markers. It also explores their advantages, disadvantages, and development prospects in tumor diagnosis and treatment.[25,27] CTCs refer to various tumor cells present in peripheral blood, which can be captured and collected based on their physical and biological characteristics.[28] They are then identified based on their biological characteristics (such as the expression of epithelial cell markers and the non-expression of hematopoietic cell markers) or physical characteristics (such as cell size, morphology, density, number, invasiveness, etc.).[18,22] The detection of CTCs is generally to analyze tumor dynamics and evaluate treatment effects by monitoring trends in the changes in their type and number.[29–32] Numerous studies have explored the association between CTCs and the tumor immune microenvironment. Changes in the tumor microenvironment depend on the influence of CTCs themselves and whether infiltrating immune cells

Table 1. Comparison of different tumor-associated biomarker detection methods

Protein	Characteristic	Assay	Major parameter	Location	Cost	Application
PD-L1	–	CellSearch ® CXC kit[19]	CTC PD-L1	Blood	￥40	PD-L1(+)-CTCs, in contrast to PD-L1(+)-tumors, are linked to survival outcomes in metastatic breast cancer
CEA, NSE, Cyfra21-1 from exosome	Linear ranges were from 10^{-3} to 10 ng/mL for CEA, 10^{-4} to 10^{2} ng/mL for NSE, and 10^{-3} to 10^{2} ng/mL for Cyfra21-1	A tri-channel electrochemical immunobiosensor[20]	LOD:10^{-4} ng/mL	Exosome	–	Quantification of exosomal markers in specific diseases
YES1 and CEA	Linear from 0.01 to 50 ng/mL	Electrochemical immunosensors[21]	LOD: 0.0022 and 0.0034 ng/mL	Blood	–	Multiplex immunosensor
CTCs	Linear from 5–5 × 10^{5} cell/mL	DNA walker powered by a DNA circuit and MOF-on-MOF nanozyme[22]	The accuracy of the DNA walker driven by a DNA circuit and MOF-on-MOF nanozyme synergies signal amplification strategy	Blood	–	CTC-related diagnosis and individualized treatment
CTCs	A deep-learning-based, peak detection and classification model	Confocal backscatter and fluorescence flow cytometry have been used for label-free detection of CTCCs in whole blood based on machine learning-enabled peak classification[18]	A high Pearson correlation coefficient of 0.943	Blood	-	*In vivo* detection of CTCCs

CEA, carcino-embryonic antigen; CTCCs, circulating tumor cell cluster; CTCs, circulating tumor cell; Cyfra21-1, cytokeratin 19 fragments; LOD, limit of detection; MOF, metal–organic frameworks; NSE, neuron-specific enolase; PD-L1, programmed death ligand 1; YES1, yamaguchi sarcoma viral oncogene homolog 1.

have sufficient anti-tumor ability.[33,34] For instance, patients with detectable CTCs exhibited markedly higher regulatory T cell infiltration in tumor tissues compared to those without detectable CTCs, indicating an immunosuppressive phenotype.

In recent years, swift technological progress has facilitated in-depth functional analyses of CTCs, offering robust tools for investigating predictive biomarkers in ICI therapy for lung cancer. In particular, single-cell proteomics, transcriptomics, and metabolomics of CTCs can reveal multidimensional molecular features linked to therapeutic outcomes. Proteomics analyses can identify specific CTC subpopulations linked to immunotherapy sensitivity or resistance[35]; transcriptomic analyses can uncover gene expression patterns associated with immune evasion mechanisms; and metabolomics provides insights into the impact of immunometabolic phenotypes on treatment efficacy.[36] Applying single-cell multi-omics to CTCs offers a more comprehensive understanding of tumor heterogeneity. For example, treatment strategies that target converging molecular pathways may fail if different cells within the tumor exhibit distinct molecular alterations.[28] In such cases, it might be necessary to target each abnormality individually. Therefore, single-cell analysis of CTCs can provide a clearer picture of the molecular landscape across the entire cancer.[27] Furthermore, the dynamic evaluation of CXCR4 expression levels on CTCs using single-cell assays could aid in tracking immunoregulatory processes.[37] In conclusion, ongoing technological advancements have made comprehensive CTC research achievable, establishing CTCs as a promising source of biomarkers for predicting and monitoring immunotherapy efficacy in lung cancer patients, with considerable clinical application potential.[28,38]

Monitoring of CTCs has demonstrated strong prognostic value in various solid tumors.[37] However, due to the absence of conclusive clinical utility data, CTC detection has not been widely implemented for monitoring lung cancer treatment. In advanced non-small cell lung cancer (NSCLC), research on the phenotypic heterogeneity of CTCs and its relationship with treatment outcomes is still limited. CTCs can be detected in the majority of stage IV NSCLC patients. Although absolute high-definition circulating tumor cell counts do not exhibit a significant correlation with prognosis, fluctuations in CTC counts over time can predict survival in metastatic lung cancer patients receiving chemotherapy.[39] This indicates that CTC levels and dynamics reflect the biological and pharmacological characteristics of different disease stages and treatment time points, highlighting their complex role in cancer research and real clinical practice (Fig. 2a).[39] Researchers have also explored the clinical significance of CTCs and circulating tumor endothelial cells, as well as their subtypes, in patients with small cell lung cancer (SCLC) (Fig. 2b).[40] Using nanoparticle-coated Smart BioSurface® slides, which enable the fixation of intact nucleated cells without preselection, epithelial CTCs were identified in patients with early-stage NSCLC (Fig. 2c).[41] CTC detection in liquid biopsies acts as a predictive and prognostic marker for SCLC, though it presents certain analytical challenges.[42] By integrating microfluidics or density gradient-based CTC enrichment with immunofluorescence staining or quantitative polymer-

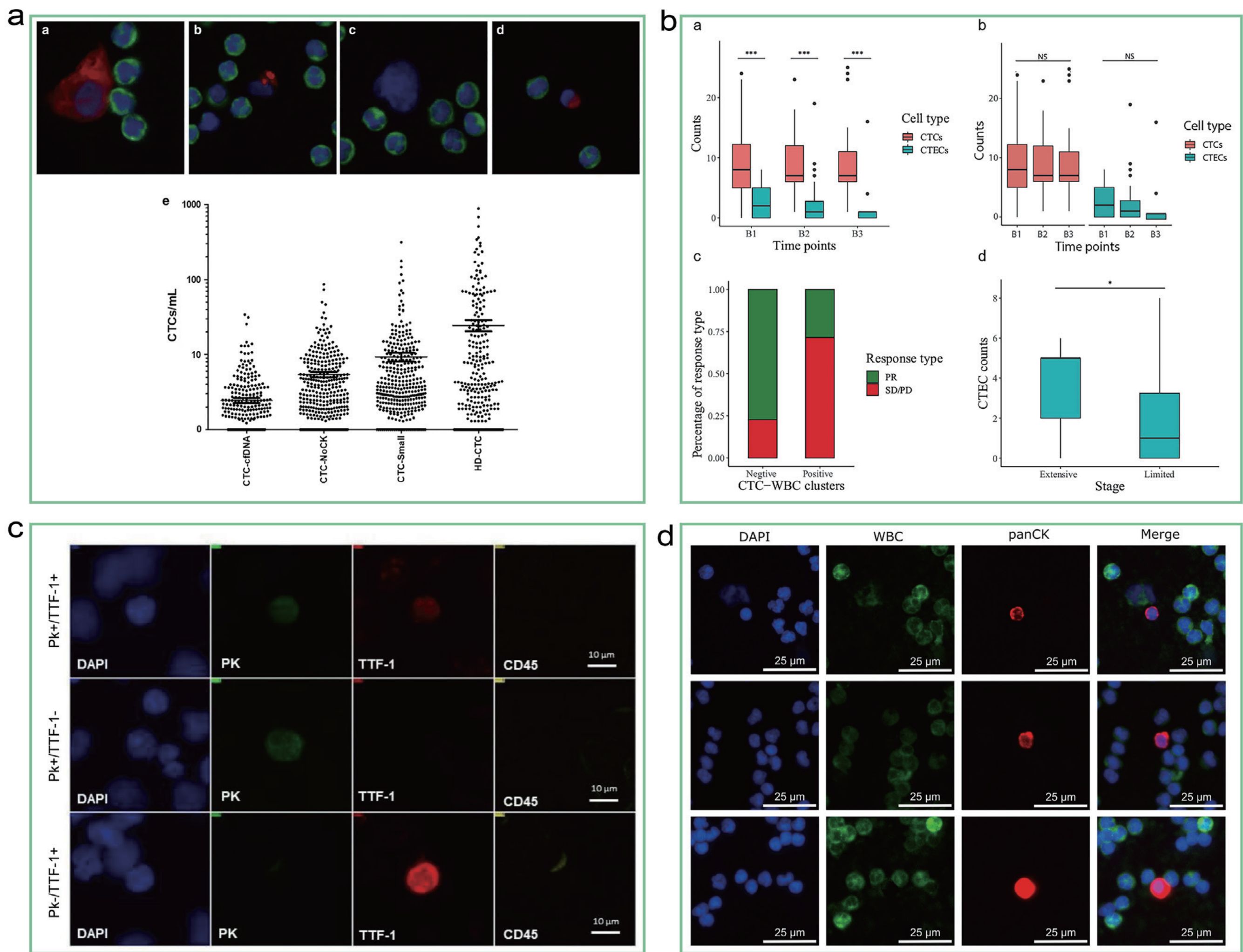

Fig. 2. Circulating tumor cells (CTCs) for detection. (a) High-definition circulating tumor cell (HD-CTC) and CTCs for stage IV non-small cell lung cancer (NSCLC) (copyright BioMed Central, 2019)[39]; (b) CTC/circulating tumor endothelial cells (CTEC) counts (copyright Wiley, 2021)[40]; (c) Subpopulations of NSCLC CTCs identified on Smart BioSurface® (SBS)-CTCs slides (copyright MDPI, 2023)[41]; (d) Representative images of three distinct CTCs identified through immunofluorescent staining of panCK (red), white blood cells (WBC) (green), and nuclear counterstaining with 4′, 6-diamidino-2-phenylindol (DAPI) (blue) (copyright Wiley-Liss Inc., 2024).[42]

ase chain reaction for CTC-related transcripts, a detection rate ranging from 60.8% to 88.0% has been attained in blood samples from SCLC patients. Epithelial and neuroendocrine transcripts, such as DLL3, are linked to shorter overall survival (OS), indicating their potential clinical value in diagnosis and treatment decisions.[43] Elevated CTC counts and the detection of CTC doublets through immunofluorescence staining are predictive of poor OS and may serve as potential markers of disease progression or treatment failure. In patients with a high CTC burden, positive results for circulating free plasma RNA and CTC-associated transcripts show strong consistency. These findings highlight the importance of CTCs and their transcripts in SCLC, stressing the clinical significance of liquid biopsies for diagnosing and managing this disease (Fig. 2d).[42] In general, there are still diverse separation and enrichment methods for CTCs in the field of LB.[44] Different methods have significant differences in sensitivity, specificity, and efficiency, and no unified standard has been established, which leads to poor comparability of test results between different experiments. Secondly, CTCs are present in low numbers in peripheral blood and are easily interfered with by other blood cells.[45] Different detection technologies have different abilities to identify low-abundance CTCs, which may lead to false negative and false positive results, especially in early cancer.[46] Thirdly, CTCs have high heterogeneity, and there is a lack of unified standards for the selection of markers (e.g., EpCAM, CK, Vimentin), which can lead to insufficient accuracy and consistency of detection. Therefore, developing unified CTC separation and enrichment standards, optimizing detection technologies (such as microfluidics and nanotechnology), and establishing a unified marker screening system are crucial to improving the detection efficiency and reliability of low-abundance CTCs in early cancer patients.[47]

Exosome associated with LB for lung cancer diagnosis and prognosis

Exosomes are round, nanoscale particles with diameters ranging from 40 to 100 nm and densities between 1.13 and 1.19 g/mL.[20]

They are released by various cell types under both physiological and pathological conditions, including immune cells, stem cells, and tumor cells.[48] These small molecules can be easily obtained from most bodily fluids, making them promising biomarkers for LB in lung cancer. EVs represent a complete intercellular communication strategy utilized by both pathogenic and non-pathogenic cells.[49] Exosomes have high stability, target specificity, good biocompatibility, and low immunogenicity, making them promising vehicles for lung cancer therapy.[50,51]

A significant body of evidence suggests that EVs play an essential role in the interactions between tumor cells and immune cells.[52] EVs originating from tumors primarily exhibit immunosuppressive functions, promoting tumor immune escape. This immune evasion mechanism includes: downregulating the expression of major histocompatibility complex class II in dendritic cells; activation of the cGAS-STING signaling pathway in dendritic cells; inducing STAT3-mediated M2 polarization in monocytes[53]; reducing the production of interferon-γ in natural killer cells[54]; and inducing T cell apoptosis.[55] Additionally, PD-L1 expression on the surface of EVs can induce T cell exhaustion, further suppressing anti-tumor immune responses.[56] On the other hand, EVs originating from tumors can also trigger immune activation by activating natural killer cells, macrophages, B lymphocytes, and T lymphocytes. Particularly, EVs related to the Hippo signaling pathway have been found to be closely associated with potent tumor clearance and immune activation.[57] Furthermore, EVs derived from antigen-presenting cells exhibit immune-stimulating effects by carrying various major histocompatibility complexes that activate T cells, although their potency in T cell activation is relatively weak.

EVs contain a variety of genetic materials, such as messenger RNA, micro RNA, long non-coding RNA, nucleic acids, and proteins, playing a crucial role in mediating cellular functions.[58] The proteins on the EV membrane have become important detection targets for various tumors and other diseases. Research has shown the potential of using EVs as immune-oncology biomarkers in melanoma patients treated with ICIs. Increased PD-L1 expression on the surface of EVs marks an adaptive immune response and can distinguish clinical responders from non-responders.[59] In patients receiving anti-CTLA-4 therapy, increased levels of PD-L1 and CD28 in EVs are related to enhanced progression-free survival and OS.[60,61] Additionally, specific EV RNA profiles correlate with the efficacy of anti-CTLA-4 treatment.[62] In conclusion, EVs, as important immune regulatory factors, have immense research and application potential in immuno-oncology. The analysis of circulating EVs as immune-related biomarkers shows great promise. However, despite the widespread detection of EVs in the blood of lung cancer patients, the lack of validated, standardized isolation techniques remains a major obstacle.[63] Addressing these technical challenges is the primary task to advance the clinical application of EV analysis.

Deep learning has been used to explore the characteristics of EVs and identify similarities in human plasma exosomes. Exosome-based deep learning-enhanced surface-enhanced Raman spectroscopy has demonstrated accurate early-stage lung cancer diagnosis. The integration of deep learning and EV analysis presents a promising approach for early LB in lung cancer (Fig. 3a).[64] The fluorescence resonance energy transfer-based magnetic aptamer sensor has been proposed for enriching EVs using aptamer magnetic affinity, followed by the detection of cancerous surface proteins using the fluorescence resonance energy transfer "lighting up" strategy.[65] Fluorescent quantum dots and aptamers are incorporated into magnetic nanoparticles. When the aptamers pair with complementary DNA on the surface of Au nanoparticles, the fluorescence emission decreases (Fig. 3b).[65] A technique integrating an automated centrifugal microfluidic disk system with functionalized membranes for exosome isolation and enrichment (Exo-CMDS) has been established, along with an original aptamer-based fluorescence system (Exo-AFS) for detecting exosome surface proteins. Exo-CMDS is efficient, rapid, and requires low sample volumes, completing a one-step separation and purification process within 8 m, with exosome concentrations reaching 5.1×10^9 particles/mL from small serum samples (<300 μL). The sensitivity of Exo-AFS for detecting PD-L1 reaches a minimum threshold of 1.58×10^5 particles/mL. In clinical trials, this method showed a diagnostic accuracy of 91% for lung cancer, outperforming exosome ELISA. Compared to traditional methods, Exo-CMDS and Exo-AFS offer significant advantages in cost, speed, purity, sensitivity, and specificity, providing an efficient and reliable tool for early cancer detection and clinical immunotherapy (Fig. 3c).[66] However, there are some potential drawbacks of Exo-CMDS and Exo-AFS, which include the complexity of fabrication, cost, sensitivity to biological variability, and exosome fouling and degradation. A targeted early diagnosis method based on a nanoparticle array for multiple lung cancer subtypes has also been proposed. By chemically conjugating amino and carboxyl groups, five targeted aptamers are modified onto mesoporous silica nanoparticles, constructing a high-specificity targeted nanoparticle array. Flow cytometry experiments have shown that even when biomarker expression levels are as low as 1.5%, this nanoparticle array could specifically identify tumor-derived exosomes in phosphate buffered saline. Transmission electron microscopy results further confirmed that the nanoparticle array could isolate tumor-derived exosomes from the blood of tumor-bearing mice, successfully detecting exosomes in the blood of mice with early-stage lung cancer (with cancer formation only seven days prior). In conclusion, the targeted nanoparticle array is a great early detection tool across multiple lung cancer subtypes (Fig. 3d).[67]

In general, in the application research of LB based on exosomes for the early diagnosis of diseases, there are still problems such as incomplete standardization of exosome separation and detection, lack of consistency in experimental results, and gaps in efficiency, purity, and repeatability of existing separation methods (e.g., ultracentrifugation, precipitation methods, microfluidic technology, filtration membrane technology, etc.), which will lead to insufficient reliability and repeatability of test results in clinical applications.[68] Second, exosomes contain complex biological molecules, including proteins, DNA, RNA, lipids, etc., and there may be significant differences in exosome markers from different sources and conditions, which will affect the difficulty of screening and verification of specific markers.[69] Third, the expression level of some exosome markers in early lung cancer is low, which can be easily interfered with by other components in the solution, leading to false positive and false negative results.[70] Finally, although a variety of potential exosome markers have been identified in research, many are still in the exploratory stage and lack large-scale clinical verification, which seriously hinders their transformation from basic research to actual clinical application. In response to the above problems, it is necessary to develop fast and efficient exosome separation technologies (e.g., nanotechnology, microfluidics, etc.) and establish unified technical standards and guidelines, with the aim of improving the purity and consistency of exosome separation and laying the foundation for large-scale clinical application.[71] Secondly, it is possible to combine the information of various biomolecules (such as proteins, micro RNAs, DNA mutations, etc.) in exosomes to

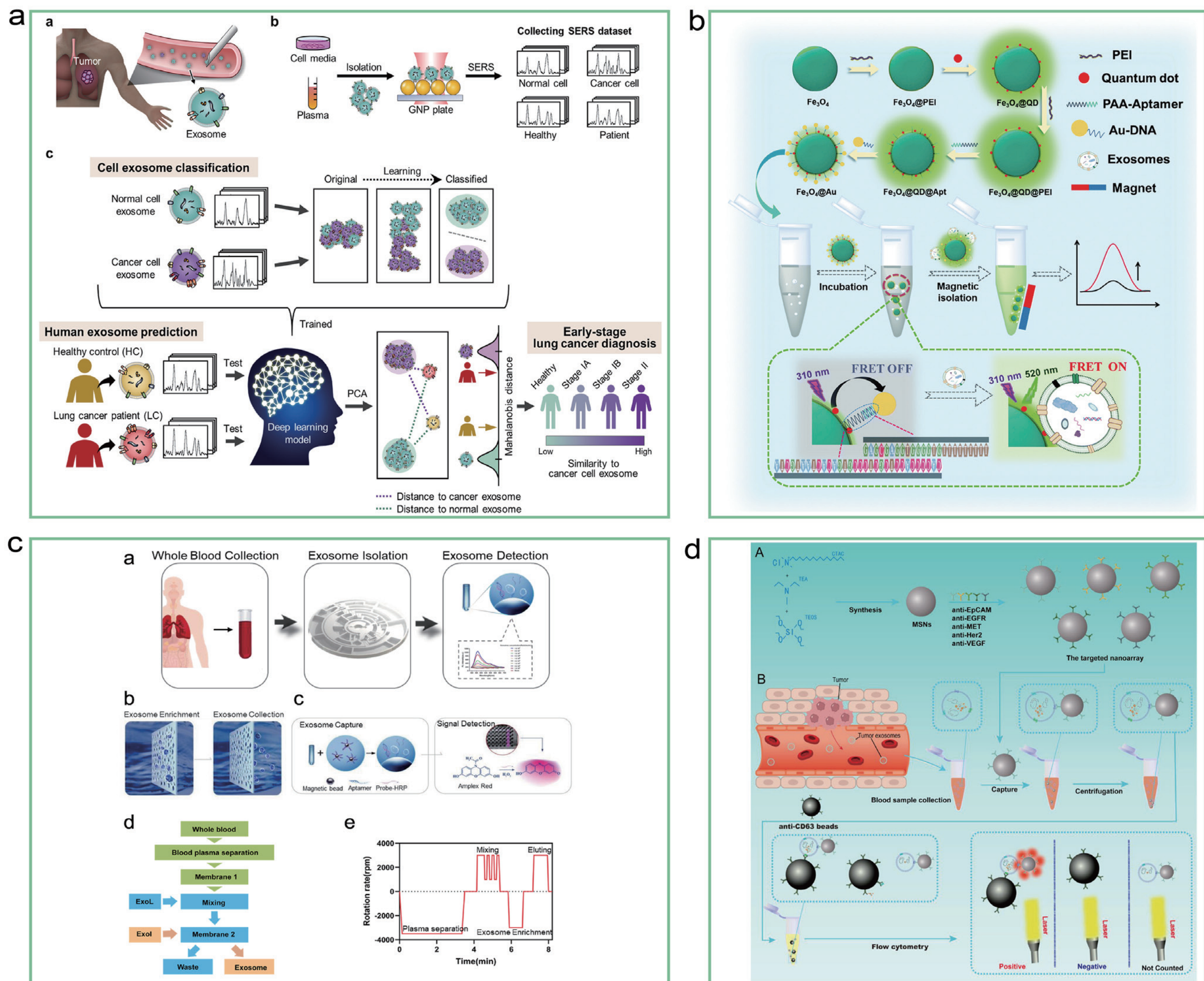

Fig. 3. Schematic overview of the deep learning-based circulating exosome analysis for lung cancer detection. (a) Deep learning-based circulating exosome analysis for lung cancer detection (copyright American Chemical Society, 2024)[64]; (b) The operational mechanism of the Förster resonance energy transfer (FRET) strategy (copyright Royal Society of Chemistry, 2021)[65]; (c) The process of exosome isolation and detection (copyright Elsevier, 2022)[66]; (d) Diagrammatic representation of the fabricated targeted nanoarray and its application in detecting tumor exosomes from the blood of lung cancer patients (copyright Elsevier, 2024).[67] PAA, polyacrylic acid; PCA, principal component analysis; PEI, poly (ethylene imine); SERS, surface-enhanced Raman spectroscopy.

build a multi-marker detection model to improve diagnostic sensitivity and specificity, especially in the early detection of lung cancer. Third, using machine learning and artificial intelligence (AI) algorithms, we can explore the potential features in exosome data, optimize marker screening and result interpretation, thereby improving the accuracy of diagnosis and prognosis, and promoting personalized treatment.[72–74] Finally, we can combine exosome research with other LB technologies (e.g., CTC, free DNA detection, etc.) to conduct multi-omics data integration analysis, aiming to provide a more comprehensive cancer diagnosis and dynamic detection solution.

The role of protein in lung cancer diagnosis and prognosis

PD-L1, an immune checkpoint protein also known as CD247 or B7-H1, is expressed on the membranes of tumor cells and antigen-presenting cells.[75] PD-L1 interacts with its corresponding receptor PD-1 on T cells, suppressing T cell activation and weakening anti-tumor immune responses. Monoclonal antibodies targeting the PD-1/PD-L1 pathway demonstrate clinical efficacy in patients with high PD-L1 expression, as determined by IHC staining of tumor biopsies.[76] Nevertheless, some patients with low tumor PD-L1 expression still respond to ICIs, likely due to the dynamic and complex nature of immune regulation that static IHC evaluations cannot fully capture. Furthermore, PD-L1 expression on both malignant cells and infiltrating leukocytes adds to the challenge of accurately interpreting PD-L1 levels in tumor tissues. The inconsistency in antibody performance for detecting PD-L1 poses an ongoing challenge, impeding the standardization of PD-L1 IHC methodologies. Additionally, the heterogeneous expression of PD-L1 between primary and metastatic lesions reduces the reliability of tissue-based assessments. Analyzing PD-L1 status in the blood through circulating markers such as CTCs, EVs, and PBMCs of-

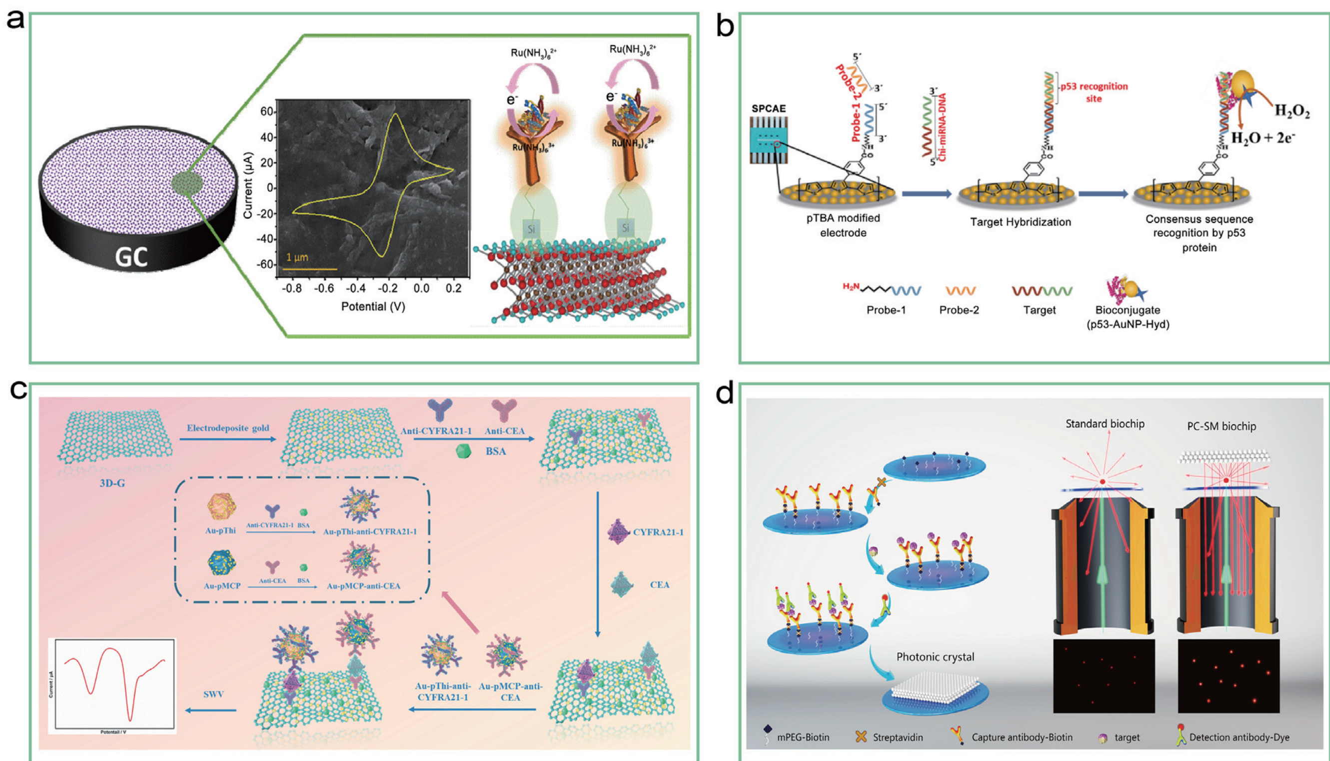

Fig. 4. Protein detection. (a) Carcinoembryonic antigen (CEA) detection (copyright Elsevier, 2018)[88]; (b) Electrochemical detection mechanism for target micro RNAs (miRNAs) (Chi-miRNA-DNA) on a sensing probe modified screen-printed carbon array electrode (SPACE) using a p53-AuNP-Hyd bioconjugate (copyright Elsevier, 2022)[89]; (c) Immunoprobe preparation process and the construction of the sandwich immunosensor (copyright Elsevier, 2021)[82]; (d) PC-SM biochip and the mechanism underlying fluorescence signal enhancement (copyright American Chemical Society, 2024).[90] BSA, Bovine Serum Albumin; CYFRA, cytokeratin 19 fragment 21-1; GC, glassy carbon; mPEG, methoxy polyethylene glycol; pTBA, poly (2,2′: 5′, 2″ -terthiophene-3′ -(p-benzoic acid)); SWV, square wave voltammetry.

fers a complementary approach to mitigate some limitations associated with tissue biopsies.[5]

Researchers have developed numerous ultrasensitive LB technologies to detect lung cancer-related biomarkers.[21,77–80] LB technology mainly relies on various metals, semiconductors, metal skeleton compounds, and other materials.[48,80–87] For example, ultra-thin Ti^3C^2-MXene nanosheets are synthesized using a minimal intensity layer exfoliation method and uniformly functionalized with aminopropyl silane (f-Ti^3C^2-MXene) to provide covalent bonding sites (anti-carcino-embryonic antigen (CEA)) for label-free ultrasensitive detection of cancer biomarkers, such as CEA (Fig. 4a).[88] An exosome microRNA array sensor, with biological conjugates consisting of p53 protein and hydrazine, selectively captures targets in p53 recognition with excellent specificity and outstanding analytical performance (Fig. 4b).[89] An electrochemical biosensor utilizing multiple signal amplification strategies for the concurrent detection of two lung cancer biomarkers: cytokeratin 19 fragment 21-1 and CEA (Fig. 4c).[82] The PC-SM biosensor chip enhances the signal-to-noise ratio of a single molecule approximately threefold by coupling the energy of a single-molecule photon with the optical bandgap of a photonic crystal. This chip was effectively employed to detect low-abundance leukemia inhibitory factors in the blood of both pancreatic cancer patients and healthy individuals (Fig. 4d).[90] In real scenarios, there may be situations where protein markers are distributed on exosomes and in free form. In such cases, we can pre-treat the sample before testing, separate free proteins and exosomal proteins, and then perform group testing. It is also possible to design different detection channels in the same system and perform dual-channel simultaneous testing. Finally, protein detection can also be combined with other omics data (RNA, DNA mutations, or metabolomics) to reveal the biological significance of free and exosomal proteins from multiple dimensions, thereby enhancing the accuracy and comprehensiveness of disease diagnosis.[91,92]

Challenges and future directions

LB holds significant promise for diagnosing, prognosticating, and predicting lung cancer treatment response. However, it continues to encounter challenges, such as detecting rare CTCs. Analyzing CTCs may provide a broader understanding of the genomic landscape of the cancer as a whole, while also presenting a safer and more cost-effective alternative to biopsying multiple tumor sites. However, evaluating tumor heterogeneity lacks standardized protocols. Although existing evidence indicates that CTCs can offer molecular insights into the overall cancer, it remains uncertain whether metastatic lesions in different organs release CTCs in a uniform manner.[93] The sensitivity and accuracy of CTC detection are limited, necessitating more advanced microfluidic and imaging technologies, as well as machine learning methods, to enhance capture and analysis capabilities. Another challenge is that some biomarkers are delicate and demand careful handling before analysis. Factors such as the sample type and amount, storage condi-

tions, timing of collection, clinical variables, and individual patient biological characteristics must be considered.[94] Furthermore, isolating and analyzing these biomarkers requires highly specific and sensitive techniques, particularly due to the low concentration of certain molecules in bodily fluids. Additionally, the analytical performance (such as sensitivity, specificity, detection limits, and stability) of various LB techniques requires further assessment and optimization.

Emerging technologies like AI will play a key role in deciphering the complexities of cancer and formulating treatment strategies to improve cancer management. AI holds significant promise in oncology, particularly in diagnosing, treating, and predicting the prognosis of cancers. Its key applications in healthcare encompass medical imaging, clinical decision support, and surgical robotics. The integration of LB with AI could enhance the early detection, management, and prognosis of lung cancer patients. While LB is unlikely to fully replace traditional tissue biopsy, combining LB with tissue biopsy and multidimensional data analysis can overcome the limitations of relying on a single method. Additionally, the standardization of longitudinal sampling still requires further optimization. Inter-laboratory collaboration, the development of consensus guidelines, and external quality assessment programs are key to resolving the standardization issues. These measures will enhance the reliability and reproducibility of LB, promoting its widespread application in clinical practice and providing a more robust foundation for precision lung cancer treatment.

Conclusions

Ultrasensitive cancer biomarker detection provides precise, early, and dynamic information, significantly enhancing the success rate of cancer immunotherapy and improving patients' quality of life. It not only facilitates the early identification of cancer but also plays a supervisory and guiding role during treatment, enabling more personalized and optimized therapeutic strategies. CTCs, exosomes, and proteins are more easily and cost-effectively collected than tissue biopsies and, in many instances, offer real-time molecular insights and response data—particularly for patients where tumor tissue biopsies are challenging to obtain. These advanced LB technologies can be combined with tissue DNA analysis to guide molecularly targeted therapies. Additionally, they can be used alongside imaging to provide important information on clinical response, drug resistance, and prognosis. Numerous promising directions for the future of liquid biopsies are emerging, including the use of alternative fluids (cerebrospinal fluid, ascites, effusions, urine, etc.) and the exploration of additional biomarkers like circulating tumor RNA and cell-free micro-RNA. Another key area of active and potentially groundbreaking research is the early detection of cancer when it is still treatable. Overall, LB is proving to be a versatile and impactful tool for enhancing oncology management and patient outcomes.

Furthermore, LB has significant potential in the immunotherapy of lung cancer. As a simple, rapid, and minimally invasive tool, LB provides valuable sources of immune-related biomarkers, which can guide precision treatment. Its advantages include the ability for repeat sampling, dynamic monitoring, and follow-up of treatment responses, especially in cases where ICI therapy induces substantial tumor changes. By capturing specific or multiple biomarker combinations at the optimal time, LB helps in formulating personalized treatment strategies, improving treatment outcomes and patient quality of life. While not yet incorporated into treatment guidelines, current evidence indicates that LB may be valuable for the continuous monitoring and optimization of lung cancer immunotherapy, offering a promising pathway for achieving precision medicine.

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Conflict of interest

One of the authors, Chuan Xu, has been an Associate Editor of *Oncology Advances* since May 2024. The authors have no other conflicts of interest to note.

Author contributions

Drafting, validation (YL, CL), conceptualization, and writing - review & editing (CJ, CX). The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

References

[1] Zhou Y, Li M, Wang H, Sun S. Dual-signal amplified electrochemical biosensor based on eATRP and PEI for early detection of lung cancer. Bioelectrochemistry 2022;148:108224. doi:10.1016/j.bioelechem.2022.108224, PMID:36029762.

[2] Amirhosein S, Shahnam S, Saghar M, Mobina M, Kiarash M, Samira S, *et al*. The role of PD-1/PD-L1 signaling pathway in cancer pathogenesis and treatment: a systematic review. J Cancer Metastasis Treat 2024;10:19. doi:10.20517/2394-4722.2024.15.

[3] Zdrenka M, Kowalewski A, Ahmadi N, Sadiqi RU, Chmura Ł, Borowczak J, *et al*. Refining PD-1/PD-L1 assessment for biomarker-guided immunotherapy: A review. Biomol Biomed 2024;24(1):14–29. doi:10.17305/bb.2023.9265, PMID:37877810.

[4] Pandey P, Khan F, Qari HA, Upadhyay TK, Alkhateeb AF, Oves M. Revolutionization in Cancer Therapeutics via Targeting Major Immune Checkpoints PD-1, PD-L1 and CTLA-4. Pharmaceuticals (Basel) 2022;15(3):335. doi:10.3390/ph15030335, PMID:35337133.

[5] Yang Y, Liu H, Chen Y, Xiao N, Zheng Z, Liu H, *et al*. Liquid biopsy on the horizon in immunotherapy of non-small cell lung cancer: current status, challenges, and perspectives. Cell Death Dis 2023;14(3):230. doi:10.1038/s41419-023-05757-5, PMID:37002211.

[6] Shiravand Y, Khodadadi F, Kashani SMA, Hosseini-Fard SR, Hosseini S, Sadeghirad H, *et al*. Immune Checkpoint Inhibitors in Cancer Therapy. Curr Oncol 2022;29(5):3044–3060. doi:10.3390/curroncol29050247, PMID:35621637.

[7] Centanni M, Moes DJAR, Trocóniz IF, Ciccolini J, van Hasselt JGC. Clinical Pharmacokinetics and Pharmacodynamics of Immune Checkpoint Inhibitors. Clin Pharmacokinet 2019;58(7):835–857. doi:10.1007/s40262-019-00748-2, PMID:30815848.

[8] Vranic S, Gatalica Z. PD-L1 testing by immunohistochemistry in immuno-oncology. Biomol Biomed 2023;23(1):15–25. doi:10.17305/bjbms.2022.7953, PMID:35964287.

[9] Incorvaia L, Fanale D, Badalamenti G, Barraco N, Bono M, Corsini LR, *et al*. Programmed Death Ligand 1 (PD-L1) as a Predictive Biomarker for Pembrolizumab Therapy in Patients with Advanced Non-Small-Cell Lung Cancer (NSCLC). Adv Ther 2019;36(10):2600–2617. doi:10.1007/s12325-019-01057-7, PMID:31432460.

[10] Sanguedolce F, Zanelli M. Assessing PD-L1 Expression in Different Tumor Types. In: Rezaei N (ed). Handbook of Cancer and Immunology. Cham: Springer International Publishing; 2022:1–21.

[11] Zhou KI, Peterson B, Serritella A, Thomas J, Reizine N, Moya S, *et al*. Spatial and Temporal Heterogeneity of PD-L1 Expression and Tumor Mutational Burden in Gastroesophageal Adenocarcinoma at Baseline Diagnosis and after Chemotherapy. Clin Cancer Res 2020;26(24):6453–6463. doi:10.1158/1078-0432.CCR-20-2085, PMID:32820017.

[12] Haragan A. Immunohistochemistry for prediction of response to immunotherapy. Diagn Histopathol 2024;30(11):604–612. doi:10.1016/j.mpdhp.2024.08.002.

[13] Rimm DL, Han G, Taube JM, Yi ES, Bridge JA, Flieder DB, *et al*. A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer. JAMA Oncol 2017;3(8):1051–1058. doi:10.1001/jamaoncol.2017.0013, PMID:28278348.

[14] Bao Y, Zhang D, Guo H, Ma W. Beyond blood: Advancing the frontiers of liquid biopsy in oncology and personalized medicine. Cancer Sci 2024;115(4):1060–1072. doi:10.1111/cas.16097, PMID:38308498.

[15] Chen K, He Y, Wang W, Yuan X, Carbone DP, Yang F. Development of new techniques and clinical applications of liquid biopsy in lung cancer management. Sci Bull (Beijing) 2024;69(10):1556–1568. doi:10.1016/j.scib.2024.03.062, PMID:38641511.

[16] Fasano R, Serratì S, Rafaschieri T, Longo V, Di Fonte R, Porcelli L, *et al*. Small-Cell Lung Cancer: Is Liquid Biopsy a New Tool Able to Predict the Efficacy of Immunotherapy? Biomolecules 2024;14(4):396. doi:10.3390/biom14040396, PMID:38672414.

[17] Ren F, Fei Q, Qiu K, Zhang Y, Zhang H, Sun L. Liquid biopsy techniques and lung cancer: diagnosis, monitoring and evaluation. J Exp Clin Cancer Res 2024;43(1):96. doi:10.1186/s13046-024-03026-7, PMID:38561776.

[18] Vora N, Shekar P, Hanulia T, Esmail M, Patra A, Georgakoudi I. Deep learning-enabled detection of rare circulating tumor cell clusters in whole blood using label-free, flow cytometry. Lab Chip 2024;24(8):2237–2252. doi:10.1039/d3lc00694h, PMID:38456773.

[19] Jacot W, Mazel M, Mollevi C, Pouderoux S, D'Hondt V, Cayrefourcq L, *et al*. Clinical Correlations of Programmed Cell Death Ligand 1 Status in Liquid and Standard Biopsies in Breast Cancer. Clin Chem 2020;66(8):1093–1101. doi:10.1093/clinchem/hvaa121, PMID:32712650.

[20] Fan C, Jiang B, Shi W, Chen D, Zhou M. Tri-Channel Electrochemical Immunobiosensor for Combined Detections of Multiple Exosome Biomarkers of Lung Cancer. Biosensors (Basel) 2022;12(7):435. doi:10.3390/bios12070435, PMID:35884238.

[21] Kiio LK, Onyatta JO, Ndangili PM, Oloo F, Santamaria C, Montuenga LM, *et al*. Ultrasensitive immunosensor for multiplex detection of cancer biomarkers carcinoembryonic antigen (CEA) and yamaguchi sarcoma viral oncogene homolog 1 (YES1) based on eco-friendly synthesized gold nanoparticles. Talanta 2024;266(Pt 1):124934. doi:10.1016/j.talanta.2023.124934, PMID:37454512.

[22] Zhao J, Long Y, He C, Leng Y, Huang Z, Ma Y, *et al*. Ultrasensitive detection of circulating tumor cells via DNA walker driven by a DNA circuit synergized with MOF-on-MOF nanozyme. Chem Eng J 2024;479:147513. doi:10.1016/j.cej.2023.147513.

[23] Pascual J, Attard G, Bidard FC, 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, PMID:35809752.

[24] Liu X, Wang F, Meng Y, Zhao L, Shi W, Wang X, *et al*. Electrochemical/visual microfluidic detection with a covalent organic framework supported platinum nanozyme-based device for early diagnosis of pheochromocytoma. Biosens Bioelectron 2022;207:114208. doi:10.1016/j.bios.2022.114208, PMID:35344731.

[25] Kou Q, Yuan H, Zhao G, Li Q, Zhang J, Li M, *et al*. Ultrasensitive detection of circulating tumor cells in clinical blood samples by a three-dimensional network nanovehicle-based aptasensor platform. Sensor Actuat B-Chem 2024;404:135172. doi:10.1016/j.snb.2023.135172.

[26] Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic - implementation issues and future challenges. Nat Rev Clin Oncol 2021;18(5):297–312. doi:10.1038/s41571-020-00457-x, PMID:33473219.

[27] Lv W, Fu B, Liu W, Huang W, Li M, Liu Y, *et al*. Efficient detection of single circulating tumor cell in blood using Raman mapping based on Aptamer-SERS bio-probe coupled with micropore membrane filtration. Talanta 2024;267:125220. doi:10.1016/j.talanta.2023.125220, PMID:37783108.

[28] Smit DJ, Pantel K. Circulating tumor cells as liquid biopsy markers in cancer patients. Mol Aspects Med 2024;96:101258. doi:10.1016/j.mam.2024.101258, PMID:38387225.

[29] Lin HC, Hsu HC, Hsieh CH, Wang HM, Huang CY, Wu MH, *et al*. A negative selection system PowerMag for effective leukocyte depletion and enhanced detection of EpCAM positive and negative circulating tumor cells. Clin Chim Acta 2013;419:77–84. doi:10.1016/j.cca.2013.01.018, PMID:23415697.

[30] Dianat-Moghadam H, Mahari A, Heidarifard M, Parnianfard N, Pourmousavi-Kh L, Rahbarghazi R, *et al*. NK cells-directed therapies target circulating tumor cells and metastasis. Cancer Lett 2021;497:41–53. doi:10.1016/j.canlet.2020.09.021, PMID:32987138.

[31] Li X, Li X, Chen S, Wu Y, Liu Y, Hu T, *et al*. TRAP1 Shows Clinical Significance in the Early Diagnosis of Small Cell Lung Cancer. J Inflamm Res 2021;14:2507–2514. doi:10.2147/JIR.S313440, PMID:34163209.

[32] Szczerba BM, Castro-Giner F, Vetter M, Krol I, Gkountela S, Landin J, *et al*. Neutrophils escort circulating tumour cells to enable cell cycle progression. Nature 2019;566(7745):553–557. doi:10.1038/s41586-019-0915-y, PMID:30728496.

[33] Wang Y, Chen R, Wa Y, Ding S, Yang Y, Liao J, *et al*. Tumor Immune Microenvironment and Immunotherapy in Brain Metastasis From Non-Small Cell Lung Cancer. Front Immunol 2022;13:829451. doi:10.3389/fimmu.2022.829451, PMID:35251014.

[34] Li W, Li F, Zhang X, Lin HK, Xu C. Insights into the post-translational modification and its emerging role in shaping the tumor microenvironment. Signal Transduct Target Ther 2021;6(1):422. doi:10.1038/s41392-021-00825-8, PMID:34924561.

[35] Kotsifaki A, Maroulaki S, Armakolas A. Exploring the Immunological Profile in Breast Cancer: Recent Advances in Diagnosis and Prognosis through Circulating Tumor Cells. Int J Mol Sci 2024;25(9):4832. doi:10.3390/ijms25094832, PMID:38732051.

[36] Morozov D, Mironov V, Moryachkov RV, Shchugoreva IA, Artyushenko PV, Zamay GS, *et al*. The role of SAXS and molecular simulations in 3D structure elucidation of a DNA aptamer against lung cancer. Mol Ther Nucleic Acids 2021;25:316–327. doi:10.1016/j.omtn.2021.07.015, PMID:34458013.

[37] Roumeliotou A, Strati A, Chamchougia F, Xagara A, Tserpeli V, Smilkou S, *et al*. Comprehensive Analysis of CXCR4, JUNB, and PD-L1 Expression in Circulating Tumor Cells (CTCs) from Prostate Cancer Patients. Cells 2024;13(9):782. doi:10.3390/cells13090782, PMID:38727318.

[38] Balakrishnan SG, Ahmad MR, Koloor SSR, Petrů M. Separation of ctDNA by superparamagnetic bead particles in microfluidic platform for early cancer detection. J Adv Res 2021;33:109–116. doi:10.1016/j.jare.2021.03.001, PMID:34603782.

[39] Shishido SN, Carlsson A, Nieva J, Bethel K, Hicks JB, Bazhenova L, *et al*. Circulating tumor cells as a response monitor in stage IV non-small cell lung cancer. J Transl Med 2019;17(1):294. doi:10.1186/s12967-019-2035-8, PMID:31462312.

[40] Zhu HH, Liu YT, Feng Y, Zhang LN, Zhang TM, Dong GL, *et al*. Circulating tumor cells (CTCs)/circulating tumor endothelial cells (CTECs) and their subtypes in small cell lung cancer: Predictors for response and prognosis. Thorac Cancer 2021;12(20):2749–2757. doi:10.1111/1759-7714.14120, PMID:34423906.

[41] Mastromarino MG, Parini S, Azzolina D, Habib S, De Marni ML, Luise C, *et al*. Liquid Biopsy Detecting Circulating Tumor Cells in Patients with Non-Small Cell Lung Cancer: Preliminary Results of a Pilot Study. Biomedicines 2023;11(1):153. doi:10.3390/biomedicines11010153, PMID:36672660.

[42] Welsch E, Holzer B, Schuster E, Fabikan H, Weinlinger C, Hauptmann-Repitz E, *et al*. Prognostic significance of circulating tumor cells and tumor related transcripts in small cell lung cancer: A step further to clinical implementation. Int J Cancer 2024;154(12):2189–2199. doi:10.1002/ijc.34886, PMID:38353516.

[43] Li W, Liu JB, Hou LK, Yu F, Zhang J, Wu W, *et al*. Liquid biopsy in lung cancer: significance in diagnostics, prediction, and treatment monitoring. Mol Cancer 2022;21(1):25. doi:10.1186/s12943-022-01505-z, PMID:35057806.

[44] Wang X, Wang L, Lin H, Zhu Y, Huang D, Lai M, *et al*. Research progress of CTC, ctDNA, and EVs in cancer liquid biopsy. Front Oncol 2024;14:1303335. doi:10.3389/fonc.2024.1303335, PMID:38333685.

[45] Hugenschmidt H, Labori KJ, Brunborg C, Verbeke CS, Seeberg LT, Schirmer CB, *et al*. Circulating Tumor Cells are an Independent Predictor of Shorter Survival in Patients Undergoing Resection for Pancreatic and Periampullary Adenocarcinoma. Ann Surg 2020;271(3):549–558. doi:10.1097/SLA.0000000000003035, PMID:30216219.

[46] Mahuron KM, Fong Y. Applications of Liquid Biopsy for Surgical Patients With Cancer: A Review. JAMA Surg 2024;159(1):96–103. doi:10.1001/jamasurg.2023.5394, PMID:37910091.

[47] Neves RPL, Ammerlaan W, Andree KC, Bender S, Cayrefourcq L, Driemel C, *et al*. Proficiency Testing to Assess Technical Performance for CTC-Processing and Detection Methods in CANCER-ID. Clin Chem 2021;67(4):631–641. doi:10.1093/clinchem/hvaa322, PMID:33491069.

[48] Liang K, Liu F, Fan J, Sun D, Liu C, Lyon CJ, *et al*. Nanoplasmonic Quantification of Tumor-derived Extracellular Vesicles in Plasma Microsamples for Diagnosis and Treatment Monitoring. Nat Biomed Eng 2017;1:0021. doi:10.1038/s41551-016-0021, PMID:28791195.

[49] Mao X, Xu J, Wang W, Liang C, Hua J, Liu J, *et al*. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Mol Cancer 2021;20(1):131. doi:10.1186/s12943-021-01428-1, PMID:34635121.

[50] Cui S, Cheng Z, Qin W, Jiang L. Exosomes as a liquid biopsy for lung cancer. Lung Cancer 2018;116:46–54. doi:10.1016/j.lungcan.2017.12.012, PMID:29413050.

[51] Yan S, Jiang C, Janzen A, Barber TR, Seger A, Sommerauer M, *et al*. Neuronally Derived Extracellular Vesicle α-Synuclein as a Serum Biomarker for Individuals at Risk of Developing Parkinson Disease. JAMA Neurol 2024;81(1):59–68. doi:10.1001/jamaneurol.2023.4398, PMID:38048087.

[52] Liu Y, Cai C, Xu W, Li B, Wang L, Peng Y, *et al*. Interpretable Machine Learning-Aided Optical Deciphering of Serum Exosomes for Early Detection, Staging, and Subtyping of Lung Cancer. Anal Chem 2024;96(41):16227–16235. doi:10.1021/acs.analchem.4c02914, PMID:39361049.

[53] Xia T, Zhang M, Lei W, Yang R, Fu S, Fan Z, *et al*. Advances in the role of STAT3 in macrophage polarization. Front Immunol 2023;14:1160719. doi:10.3389/fimmu.2023.1160719, PMID:37081874.

[54] Wu H, Liu Q, Li J, Leng X, He Y, Liu Y, *et al*. Tumor-Resident Microbiota-Based Risk Model Predicts Neoadjuvant Therapy Response of Locally Advanced Esophageal Squamous Cell Carcinoma Patients. Adv Sci (Weinh) 2024;11(41):e2309742. doi:10.1002/advs.202309742, PMID:39268829.

[55] Zheng L, Fisher G, Miller RE, Peschon J, Lynch DH, Lenardo MJ. Induction of apoptosis in mature T cells by tumour necrosis factor. Nature 1995;377(6547):348–351. doi:10.1038/377348a0, PMID:7566090.

[56] Gupta P, Kadamberi IP, Mittal S, Tsaih SW, George J, Kumar S, *et al*. Tumor Derived Extracellular Vesicles Drive T Cell Exhaustion in Tumor Microenvironment through Sphingosine Mediated Signaling and Impacting Immunotherapy Outcomes in Ovarian Cancer. Adv Sci (Weinh) 2022;9(14):e2104452. doi:10.1002/advs.202104452, PMID:35289120.

[57] Wang X, Jian Q, Zhang Z, Gu J, Wang X, Wang Y. Effect of tumor-derived extracellular vesicle-shuttled lncRNA MALAT1 on proliferation, invasion and metastasis of triple-negative breast cancer by regulating macrophage M2 polarization via the POSTN/Hippo/YAP axis. Transl Oncol 2024;49:102076. doi:10.1016/j.tranon.2024.102076, PMID:39222611.

[58] Zhang F, Guo J, Zhang Z, Duan M, Wang G, Qian Y, *et al*. Application of engineered extracellular vesicles for targeted tumor therapy. J Biomed Sci 2022;29(1):14. doi:10.1186/s12929-022-00798-y, PMID:35189894.

[59] Kjeldsen JW, Lorentzen CL, Martinenaite E, Ellebaek E, Donia M, Holmstroem RB, *et al*. A phase 1/2 trial of an immune-modulatory vaccine against IDO/PD-L1 in combination with nivolumab in metastatic melanoma. Nat Med 2021;27(12):2212–2223. doi:10.1038/s41591-021-01544-x, PMID:34887574.

[60] Zhang C, Fan Y, Che X, Zhang M, Li Z, Li C, *et al*. Anti-PD-1 Therapy Response Predicted by the Combination of Exosomal PD-L1 and CD28. Front Oncol 2020;10:760. doi:10.3389/fonc.2020.00760, PMID:32528882.

[61] Brüggemann C, Kirchberger MC, Goldinger SM, Weide B, Konrad A, Erdmann M, *et al*. Predictive value of PD-L1 based on mRNA level in the treatment of stage IV melanoma with ipilimumab. J Cancer Res Clin Oncol 2017;143(10):1977–1984. doi:10.1007/s00432-017-2450-2, PMID:28616701.

[62] Sun Z, Tong Y, Zhao L, Li J, Gao F, Wang C, *et al*. MoS2@Ti3C2 nanohybrid-based photoelectrochemical biosensor: A platform for ultrasensitive detection of cancer biomarker exosomal miRNA. Talanta 2022;238(Pt 2):123077. doi:10.1016/j.talanta.2021.123077, PMID:34814060.

[63] Liang H, Zhang L, Zhao X, Rong J. The therapeutic potential of exosomes in lung cancer. Cell Oncol (Dordr) 2023;46(5):1181–1212. doi:10.1007/s13402-023-00815-8, PMID:37365450.

[64] Shin H, Oh S, Hong S, Kang M, Kang D, Ji YG, *et al*. Early-Stage Lung Cancer Diagnosis by Deep Learning-Based Spectroscopic Analysis of Circulating Exosomes. ACS Nano 2020;14(5):5435–5444. doi:10.1021/acsnano.9b09119, PMID:32286793.

[65] Zhu N, Li G, Zhou J, Zhang Y, Kang K, Ying B, *et al*. A light-up fluorescence resonance energy transfer magnetic aptamer-sensor for ultra-sensitive lung cancer exosome detection. J Mater Chem B 2021;9(10):2483–2493. doi:10.1039/d1tb00046b, PMID:33656037.

[66] Zhao L, Wang H, Fu J, Wu X, Liang XY, Liu XY, *et al*. Microfluidic-based exosome isolation and highly sensitive aptamer exosome membrane protein detection for lung cancer diagnosis. Biosens Bioelectron 2022;214:114487. doi:10.1016/j.bios.2022.114487, PMID:35780540.

[67] Lian S, Wang Q, Liu Y, Lu Y, Huang L, Deng H, *et al*. Multi-targeted nanoarrays for early broad-spectrum detection of lung cancer based on blood biopsy of tumor exosomes. Talanta 2024;276:126270. doi:10.1016/j.talanta.2024.126270, PMID:38761662.

[68] Jiang C, Zhang N, Hu X, Wang H. Tumor-associated exosomes promote lung cancer metastasis through multiple mechanisms. Mol Cancer 2021;20(1):117. doi:10.1186/s12943-021-01411-w, PMID:34511114.

[69] Lapitz A, Arbelaiz A, Olaizola P, Aranburu A, Bujanda L, Perugorria MJ, *et al*. Extracellular Vesicles in Hepatobiliary Malignancies. Front Immunol 2018;9:2270. doi:10.3389/fimmu.2018.02270, PMID:30369925.

[70] Wen SW, Lima LG, Lobb RJ, Norris EL, Hastie ML, Krumeich S, *et al*. Breast Cancer-Derived Exosomes Reflect the Cell-of-Origin Phenotype. Proteomics 2019;19(8):e1800180. doi:10.1002/pmic.201800180, PMID:30672117.

[71] Tang H, Yu D, Zhang J, Wang M, Fu M, Qian Y, *et al*. The new advance of exosome-based liquid biopsy for cancer diagnosis. J Nanobiotechnology 2024;22(1):610. doi:10.1186/s12951-024-02863-0, PMID:39380060.

[72] Wu N, Zhang XY, Xia J, Li X, Yang T, Wang JH. Ratiometric 3D DNA Machine Combined with Machine Learning Algorithm for Ultrasensitive and High-Precision Screening of Early Urinary Diseases. ACS Nano 2021;15(12):19522–19534. doi:10.1021/acsnano.1c06429, PMID:34813275.

[73] Lei Y, Fei X, Ding Y, Zhang J, Zhang G, Dong L, *et al*. Simultaneous subset tracing and miRNA profiling of tumor-derived exosomes via dual-surface-protein orthogonal barcoding. Sci Adv 2023;9(40):eadi1556. doi:10.1126/sciadv.adi1556, PMID:37792944.

[74] Diao X, Li X, Hou S, Li H, Qi G, Jin Y. Machine Learning-Based Label-

Free SERS Profiling of Exosomes for Accurate Fuzzy Diagnosis of Cancer and Dynamic Monitoring of Drug Therapeutic Processes. Anal Chem 2023;95(19):7552–7559. doi:10.1021/acs.analchem.3c00026, PMID:37139959.
[75] Cheng Y, Li H, Zhao D, Liu Y, Wang Y, Liu X, *et al*. Method development for detection of PD-L1 in circulating tumor cells in small-cell lung cancer patients. J Clin Oncol 2017;35(15_suppl):e20012–e20012. doi:10.1200/JCO.2017.35.15_suppl.e20012.
[76] Wang Y, Shen C, Zeng X, Xiong Y, Li K, Huang K, *et al*. Tandem hybridization chain reaction and selective coordination enable fluorescence detection of exosomes in lung cancer. Sensor Actuat B-Chem 2024;410:35722. doi:10.1016/j.snb.2024.135722.
[77] Xianyu Y, Wu J, Chen Y, Zheng W, Xie M, Jiang X. Controllable Assembly of Enzymes for Multiplexed Lab-on-a-Chip Bioassays with a Tunable Detection Range. Angew Chem Int Ed Engl 2018;57(25):7503–7507. doi:10.1002/anie.201801815, PMID:29480958.
[78] Zhang W, Lu Y, Su C, Wang Y, Wang YF, Zhang B, *et al*. Confinement-guided ultrasensitive optical assay with artificial intelligence for disease diagnostics. Innov Med 2023;1(2):100023. doi:10.59717/j.xinnmed.2023.100023.
[79] Zhang J, Li Y, Chai F, Li Q, Wang D, Liu L, *et al*. Ultrasensitive point-of-care biochemical sensor based on metal-AIEgen frameworks. Sci Adv 2022;8(30):eabo1874. doi:10.1126/sciadv.abo1874, PMID:358 95821.
[80] Zhou S, Tu D, Liu Y, You W, Zhang Y, Zheng W, *et al*. Ultrasensitive Point-of-Care Test for Tumor Marker in Human Saliva Based on Luminescence-Amplification Strategy of Lanthanide Nanoprobes. Adv Sci (Weinh) 2021;8(5):2002657. doi:10.1002/advs.202002657, PMID:33717839.
[81] Cheng H, Sun W, Lu Y, Li H, Su W, Zhang J, *et al*. Hot electrons in carbon nitride with ultralong lifetime and their application in reversible dynamic color displays. Cell Rep Phys Sci 2021;2(8):100516. doi:10.1016/j.xcrp.2021.100516.
[82] Yang H, Bao J, Huo D, Zeng Y, Wang X, Samalo M, *et al*. Au doped polythionine and poly-m-Cresol purple: Synthesis and their application in simultaneously electrochemical detection of two lung cancer markers CEA and CYFRA21-1. Talanta 2021;224:121816. doi:10.1016/j.talanta.2020.121816, PMID:33379041.
[83] Lu Y, Chen H, Cheng H, Qiu H, Jiang C, Zheng Y. Plasmonic Physical Unclonable Function Labels Based on Tricolored Silver Nanoparticles: Implications for Anticounterfeiting Applications. ACS Appl Nano Mater 2022;5(7):9298–9305. doi:10.1021/acsanm.2c01622.
[84] Lu Y, Cheng H, Li GC, Han F, Jiang C, Lo TW, *et al*. Dynamic Cryptography through Plasmon-Enhanced Fluorescence Blinking. Adv Funct Mater 2022;32(30):2201372. doi:10.1002/adfm.202201372.
[85] Xu H, Chen X, Wang H, Wang C, Guo Y, Lin Y, *et al*. Utilization of aggregation-induced emission materials in urinary system diseases. Aggregate 2024;5(5):e580. doi:10.1002/agt2.580.
[86] Zheng W, Zhou S, Xu J, Liu Y, Huang P, Liu Y, *et al*. Ultrasensitive Luminescent In Vitro Detection for Tumor Markers Based on Inorganic Lanthanide Nano-Bioprobes. Adv Sci (Weinh) 2016;3(11):1600197. doi:10.1002/advs.201600197, PMID:27980996.
[87] Zhou S, Zheng W, Chen Z, Tu D, Liu Y, Ma E, *et al*. Dissolution-Enhanced Luminescent Bioassay Based on Inorganic Lanthanide Nanoparticles. Angew Chem 2014;126(46):12706–12710. doi:10.1002/ange.201405937.
[88] Kumar S, Lei Y, Alshareef NH, Quevedo-Lopez MA, Salama KN. Biofunctionalized two-dimensional Ti(3)C(2) MXenes for ultrasensitive detection of cancer biomarker. Biosens Bioelectron 2018;121:243–249. doi:10.1016/j.bios.2018.08.076, PMID:30219724.
[89] Mahmudunnabi RG, Umer M, Seo KD, Park DS, Chung JH, Shiddiky MJA, *et al*. Exosomal microRNAs array sensor with a bioconjugate composed of p53 protein and hydrazine for the specific lung cancer detection. Biosens Bioelectron 2022;207:114149. doi:10.1016/j.bios.2022.114149, PMID:35290882.
[90] Liu S, Ma B, Qi L, Ping J, Che Y, Zhang Y, *et al*. Ultrasensitive Detection of Cancer Biomarkers Using Photonic-Crystal-Enhanced Single-Molecule Imaging. Anal Chem 2024;96(33):13719–13726. doi:10.1021/acs.analchem.4c02863, PMID:39120618.
[91] Chen G, Zhang J, Fu Q, Taly V, Tan F. Integrative analysis of multi-omics data for liquid biopsy. Br J Cancer 2023;128(4):505–518. doi:10.1038/s41416-022-02048-2, PMID:36357703.
[92] Akhoundova D, Rubin MA. Clinical application of advanced multi-omics tumor profiling: Shaping precision oncology of the future. Cancer Cell 2022;40(9):920–938. doi:10.1016/j.ccell.2022.08.011, PMID:36055231.
[93] Bardelli A, Pantel K. Liquid Biopsies, What We Do Not Know (Yet). Cancer Cell 2017;31(2):172–179. doi:10.1016/j.ccell.2017.01.002, PMID:28196593.
[94] Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, *et al*. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol 2018;36(16):1631–1641. doi:10.1200/JCO.2017.76.8671, PMID:29504847.