RESEARCH ARTICLE

Editorial Process: Submission:06/04/2025 Acceptance:12/20/2025 Published:12/26/2025

Innovations in Oncology Diagnostics: Genetic Research and Liquid Biopsy as Tools of Personalised Medicine

Lazzat Niyazbekova[1]*, Ervin Marku[2], Plamen Petkov[3], Nazgul Karimova[4], Firdavs Ulmasov[5]

Abstract

Objective: The aim of this study was to analyze contemporary approaches to cancer diagnosis and treatment using liquid biopsy, with a particular focus on the efficacy of oncogenic biomarker detection in various biological fluids. **Materials and Methods:** This study examined scientific literature from the last five years on the use of liquid biopsy in oncology, focusing on tumour marker detection in blood, plasma, saliva, and urine, along with their analytical significance and clinical implications. **Results:** Tumour-derived circulating nucleic acids, circulating tumour cells, and secretory vesicles isolated from biological fluids were shown to be highly informative. These enabled the detection of mutations in key oncogenic markers (*AR, KRAS, EGFR, PIK3CA, PTEN, P53*), quantification of regulatory RNAs (*BCAR4, UCA1, H19, miR-21, miR-146a-5p, miR-181a*), and assessment of translational levels of proteins such as Cfh, Muc1, Bst2, Er, and Ccne1. Transcriptomic analysis provided insights into T-lymphocyte activity, tumour resistance to gefitinib and tyrosine kinase inhibitors, and the suppression of tumour progression. The expression of proteoglycans isolated from exosomes was identified as a marker of early-stage pancreatic cancer. The titre of CTCs enabled the prediction of recurrence in 64% of patients with lung carcinoma and 100% of post-surgical cases of colorectal cancer, facilitating hormone therapy adjustments in prostate cancer patients. Mutations in the *EGFR* gene, amplified from persistent deoxyribonucleic acid, were used to adjust chemotherapy regimens throughout the course of treatment. **Conclusion:** The analysis of material obtained from the biological fluids of oncology patients enabled disease prognosis, risk assessment of progression, and the determination of optimal approaches to personalised therapy, ultimately improving treatment efficacy. The collected data may serve as a foundation for clinically valuable laboratory studies and practical research.

Keywords: exosomes- tumour markers- sequencing- mutation- epigenetic changes

Asian Pac J Cancer Prev, **26 (12)**, 4549-4561

Introduction

As of today, the majority of cancer types remain incurable, with fewer than 25% of patients with metastases surviving for five years or longer [1]. As treatment strategies advance, tumours demonstrate an increasing capacity to adapt to therapy. While the majority of cancer cells sensitive to a given drug are eliminated, a small fraction acquires resistance, survives, proliferates, and migrates, leading to the formation of new tumour foci [2]. From 2004 to 2024, the field of oncology has seen rapid progress in the development of personalized cancer detection and treatment approaches, which involve "sequencing" each tumour on an individual basis. Sequencing is a laboratory technique employed to determine the exact arrangement of nucleotides, the fundamental components of DNA and RNA, within a specific DNA or RNA molecule. In the realm of liquid biopsy, sequencing facilitates the examination of genetic mutations, chromosomal abnormalities, and epigenetic modifications within tumor-derived genetic material present in biological fluids such as blood, saliva, or urine [3]. Tissue biopsy remains the conventional method for obtaining molecular markers. However, this approach is associated with several limitations, including its invasive nature, restricted access to the site of transformation, frequently insufficient sample volume, risk of tissue damage and infectious contamination, and the potential for haemorrhage [4]. Tumour cells within a single lesion often exhibit genetic heterogeneity, making it challenging

*[1]Department of Pathological Physiology named after Professor A.N. Nurmukhambetov, Kazakh State Medical University named after S.D. Asfendiyarov, Almaty, Republic of Kazakhstan. [2]Department of Pre-Clinical Studies, University of Medicine, Tirana Tirana, Albania. [3]Alexandrovska Hospital, Sofia, Bulgaria. [4]Department of Clinical Disciplines No. 2, Osh State, University Osh, Kyrgyz Republic. [5]Department of Oncology, Samarkand State Medical University, Samarkand, Uzbekistan. *For Correspondence: niyazbekovalazzat68@gmail.com*

to capture the full spectrum of oncogenic markers via biopsy. In metastatic cases, multiple biopsies are often required due to the tumour's adaptive capacity and the increasing number of growth foci [5].

One innovative approach to cancer diagnostics that has gained attention since 2010 is liquid biopsy [6]. It has been established that during tumour progression, cancer cells infiltrate biological fluids such as blood, lymph, cerebrospinal fluid, urine, saliva, and ascitic fluid, making these fluids viable sources for oncological characterization alongside tissue samples. Studies have demonstrated that liquid biopsy enables biomarker screening in cases where obtaining tissue samples is challenging, such as in malignant brain tumours. Researchers have found that for molecular profiling of brain tumours, only a few millilitres of cerebrospinal fluid, obtained via lumbar puncture, are sufficient. This fluid contains circulating tumour DNA (ctDNA) or extracellular vesicles harbouring tumour RNA [7]. It has also been observed that RNA encapsulated in exosomes retains stability, whereas extracellular RNA is rapidly degraded by ribonucleases. Nanopore sequencing, among other techniques, has been employed to assess copy number variations (CNV) and methylation profiles.

Scientific studies have shown that ctDNA can be detected not only during cancer progression but also in remission and recurrence stages. This method allows for precise tumour classification. Additionally, it has been established that the presence of a tumour elicits the emergence of antigen-presenting leukocyte subpopulations expressing specific tumour antigens on their membranes. These cells can be isolated from whole blood and utilized for biomarker screening [8].

Research has demonstrated that liquid biopsy offers several advantages over conventional tissue biopsy. It is characterized by high sensitivity, the ability to dynamically monitor treatment efficacy due to the repeated accessibility of biological material, and the feasibility of outpatient procedures without hospitalization [9]. Furthermore, liquid biopsy requires only local anaesthesia, eliminating the need for general anaesthesia, reducing complication risks, and enabling early cancer detection. However, researchers have noted that ctDNA released into biological fluids degrades rapidly, resulting in low concentrations in blood, saliva, or cerebrospinal fluid [10]. Other limitations of this method include the limited quantity of tumour DNA in fluids, nucleotide heterogeneity within marker fragments even within the same tumour, and the potential overlap between oncogenic and native DNA sequences [11].

The personalized approach incorporating liquid biopsy, as reported in previous studies, allows for the escalation or de-escalation of therapy based on oncogenic markers detected in biological fluids (blood, lymph, saliva, etc.). This strategy has been successfully implemented in clinical practice for colorectal cancer treatment [12].

In recent years, various technologies have been introduced for detecting tumour markers in biological fluids. Researchers have reported the use of systems such as AndaTest, EPISPOT (enzyme-linked immunosorbent assay), and RosetteSep, as well as selective amplification methods for specific DNA fragments. These approaches facilitate the identification of adhesion molecules in transformed cells, specific proteases, tumour antigens, and alterations in cell membrane structures [13]. The analysis of circulating tumour cells (CTCs) has enabled the diagnosis of prostate, breast, and colorectal cancers.

Extracellular tumour DNA has been identified using technologies such as DEPArray, paired-end reassembly analysis (PEAR), and methylation-specific targeted amplification and sequencing [14]. Mutant allele fraction detection methods have been applied in the diagnosis of melanoma, ovarian cancer, lung cancer, breast cancer, colorectal cancer, and hepatocellular carcinoma.

Additionally, studies have demonstrated the efficacy of extracellular tumour vesicle isolation methods, including preparative exclusion chromatography, agglutination, and magnetic immunoselection. These approaches have enabled the extraction of fractions of lectin, CD63, caveolin-1, and prostate-specific transglutaminase, facilitating the identification of prostate cancer, ovarian cancer, melanoma, and acute myeloid leukaemia [15, 16]. MicroRNA analysis has also been employed to assess tumour sensitivity to chemotherapy in lung cancer patients [17].

Despite significant advancements in liquid biopsy applications, a review of the literature highlights certain research gaps. Specifically, most studies have focused on the validation of oncogenic marker detection methods, while their clinical significance remains underexplored. Furthermore, reports indicate that in some countries, including Bulgaria and Kazakhstan, liquid biopsy has already been utilized to detect mutations in genes such as *PIK3A, BRCA1, BRCA2, PALB2*, and endometrial cancer markers. However, active research in this area has not been conducted in these regions.

This study aims to evaluate the efficacy and potential of liquid biopsy in cancer diagnostics and customized therapy, concentrating on biomarker detection, genetic mutation identification, and treatment monitoring through biological fluids. Objectives were defined:

1) Assess the efficacy of liquid biopsy methodologies for identifying tumour biomarkers in biological fluids.

2) Examine the function of liquid biopsy in detecting significant genetic alterations pertinent to individualized cancer treatments.

3) Evaluate the efficacy of liquid biopsy in monitoring cancer advancement and treatment response.

4) Investigate technology innovations in liquid biopsy, including next-generation sequencing and digital droplet polymerase chain reaction, for clinical application.

5) Identify the clinical limits of liquid biopsy and suggest measures to enhance accuracy and sensitivity.

Materials and Methods

As this study is theoretical in nature, no clinical experiments were conducted. The results section consolidates information obtained from previously conducted clinical and laboratory studies on the isolation and analysis of tumour markers from biological fluids, including plasma, lymph, urine, saliva, and cerebrospinal fluid. The methodological approaches, their relevance for

personalized diagnostics, therapy selection, prognosis prediction, and recurrence probability assessment were thoroughly examined. The study incorporates research conducted over the past ten years and published within the last five years (2021-2025), including laboratory and clinical experiments, reviews, and meta-analyses. Only studies published in English and indexed in PubMed, Scopus, Elsevier, and Web of Knowledge were considered. The literature search was conducted in English using key terms such as "liquid biopsy", "breast cancer", "ovarian cancer", "non-small cell lung cancer", "colorectal cancer", "squamous cell carcinoma", "prostate cancer", "tumour vesicles", "exosomes", "tumour markers", "regulatory microRNA", "saliva diagnostics", "*KRAS*", "*EGFR*", and "stratification of patients for therapy", among others. The inclusion criteria encompassed studies describing the role of CTCs, ctDNA, and tumour-derived vesicles in carcinogenesis, either in humans or laboratory animals. The exclusion criteria included "case reports", tissue biopsy results, conference abstracts, and experiments conducted before or during 2020.

Articles that passed the initial screening and met the inclusion criteria underwent full-text analysis following the review of titles and abstracts. Each selected study was assessed based on the name of the first author, publication year, study design, and methodological approach. If multiple independent studies on the same topic, conducted in different locations and at different times, reported varying statistical data, additional data processing was performed. A total of 65 sources were analysed, of which 15 were excluded (Figure 1). Consequently, 50 publications were included in the final analysis. The experiments under review were conducted in clinical and laboratory settings in Germany, China, the United States, Italy, Turkey, Japan, Poland, Spain, and India. Systematization of the findings was performed according to thematic context, grouping studies that provided empirically substantiated data on the role of specific tumour markers in oncogenesis.

When processing numerical data, probability values (P) were determined to confirm statistical reliability (software used: GraphPad Prism 9.0 and Microsoft Excel). Differences between sample indicators were considered statistically significant if the probability value was less than or equal to 0.5 ($P \leq 0.5$). Based on the review results, recommendations were formulated for countries such as Albania, Bulgaria, Kazakhstan, Kyrgyzstan, and Uzbekistan, taking into account the specific characteristics of their healthcare systems, medical infrastructure development levels, and access to advanced technologies. All five countries demonstrate an interest in attracting international investments for the advancement of biotechnology. Liquid biopsy has the potential to serve not only as a medical tool but also as an economic driver by fostering the growth of biomedical start-ups, attracting capital, and facilitating collaborative scientific projects.

Results

This study examines various aspects of liquid biopsy based on the types of tumour-derived materials that infiltrate biological fluids, as well as the prospects and cutting-edge technologies for utilizing this approach in diagnostics and therapeutic adjustment. Biological fluids can serve as a source of intact tumour cells, ctDNA, RNA, and proteins. DNA profiling enables the detection of specific genetic mutations (nonsense, missense, neutral, transitions, transversions, deletions), chromosomal aberrations (translocations, CNV in marker sequences, gene rearrangements), and epigenetic modifications (methylation status) (Figure 2).

Both coding (spliced) and non-coding RNA serve as templates for molecular diagnostics through reverse transcription-based assays. The morphology of CTCs provides insights into cancer subtypes, while molecular screening facilitates a personalized approach to treatment.

Circulating DNA as an analytical template

ctDNA originates from necrotic or apoptotic tumour

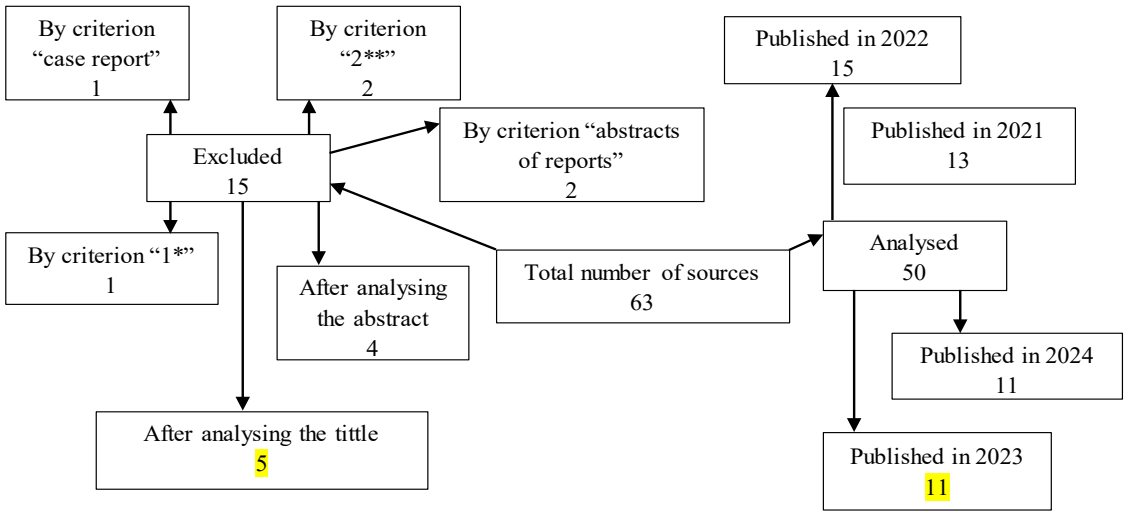

Figure 1. Selection of Literary Sources for Data Processing on Liquid Biopsy. Note: criterion 1* – "studies on tumour markers obtained via tissue biopsy"; criterion 2** – "experiments conducted up to and including 2020". Source: compiled by the authors.

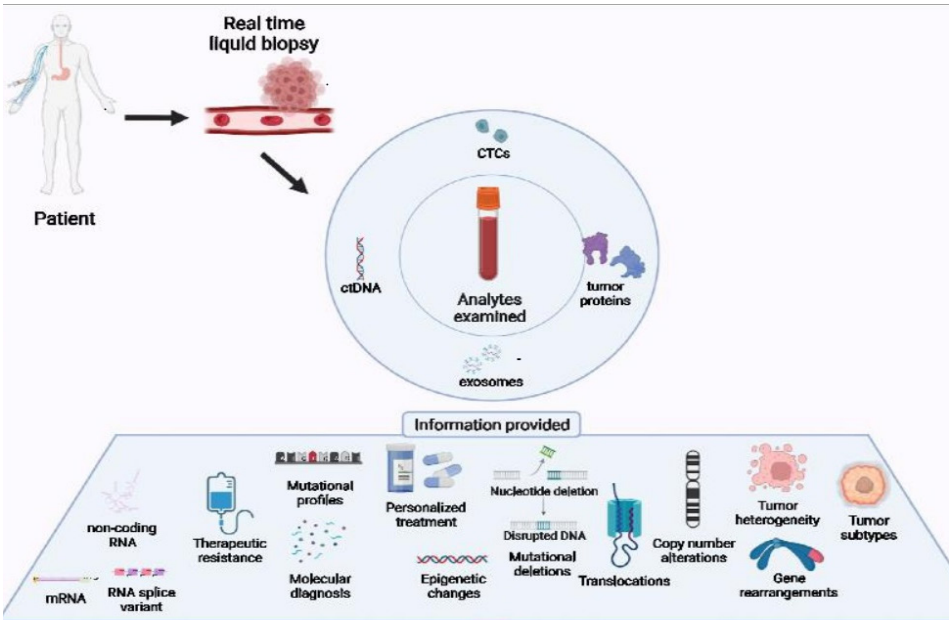

Figure 2. Informative Value of Tumour Biomaterial Derived from Body Fluids. Source: compiled by the authors based on data from [18].

cells. As these cells undergo lysis, their nucleic acids are released into the bloodstream, lymphatic system, cerebrospinal fluid, urine, pleural fluid, and other biological fluids. ctDNA is often encapsulated within nucleosomes, forming sequences approximately 150 base pairs in length, with an average concentration of 100 ng/mL. The fragment size varies depending on tumour pathology: longer fragments are prevalent in necrotic tumours, whereas shorter fragments dominate in tumours characterized by apoptotic processes. In circulation, ctDNA remains in its native state for no more than three hours, as DNase activity in the blood rapidly degrades it. Consequently, the concentration of ctDNA in biological fluids is typically very low. Blood plasma is the preferred medium for biomarker analysis because it contains minimal background DNA from non-transformed cells [8, 19]. One of the key diagnostic markers in tumour-derived ctDNA is the *KRAS* gene mutation (Kirsten rat sarcoma viral oncogene homolog). The ctDNA titter, and consequently the expression levels of tumour markers, fluctuates depending on cancer stage, tumour size, and treatment efficacy. Polymerase chain reaction (PCR) allows for the detection of tumour-specific mutations, assessment of CNV, and tracking of copy number changes in response to disease progression or therapy [20]. Notably, mutation spectra in metastases often differ from those in the primary tumour, enabling liquid biopsy to determine whether a given tumour is metastasizing [21]. Many mutations that remain undetected in conventional tissue biopsies can be identified through genetic analysis of ctDNA obtained from biological fluids. The clinical applications of liquid biopsy-based ctDNA analysis are summarized in Table 1.

Discussion

If certain mutations or specific fragment copy numbers are associated with recurrence, their disappearance or decreased expression (as determined by real-time PCR) can allow for a reduction in chemotherapy burden on the patient [23]. In addition to the emergence of mutations, an epigenetic prognostic marker of free ctDNA is the degree of methylation in specific regions (so-called hypermethylated "CpG islands"), which is detected fluorescently during real-time PCR [24].

After collecting whole blood for tumour DNA analysis, it is critical to perform extraction within 6 hours, as prolonged storage leads to leukocyte membrane degradation, resulting in sample contamination with nucleic acids from cellular elements. Standardized approaches for tumour DNA extraction include silica gel column enrichment, centrifugation, immunomagnetic sorption, and phenol/chloroform extraction. Tumour type identification is based on detecting mutations or rearrangements in specific regions associated with a given type or conducting whole-genome screening of tumour DNA for nucleotide substitutions and chromosomal aberrations. Identification methods include massive parallel sequencing, digital droplet PCR, real-time PCR, hypermethylation analysis, and mass spectrometry [25]. Digital droplet PCR stands out for its simplicity, high sensitivity, and short turnaround time; however, it is essential to know the specific genetic or epigenetic alterations associated with particular tumour types (e.g., RAS, RAF (rapidly progressing fibrosarcoma), *EGFR*, deletions in exon 19, etc.). To achieve this, specific gene panels are used. This approach has enabled the diagnosis of breast cancer, prostate cancer, and colorectal cancer at later stages in 75% of patients [23]. Mass parallel sequencing (with luminescence) from plasma samples provides a comprehensive tumour genome profile, revealing the copy number of altered sequences and identifying chromosomal aberrations. This diagnostic method has detected gene fusions such as TMPRSS2-ERG, gaps or loss of the short

arm of chromosome 8 in prostate cancer patients (different results were observed in patients with castration-sensitive versus castration-resistant tumours). This discrepancy is due to additional mutations that induce resistance to the treatment. Similar changes have been diagnosed in breast cancer and colorectal cancer patients.

Deep sequencing of extracellular cancer nucleosome material revealed a correlation between their quantity, DNA fragment length, and treatment effectiveness: the fewer circulating nucleosomes and the longer their marker sequences, the better the tumour's sensitivity to chemotherapy in colorectal cancer. Conversely, the appearance of numerous short fragments indicates recurrence [26].

PEAR is based on a PCR method with primers complementary to the sequence near the break point. The detection limit for this approach is reduced to 0.001% (in breast cancer and colorectal cancer patients). Therefore, the low concentration of DNA in biological fluids is not a problem in this case. Paired-end reassembly analysis is used for developing new markers specific to solid tumours and for monitoring disease severity [18].

In contrast to PCR, sequencing allows the detection of new mutations associated with specific cancer types, and through improvements, it achieves a detection limit of 0.01% [2]. Deep sequencing of tagged amplicons (Tam-Seq) offers a sensitivity of over 97% and allows the detection of alleles with a frequency of less than 2%. In the first stage, larger amplicons are accumulated, containing tagged fragments (to enrich the matrix with the maximum possible allele diversity), followed by the targeted amplification of required alleles using tagged primers, which are then sequenced. This method successfully identified mutations in the *EGFR* gene and the tumour suppressor *P53*; DNA was isolated from plasma samples of ovarian cancer patients. However, these mutations could not be detected in biopsy material. The Tam-Seq method allows real-time monitoring of tumour mutations over several months by isolating DNA from plasma [18]. Since the titre of free circulating tumour nucleic acids is very low, whole-genome or complete sequence amplification is typically performed before sequencing. During amplification, DNA polymerase may introduce "errors", distorting the matrix. Thus, new enrichment methods are being explored to avoid whole-genome amplification.

ctDNA does not always contain distinctive oncogenic markers; many of its sequences overlap with native DNA sequences. Moreover, its concentration is significantly lower than that of circulating free DNA from normal cells. During amplification, primers may bind to complementary fragments of non-tumour DNA, leading to false quantitative results [24]. To minimize native DNA background, repeated plasma centrifugation and strict adherence to temperature and incubation time intervals are employed.

Variations in ctDNA fragment sizes directly influence diagnostic precision in liquid biopsy applications. Shorter ctDNA fragments, generally arising from apoptosis, are more prevalent in early-stage tumours or those exhibiting elevated apoptotic activity. These fragments are frequently more challenging to differentiate from background

Table 1. Application of Free ctDNA Analysis in Clinical Practice

Cancer type	Genes	Diagnosis/prognosis
B-cell lymphoma	Whole-genome sequencing	Tumour type identification and therapy personalization according to tumour type
Colorectal cancer	Whole-genome sequencing	Monitoring clonal variations in tumour cells and response to treatment (resistance to EGFR inhibitors); recurrence development
Colorectal cancer	BRAF, NRAS, KRAS mutations	Response to cetuximab and panitumumab
Breast cancer, colorectal cancer	Whole-genome sequencing	Recurrence development
Breast cancer	PCR technologies, PIC3A mutations	Diagnostic marker
Lung cancer	Epidermal growth factor receptor (EGFR) mutations	Poor prognosis
Prostate cancer	Androgen receptor mutations	Response to prednisolone and abiraterone; tumour heterogeneity due to different mechanisms of drug resistance
Solid tumours	PIK3CA, EGFR, GAS6, MED1, RB1 mutations	Response to lapatinib, paclitaxel, tamoxifen, cisplatin, gefitinib

Source: compiled by the authors based on data from [18, 22].

circulating free DNA originating from normal cells, due to overlapping lengths. This overlap diminishes specificity and heightens the likelihood of false-positive or false-negative outcomes, especially when employing PCR-based detection techniques that may mistakenly amplify non-tumour DNA. In contrast, longer ctDNA fragments, usually associated with dying tumour cells, tend to include complete sections of DNA that have specific mutations or changes. These extended snippets enhance mutation detection and promote the application of next-generation sequencing (NGS) and other enrichment methodologies. The presence of longer, complete sequences makes it easier and more reliable to find changes specific to tumours, like single nucleotide variants, CNVs, and gene fusions. Extensive sequencing of nucleosome-bound ctDNA has demonstrated that elongated marker sequences are associated with improved treatment response in colorectal cancer, while a prevalence of short pieces may signify disease recurrence. Consequently, fragment length influences the technical parameters of ctDNA detection and offers predictive insights into tumour behaviour and treatment effectiveness. The variability in ctDNA fragment sizes affects both analytical efficacy and clinical interpretation.

CTCs and alternative biomaterials

During tumour growth and metastasis, intercellular connections break down; tumour cells penetrate the basal membrane and enter the bloodstream and lymphatic system (CTCs) [27, 28]. These cells acquire resistance to apoptosis and oxidative stress, activate immunosuppression cascades, and form complexes with platelets or neutrophils, enabling them to survive despite losing adhesion capability and facilitating their rapid dissemination throughout the body. The CTC concentration in bodily fluids is relatively low: one cell per 106 blood elements. Even though they originate from the same clone, their morphology varies depending on tumour growth stage. A direct correlation has been observed between oncogene expression levels, the number of free tumour cells in the blood, and metastasis growth rates. Therefore, these cells (or more precisely, their transcriptome, proteome, genome, and secretome) serve as diagnostic indicators. Unlike "standard" blood biomarkers, CTC characteristics change more dynamically, allowing for more accurate tumour progression monitoring and early cancer detection [29].

The spectrum of mutations in oncogenes in CTC serves as a prognostic marker for treatment response and tumour characteristics. Specifically, the substitution of threonine with methionine at position 790 in *EGFR* results in resistance of lung carcinoma to erlotinib and gefitinib and is associated with recurrences, whereas it indicates sensitivity to tyrosine kinase inhibitors. Similar missense mutations in oncogenic markers *KRAS* and *PIC3A* correlate with drug response in colorectal cancer patients. Since the molecular profile of a tumour changes during its progression, CTCs extracted from biological fluids allow for continuous real-time monitoring.

Epigenetic changes in oncogenic markers can be studied using circulating cell material. Promoter methylation inhibits gene expression. Methylation of the operator regions of tumour suppressors such as *CST6, BRMS1,* and *SOX17* correlates with active metastasis in breast cancer. Methylation of promoters of angiogenesis regulators in CTCs (*SFRP2, VEGF*) has been observed in patients with colorectal cancer and prostate cancer. In breast cancer, methylation of the estrogen receptor promoter is a prognostic marker of chemotherapy resistance (to exemestane and everolimus) [30].

Non-coding microRNAs isolated from CTCs are also associated with oncogenesis. Accumulation of methylated forms of miR-200 has been detected in circulating cancer cells in prostate cancer, and it is linked to epithelial-to-mesenchymal transition. Therefore, epigenetic changes in CTCs allow for the prediction of metastasis development or drug sensitivity/resistance. Isolated CTCs from biological fluids can be used in in vivo and in vitro experiments. Human breast cancer CTCs were transplanted into rodents, and active metastasis was observed in the lungs, liver, and bones. The greater the number of marker receptors on the surface of CTCs (MET+VE, CD44+, EPCAM+, CD47+), the more metastasis points were detected in the model organisms, and, correspondingly, survival rates decreased. This allows for the prediction of potential disease dynamics in patients. Since CTCs typically circulate in low titters in biological fluids, they are "artificially enriched" by isolating the cell line and cultivating it, after which they are implanted into animals for in vivo experiments or tested for drug efficacy in vitro (developed for CTC-MCC-41) [31].

Due to the low titre of CTC in the blood (up to 10 per 1 ml of peripheral blood), a challenge arises in their isolation and concentration. Cells are isolated based on physical characteristics (size, shape, density, surface charge) and the expression of surface receptors. A convenient method for concentration is magneto-affinity selection: positive selection uses antibodies (or primers) to antigens or transcripts of cancer cells (e.g., adhesion molecules like EpCAM), while negative selection uses antibodies to surface molecules of blood components (e.g., leukocyte marker CD45). The concentrated cells are labelled with a fluorescent dye and counted (based on fluorescence signal) under a microscope. Test systems such as AndaTest, CellSearch, CTC-Chips, and EPISPOT operate on this technology. EPISPOT, in particular, captures specific proteins synthesized by cancer cells in a nutrient medium over 1-2 days of culture and is successfully used for diagnosing melanoma, breast cancer, prostate cancer, and colorectal cancer. CTC screening is performed using real-time PCR (for genes, transcripts) or blot tests for protein expression [32]. Negative selection has some drawbacks, as different background cells carry different surface markers. If working with a limited marker (e.g., CD45), only the leukocyte background can be excluded. However, on the residual endothelial background, some CTCs might be lost [33].

In addition to plasma and serum, diagnostic molecules are also extracted from urine and saliva in liquid biopsies. Saliva as a biomaterial offers the advantages of non-invasiveness, simplicity, and low cost of collection.

Nucleotide sequences are analysed using the EFIRM technology – DNA separation in an electric field, hybridization with specific marker probes, and detection of bound molecules via fluorescence. According to data Nonaka and Wong [34], this method successfully identified mutations in the tyrosine kinase domain of *EGFR* in lung carcinoma; mutations in the tumour suppressor FOXP1 and in the signalling G-protein gene GNG2. Spectroscopic techniques can identify free metabolites in saliva, notably, increased porphyrin concentration is associated with squamous cell carcinoma of the oral cavity [35]. In urogenital oncology, most signalling cancer molecules are secreted into the urogenital tract, making urine a valuable biomaterial for monitoring. According to Tosoian et al. [36], markers for prostate cancer were identified in urine, such as TMPRSS2-ERG gene fusion, nuclear matrix protein Nmp22, prostate cancer gene 3 (PCA3), long non-coding RNAs MALAT1, UCA1, FR0348383. These RNAs proved to be more specific markers for prostate cancer than plasma antigens. New circulating nucleic acids, including *PIRMT5, UBE2C*, and *IQGAP3*, have been identified in urine, expanding the diagnostic library for bladder cancer malignancies. Piwi-RNA (piRNA-823), which silences transposons in complex with piwi-protein subfamilies, has become a diagnostic marker for kidney carcinoma. Alongside RNA, DNA markers associated with kidney cancer (*TP53 249 T* substitution, *RASSF1A* mutation, *GSTP1* methylation) were identified in urine. Circular RNA PIRMT5 binds regulatory microRNAs, inducing epithelial-mesenchymal transition [37, 38]. Its levels correlate with late-stage urothelial carcinoma and decreased patient survival. Even non-neurological cancers (gastrointestinal, lung, breast) have been identified from urinary molecules (epigenetic changes in DNA methylation). The sensitivity for detecting markers from tissues and urine was almost identical, with around 75% of *EGFR* mutations being detectable.

Extracellular vesicles (exosomes) as a matrix for analysis

Extracellular vesicles, surrounded by a bilayer lipid membrane and ranging in size from 35 to 150 nm, are released by cancer cells into the external environment. These vesicles are conventionally classified into exosomes, apoptotic bodies, and microvesicles (ectosomes). They contain a pool of proteins, RNAs, and DNAs, which can be used to characterize the state of a tumour. Due to this, they can be employed to evaluate the effectiveness of treatment and disease prognosis. Molecules secreted by tumours stimulate angiogenesis, contribute to immune protection, and promote metastasis growth. Since cancer cells not only release but also internalize exosomes, these specific intercellular interactions can be useful for the delivery of anti-tumour agents [39].

Extracellular vesicles differ in size, shape, and density, allowing their isolation by methods such as dialysis, gradient ultracentrifugation, using nanomembrane "molecular sieves" (to prevent damage or deformation by centrifugal force), exclusion chromatography, immunomagnetic or polymer sorption. Afterward, RNA from exosomes is isolated and real-time reverse transcription PCR is performed. For DNA analysis, sequencing or PCR is conducted, while proteins are analysed via Western blotting or enzyme-linked immunosorbent assay. The practical significance of a range of vesicular markers is summarized in Table 2.

During carcinogenesis, immune cells also secrete exosomes, the content of which changes in response to the tumour. An increase in the surface receptors CD28 and PD-1 on the exosomes of dendritic cells indicates the clinical efficacy of the chemotherapy drug ipilimumab in the treatment of melanoma [18]. Non-coding tumour RNA is considered one of the most complex biomarkers to analyse. Its content in bodily fluids is critically low; it is highly sensitive to RNase activity, which is abundant in the surrounding environment; it is heterogeneous in sequence across different patients, and there are no standard amplicons for non-coding RNAs [31]. Some of these biomarkers are unstable, making them difficult to isolate. Regarding plasma isolation, critical selectivity and specificity are required. Currently, no routine protocols have been developed. As for exosomes, methods for enrichment (concentration) need to be further refined. To identify concentrated exosomes, associated tumour RNA and proteins are examined. As indicated by Haupts et al. [26], false results are more frequently observed in patients with skin tumours. During the epithelial-to-mesenchymal transition, the expression of oncogenes is strongly inhibited, meaning that, during screening, the tumour may be classified as benign. The study by Oshi et al. [51] provide evidence of the periodic occurrence of false-negative and false-positive results in fluid biopsies. Occasionally, blood or urine can be contaminated by external agents, so in such cases, molecular confirmation of the phenotype is necessary to correctly adjust the treatment. In this regard, classical biopsy is used in conjunction with liquid biopsy.

When comparing the four "information sources" in liquid biopsy – cells, DNA, RNA, and exosomes – the latter have the advantage. Exosomes are easier to concentrate from blood than other types of material; their contents remain stable in the surrounding environment due to the hydrophobic lipid membrane. Unlike DNA, which is merely a template molecule with sequences, exosomes carry information about how genes are expressed in the phenotype – in the RNA transcriptome pool or the proteome [52].

Exosomes are utilised for biomarker analysis in liquid biopsy owing to their distinctive biological and biophysical characteristics, rendering them extremely appropriate for non-invasive cancer diagnoses [53]. In contrast to other extracellular vesicles like microvesicles or apoptotic bodies, exosomes derive from the endosomal pathway and are released in a regulated fashion, containing a selectively enriched cargo of proteins, lipids, and nucleic acids (including microRNAs, long non-coding RNAs, and DNA fragments) that accurately represent the molecular and phenotypic characteristics of their source tumour cells [54]. Their small size (35-150 nm) and encapsulation inside a stable lipid bilayer preserve their contents from enzymatic breakdown in bodily fluids, facilitating accurate detection and measurement. Furthermore, exosomes circulate throughout the body

Table 2. Application of Exosomal Biomolecule Analysis in Clinical Practice

No.	Cancer type	Biomolecules	Results
1	Pancreatic ductal adenocarcinoma	Alkaline Phosphatase AlppL2, Intercellular Junction Protein Thrombospondin Thbs2	Diagnostic markers in 26 patients *
2	Pancreatic cancer	5 lopids, 10 proteins	Cancer diagnosis in 60 patients**
3	Pancreatic ductal adenocarcinoma	Extracellular and Exosomal microRNAs	Diagnostic markers in 523 patients **
4	Breast cancer	miR-21, miR1260b, miR-484, miR-181a, miR106b	Diagnostic markers in 166 patients** (in 46 – at an early stage)
5	Breast cancer	miR-6076, miR-4515, miR-202-3p, miR-5195-3p, miR-4443, miR-4713-3p	Diagnosis of early-stage cancer in 58 patients* with a breast cyst
6	Breast cancer	Extracellular Matrix Protein Ecm1, Mannose Lectin Receptor Mbl2, Biotinidase Btd	Diagnostic markers
7	Colorectal cancer	Ubiquitin-Transferae Rnf208	Diagnosis in 39 patients*
8	Colorectal cancer	Cell Adhesion Molecule Cea, 5'-Nucleotidase Ecto Cd73	Diagnostic markers
9	Colorectal cancer	Increased Concentration of Long Non-Coding RNA PCA3, BCAR4	Actively expressed in tumour cells; diagnostic markers
10	Colorectal cancer	Increased Concentration of Long Non-Coding RNA UCA1	Tumour resistance to the chemotherapeutic agent cetuximab
11	Colorectal adenocarcinoma	7 Exosomal Proteins	Diagnostic markers in 84 patients*
12	Ovarian cancer	Complement Activator Cfh, Cyclin E1 Ccne1, Mucin Ca-125	Diagnostic markers in a sample of 250 individuals**
13	Ovarian cancer	Mucin Muc1, Bone Marrow Stromal Cell Antigen Bst2	Diagnosed with stage I cancer in 17* patients, stage II – in 30**, stage III – in 10*, and benign tumours in 17* individuals
14	Acute myeloid leukaemia	CD34+ receptors	Monitoring of therapy response and disease progression
15	Pancreatic cancer	Migration inhibitory factor (Mif)	Risk of liver metastases
16	Pancreatic cancer	Glypican-1, a surface proteoglycan (in conjunction with KRAS mutations)	Early-stage cancer diagnosis
17	Pancreatic cancer	miR-4306, miR-4644, miR-3976, miR-1246, CD44v6 proteins, CD104, Tspan8	Minimally invasive tumours, highly sensitive to drugs
18	Pancreatic cancer	Exosomal DNA; mutations in the P53 and KRAS genes	Diagnostic markers
19	Melanoma	Surface receptors CD63+, caveolin-1	Diagnostic markers
20	Melanoma	miR-211-5p	Increased tumour resistance to BRAF kinase inhibitors (mutation of this gene disrupts cell division and differentiation; a melanoma biomarker)
21	Prostate cancer	Transglutaminase; stem cell antigen	Active tumour growth
22	Prostate cancer	Circular RNA circ_044516	Diagnostic marker; silencing correlates with tumour growth suppression
23	Lung cancer	miR-21-5b, miR-23b-3p, miR-105b-5p	Non-invasiveness of the tumour
24	Lung cancer	Decreased transcription levels of the immunosuppressive miR-125b-5p	Patients showed improved T-lymphocyte activity and response to immunotherapy after the treatment course
25	Lung cancer	Increased concentration of the miR-146a-5p	Increased tumour sensitivity to the chemotherapeutic agent cisplatin
26	Lung cancer	Increased concentration of long non-coding RNA H19	Tumour resistance to the chemotherapeutic agent gefitinib
27	Bladder cancer	Long non-coding RNA H19	Actively expressed in tumour cells; a diagnostic marker
28	Oesophageal squamous cell carcinoma	Increased concentration of long non-coding RNA PART1	Tumour resistance to the chemotherapeutic agent gefitinib
29	Renal cell carcinoma	Increased concentration of long non-coding RNA ARSR	Tumour resistance to the chemotherapeutic agent sunitinib

Note: * p≤0.5; ** p≤0.99; Source, compiled by the authors based on data from [18, 40-50].

and can be easily extracted from blood, urine, or saliva using proven enrichment methods, rendering them a viable medium for the longitudinal assessment of cancer progression, treatment efficacy, and the development of resistance. These benefits make exosomes especially useful for detecting tumour-specific biomarkers with elevated sensitivity and specificity.

Despite the aforementioned drawbacks, liquid biopsy remains a promising method for non-invasive real-time molecular profiling of tumours. Exosomes can serve as drug delivery vehicles to recipient cells, while circulating cells provide a model for laboratory-based identification of targets that initiate metastasis growth. The future direction of this approach lies in the development of new, cost-effective test systems for the rapid identification and quantitative determination of marker DNA, RNA, and proteins, distinguished by a low detection threshold and high specificity, as well as the search for new markers in urine, saliva, and mucosal secretions.

Personalised therapy based on liquid biopsy material

The analysis of CTCs has enabled the adjustment of hormonal therapy for patients with metastatic prostate cancer. When fewer than 5 CTCs were detected in the blood of patients, the body responded positively to hormones, and the rate of cancer progression slowed. In contrast, if more than 5 CTCs were isolated from the plasma of patients, there was a higher risk of mortality [55]. Diagnostic markers for breast cancer include the CTC receptors *HER-2* and Er (oestrogen receptor). Their status changes in cases of recurrence and during disease progression. Monitoring the status of these receptors, along with *Ki67* and *Bcl2* (a marker for B-lymphocyte lymphoma-2), allows for predicting the patients' response to endocrine therapy. For colorectal cancer treatment, two modifications of bevacizumab are used. One is the standard formulation, while the other is an enhanced version, though more toxic. If patients have fewer than 3 CTCs, standard therapy is applied; if more, enhanced therapy is used. Thus, CTC analysis helps avoid the use of excessively toxic drugs when substitute therapy is possible. In addition to cell titre, monitoring mutations in oncogenes from free ctDNA is significant for treatment personalisation. Patients receiving drugs tailored to the genomic profile of their tumour experience lower rates of cancer progression [11].

The drug pembrolizumab contains an antibody complementary to the programmed cell death receptor PD-1. This protein is presented on the membrane surface of lung tumours. Signalling through this receptor and its ligand Pdl-1 in transformed cells suppresses the anti-tumour response of T-lymphocytes. The drug screens tumour receptors, blocking the PD pathway in the tumour and restoring the T-lymphocytic anti-tumour response. The lower the Pdl-1 production by CTCs in the early stages of treatment, the more resistant the tumour is to immunotherapy. Therefore, such patients are prescribed drugs with a different mechanism of action. In prostate cancer, a key marker is the androgen receptor [56]. Anti-cancer drugs such as abiraterone and enzalutamide bind to this receptor, blocking the signal transmission between oncocells. This protein is present in CTCs. The spliced variant 7 androgen receptor encodes a product that cannot bind to the aforementioned drugs. Therefore, before prescribing treatment, patients undergo a blood test for CTCs with androgen receptor splicing analysis. If a mutation is found, abiraterone or enzalutamide are not prescribed [57].

The sensitivity to the tyrosine kinase inhibitor *EGFR* (erlotinib) is determined by the *EGFR*L858R single nucleotide substitution or deletions in exon 19 (*EGFR*T790M). If these mutations are detected in ctDNA, erlotinib is replaced with other drugs in the treatment of lung cancer. Due to selective pressure from the use of *EGFR* antibodies, mutations in the GTP-ase *KRAS* gene (amino acid substitution) are generated in tumours, and these mutations are found in DNA released into the bloodstream by mutated subclones. However, after the cessation of *EGFR* antibody blockade, the *KRAS* subclone population rapidly decreases. Monitoring tumour DNA enables the periodic replacement of *EGFR* immunotherapy with drugs that have different mechanisms of action [58].

At the end of treatment, a critical moment is determining the threshold of residual markers at which the disease can still be diagnosed. Based on this data, decisions are made regarding whether to continue treatment, predict the likelihood of relapse, and introduce new drugs into the protocol. The expression of certain genes in CTCs, their methylation patterns, the quantity, shape, and size of CTCs are all factors that can predict metastasis even before clinical signs appear. If, in the early stages of lung cancer (after tumour resection), CTCs are released into the blood from the pulmonary vein, there is a high risk of recurrence [59, 60]. If the marker profile of these cells is more similar to the profile of metastases than the "PEARnt tumour", the risk of recurrence increases, particularly in lymph nodes rather than at the primary location. Tumour DNA has been detected as early as 213 days before recurrence. Recurrence occurred in 64% of patients in whom persistent DNA was found [61].

It is recommended to monitor CTC levels for several years. Statistically, after surgical treatment of colorectal cancer (during adjuvant therapy), cancer cells were still present in patients' blood, but this did not impact the outcome. However, if these cells did not disappear within 2-3 years, recurrences and metastases developed over time. Furthermore, if the cell count was 4 or higher during the first six months after surgery, the likelihood of recurrence was 100%. In breast cancer, recurrence is indicated by the simultaneous presence of both cancer DNA and cells in the blood. However, cancer DNA alone does not predict recurrence [11].

Thus, liquid biopsy, combined with advanced molecular technologies, enables the concentration and detection of cancer signalling proteins, genes, and RNAs in the context of disease dynamics. Based on the changes in their spectra, the doctor can personalize therapy for each patient.

In conclusion, thanks to active research in recent years in non-invasive diagnosis and control of oncological processes, promising methods for cancer treatment have been developed. However, liquid biopsy is not yet a

standardized and sole method for diagnosis and remains an adjunct tool alongside traditional biopsy. The biggest shortcoming of liquid biopsy today is its insufficient accuracy and sensitivity in determining tumour subtypes comPEARd to tissue sample analysis [62]. This is due to the low concentration of markers, as there is a small amount of tumour material circulating in the fluids. The isolation of exosomes can be hindered by platelets, especially if their numbers are high. Platelets "block" tumour vesicles with their density and size. Contaminants of target molecules can also include protein aggregates, lipoproteins, leukocytes, and native DNA. Tumour cells, their granules, and platelets usually differ in their nucleic acid, protein, and lipid profiles. Because of this, it is still unclear whether liquid oncological material contains information on all subclones of tumour cells from the primary site or metastases. Due to the lack of standardized protocols, there are insufficient quality control methods for liquid biopsy. Furthermore, biomarkers often degrade during the collection, processing, and storage of the material [11, 22].

According to the data Chen et al. [63], to obtain the most comprehensive picture of genetic and epigenetic transformations, changes in the expression levels of individual genes, and the acquisition of drug resistance by the tumour, single-cell analysis is conducted. Sequencing the transcriptome, epigenome, and genome of each cell (which are present in low titters in biopsy samples) allows the detection of cellular heterogeneity in tumours, which is often overlooked in routine sequencing using "mixed" samples. The low titters of CTCs, free tumour DNA, vesicles, immature and mature endothelial cells, as well as tumour-altered immune competent cells, require liquid biopsy to have an extremely low detection threshold. Due to the insufficient amount of material for more thorough investigation of a particular tumour subtype, the development of patient selection criteria based on economic feasibility is required.

Considering the achievements in the field of liquid biopsy, key recommendations can be outlined. In Albania, it is important to incorporate liquid biopsy into national oncological protocols as an auxiliary method for monitoring therapy effectiveness, particularly for colorectal cancer and lung carcinoma treatment. It is advisable to integrate digital droplet PCR and next-generation sequencing techniques into clinical practice. This would allow the detection of mutations in *KRAS* and *EGFR* genes, which are key biomarkers for personalized therapy.

In Bulgaria, given the presence of qualified laboratory specialists and strong cooperation with the European Union, the focus should be on standardizing approaches to determining the methylation levels of CpG islands in tumour DNA and using transcriptome sequencing of CTCs. This would facilitate effective monitoring of patient responses to targeted therapy, particularly in breast and prostate cancer treatment. Kazakhstan has significant potential for developing liquid biopsy. The next step should be the implementation of a comprehensive analysis of ctDNA and RNA using next-generation sequencing, which would allow for the detection of new oncogenes specific to endemic tumour types. Furthermore, the development of laboratory standards for exosomal RNA extraction is crucial, opening up possibilities for early diagnosis of aggressive forms of cancer, including hepatocellular carcinoma.

For Kyrgyzstan, it is advisable to create a national program for the integration of liquid biopsy into early diagnostic practice and therapy monitoring. The primary task is to introduce real-time PCR (qPCR) for detecting mutations in circulating DNA, particularly for lung and breast cancer. It is also essential to conduct training programs for oncologists and laboratory specialists, which will enable more effective use of the obtained results in clinical practice. In Uzbekistan, an important direction is the expansion of the use of extracellular vesicle analysis for monitoring tumour progression and assessing responses to chemotherapy. Research should be intensified on the use of long non-coding RNAs and their connection to tumour resistance to drugs, which will improve treatment strategies for patients with metastatic cancer. Thus, in each of these countries, liquid biopsy holds significant potential for improving early diagnosis and personalized therapy for oncological diseases. The integration of molecular methods, training of specialists, and expansion of international cooperation will contribute to the effective use of this tool in clinical practice and reduce cancer mortality.

In conclusions, the issue of liquid biopsy has been considered in the context of personalized diagnosis and treatment of oncological diseases. Free circulating tumour nucleic acids, secretory vesicles, and circulating cells that persist in blood plasma, lymph, cerebrospinal fluid, saliva, and urine, migrating from the site of injury, are used for marker monitoring. The most informative marker matrix is vesicles, which simultaneously contain information about the genome, transcriptome, metabolome, and proteome of transformed cells. Tumour material is isolated using immunomagnetic adsorption, preparative chromatography, selective precipitation, dialysis, and gradient centrifugation methods. Target molecules are analysed for gene mutations, chromosomal aberrations, epigenetic changes (methylation, regulatory RNAs), translational levels, and post-translational modifications (PCR in various variants, sequencing, blotting). Results of molecular screening of plasma samples revealed diagnostic markers for breast cancer (miR-21, miR-181a, miR-484, miR-12060b, and other regulatory RNAs; *Ecm1*, *HER-2*, *ER*, *Mbl2, Btd*, mutations in the *PIC3A* gene), colorectal cancer (Rnf208, ecto-Cd73, Cea, long non-coding RNAs *UCA1, PCA3, BCAR4*, mutations in *NRAS, KRAS, BRA*F genes), ovarian cancer (Muc1, Bst2, Ccne1, Cfh), prostate cancer (circular RNA circ_044516, transglutaminase, mutations in the AR gene), lung cancer (miR-146a-5p, miR-125b-5p, miR-21b-5p, long non-coding RNA H19, mutations in the *EGFR* gene), and pancreatic cancer (Mif, CD104, CD44v6, miR-4306, miR-4644, mutations in the *P53*, *KRAS* genes). Through the titration of ctDNA, personalized hormone therapy was tailored for patients with prostate cancer, and chemotherapy was selected for patients with colorectal cancer. In cases of deletions in the tyrosine kinase gene *EGFR*, patients with lung

tumours were either prescribed or had their inhibitor treatment substituted. The residual migratory ctDNA or DNA detected in blood post-treatment was found to be a prognostic factor for recurrence in lung cancer (64%) and colorectal cancer (100% recurrence when ctDNA titters exceeded 4). The study also emphasises how important liquid biopsy is for creating personalised treatment plans in certain countries, especially by adjusting targeted therapies based on common local mutation profiles like *KRAS*, *EGFR*, and AR. This enables the development of regionally customised molecular diagnostic techniques and therapy guidelines.

The advantages of liquid biopsy include non-invasiveness, easy access to biological material, outpatient feasibility, and a low risk of complications. However, the method's drawbacks include low sensitivity due to insufficient persistent cancer material, its genetic-protein heterogeneity, and the instability of isolated proteins and nucleic acids. A limitation of the current work may be the contradictory results regarding some markers obtained by different researchers at various times. Future research prospects include the development of new methods for concentrating material and convenient molecular test systems for determination.

Author Contribution Statement

Study conception and design: LN; analysis and interpretation of results: EM; draft manuscript preparation: PP, NK, FU. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgements

None.

Conflict of Interest

The authors of this manuscript declare no conflict of interest.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660.
2. Caputo V, Ciardiello F, Corte CM, Martini G, Troiani T, Napolitano S. Diagnostic value of liquid biopsy in the era of precision medicine: 10 years of clinical evidence in cancer. Explor Targeted Anti-Tumor Ther. 2023;4(1):102-38. https://doi.org/10.37349/etat.2023.00125.
3. Aleksiev V, Markov D, Bechev K. Tumor Markers in Pleural Fluid: A Comprehensive Study on Diagnostic Accuracy. Diagn. 2025;15(2):204. https://doi.org/10.3390/diagnostics15020204
4. Rodríguez J, Avila J, Rolfo C, Ruíz-Patiño A, Russo A, Ricaurte L, et al. When tissue is an issue the liquid biopsy is nonissue: A review. Oncol Ther. 2021;9(1):89-110. https://doi.org/10.1007/s40487-021-00144-6.
5. Martins I, Ribeiro IP, Jorge J, Gonçalves AC, Sarmento-Ribeiro AB, Melo JB, et al. Liquid biopsies: Applications for cancer diagnosis and monitoring. Genes. 2021;12(3):349. https://doi.org/10.3390/genes12030349.
6. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease – Latest advances and implications for cure. Nature reviews. Clin Oncol. 2019;16(7):409-24. https://doi.org/10.1038/s41571-019-0187-3.
7. Della Corte L, Russo G, Pepe F, Pisapia P, Dell'Aquila M, Malapelle U, et al. The role of liquid biopsy in epithelial ovarian cancer: State of the art. Crit Rev Oncol Hematol. 2024;194:104263. https://doi.org/10.1016/j.critrevonc.2024.104263.
8. Konkayev A, Kadralinova A, Azimova B, Tazhibayeva D, Yeltayeva A, Konkayeva M. Usage of Meropenem Continuous Infusion for Treatment of Infectious Complications in Orthopedic Elderly Patients with Anemia: A Case Series. Med Lith. 2024;60(6):929. https://doi.org/10.3390/medicina60060929
9. Nasrytdinov TS. Prognostic value of liquid biopsy in CRC: A literature review. Oncology and Radiol Kazakhstan. 2023;68(2):76-81. https://doi.org/10.52532/2521-6414-2023-2-68-76-81.
10. Mishra V, Singh A, Chen X, Rosenberg AJ, Pearson AT, Zhavoronkov A, et al. Application of liquid biopsy as multi-functional biomarkers in head and neck cancer. British J Canc. 2022;126(3):361-70. https://doi.org/10.1038/s41416-021-01626-0.
11. Kurma K, Eslami-S Z, Alix-Panabières C, Cayrefourcq L. Liquid biopsy: Paving a new avenue for cancer research. Cell Adhes Migr. 2024;18(1):1-26. https://doi.org/10.1080/19336918.2024.2395807.
12. Najafi S, Majidpoor J, Mortezaee K. Liquid biopsy in colorectal cancer. Clin Chim Acta. 2024;553:117674. https://doi.org/10.1016/j.cca.2023.117674.
13. Pantel K, Alix-Panabières C. Crucial roles of circulating tumor cells in the metastatic cascade and tumor immune escape: Biology and clinical translation. J Immunother Canc. 2022;10(12):e005615. https://doi.org/10.1136/jitc-2022-005615.
14. Stejskal P, Goodarzi H, Srovnal J, Hajdúch M, van 't Veer LJ, Magbanua MJ. Circulating tumor nucleic acids: biology, release mechanisms, and clinical relevance. Molec Canc. 2023;22(1):15. https://doi.org/10.1186/s12943-022-01710-w.
15. Sarkar D, Khan BA, Bardhan A, Ghosh A, Pal DK. Exploring the potential of BOLA3-DT as a diagnostic biomarker in prostate cancer. Urol J. 2025;92(2):267-72. https://doi.org/10.1177/03915603251314995
16. Terlizzi M, Bossi A. Much ado about nothing? Assessing the actual benefits of proton beam therapy for prostate cancer. Urol J. 2025;92(1):65-6. https://doi.org/10.1177/03915603241283296
17. Sanchez-Cabrero D, Garcia-Guede Á, Burdiel M, Pernía O, Colmenarejo-Fernandez J, Gutierrez L, et al. miR-124 as a liquid biopsy prognostic biomarker in small extracellular vesicles from NSCLC Patients. Intern J Molec Sci. 2023;24(14):11464. https://doi.org/10.3390/ijms241411464.
18. Lone SN, Nisar S, Masoodi T, Singh M, Rizwan A, Hashem S, et al. Liquid biopsy: A step closer to transform diagnosis, prognosis and future of cancer treatments. Molec Canc. 2022;21(1):79. https://doi.org/10.1186/s12943-022-01543-7.
19. Tulewicz-Marti EM, Lewandowski K, Szczubelek M, Rydzewska G. Management of anaemia in patients with inflammatory bowel disease - results of a questionnaire among Polish healthcare professionals. Przegl Gastroenterol. 2021;16(1):89-94. https://doi.org/10.5114/pg.2021.104738
20. Tulewicz-Marti E, Stępień-Wrochna B, Maciejewska K, Łodyga M, Karłowicz K, Lewandowski K, et al. Awareness

and Compliance with the Recommendations of Primary and Secondary Prevention of Cancer in Patients with Inflammatory Bowel Disease. J Pers Med. 2023;13(6):913. https://doi.org/10.3390/jpm13060913
21. Nagasawa S, Kashima Y, Suzuki A, Suzuki Y. Single-cell and spatial analyses of cancer cells: Toward elucidating the molecular mechanisms of clonal evolution and drug resistance acquisition. Inflamm Regen. 2021;41(1):22. https://doi.org/10.1186/s41232-021-00170-x.
22. Díaz Del Arco C, Fernández Aceñero MJ, Ortega Medina L. Liquid biopsy for gastric cancer: Techniques, applications, and future directions. World J Gastroent. 2024;30(12):1680-705. https://doi.org/10.3748/wjg.v30.i12.1680.
23. de Kock R, van den Borne B, Youssef-El Soud M, Belderbos H, Brunsveld L, Scharnhorst V, et al. Therapy monitoring of *EGFR*-positive non-small-cell lung cancer patients using ddPCR multiplex assays. J Molec Diagn. 2021;23(4):495-505. https://doi.org/10.1016/j.jmoldx.2021.01.003.
24. Heidrich I, Ačkar L, Mossahebi Mohammadi P, Pantel K. Liquid biopsies: Potential and challenges. Intern J Canc. 2021;148(3):528-45. https://doi.org/10.1002/ijc.33217.
25. Shin S, Woo HI, Kim JW, Yoonjung Kim MD, Lee KA. Clinical practice guidelines for pre-analytical procedures of plasma epidermal growth factor receptor variant testing. Ann Lab Med. 2022;42(2):141-9. https://doi.org/10.3343/alm.2022.42.2.141.
26. Haupts A, Vogel A, Foersch S, Hartmann M, Maderer A, Wachter N, et al. Comparative analysis of nuclear and mitochondrial DNA from tissue and liquid biopsies of colorectal cancer patients. Sci Rep. 2021;11(1):16745. https://doi.org/10.1038/s41598-021-95006-6.
27. Kaloshi G, Roji A, Seferi A, Cakani B, Bushati T, Roci E, et al. Spinal dissemination of intracranial glioblastoma in bevacizumab era: A potential bevacizumab-induced mechanism. Acta Inf Med. 2014;22(2):142-144. https://doi.org/10.5455/aim.2014.22.142-144
28. Efremov A. Psychosomatics: Communication of the Central Nervous System through Connection to Tissues, Organs, and Cells. Clin Psychopharm Neurosci. 2024;22(4):565-77. https://doi.org/10.9758/cpn.24.1197
29. Han HJ, Sung JY, Kim SH, Yun UJ., Kim H, Jang EJ, et al. Fibronectin regulates anoikis resistance via cell aggregate formation. Canc Lett. 2021;508:59-72. https://doi.org/10.1016/j.canlet.2021.03.011.
30. Gao Z, Pang B, Li J, Gao N, Fan T, Li Y. Emerging role of exosomes in liquid biopsy for monitoring prostate cancer invasion and metastasis. Front Cell Devel Biol. 2021;9:679527. https://doi.org/10.3389/fcell.2021.679527.
31. Fattore L, Ruggiero CF, Liguoro D, Castaldo V, Catizone A, Ciliberto G, et al. The promise of liquid biopsy to predict response to immunotherapy in metastatic melanoma. Front Oncol. 2021;11:645069. https://doi.org/10.3389/fonc.2021.645069.
32. Montayeva NS, Kushaliyev KZ, Shalmenov M, Gusmanov MG, Sadenov MM. Haematological, biochemical and immunomorphologic changes in dogs with melanoma and melanocytoma. Biosci Biotech Res Asia. 2015;12(1):321-6. https://doi.org/10.13005/bbra/1668
33. Templeman A, Miller MC, Cooke MJ, O'Shannessy DJ, Gurung Y, Pereira T, et al. Analytical performance of the FDA-cleared Parsortix® PC1 system. J Circul Biomark. 2023;12:26-33. https://doi.org/10.33393/jcb.2023.2629.
34. Nonaka T, Wong DT. Saliva diagnostics. Ann Rev Anal Chem. 2022;15(1):107-21. https://doi.org/10.1146/annurev-anchem-061020-123959.
35. Mumtaz M, Bijnsdorp IV, Böttger F, Piersma SR, Pham TV, Mumtaz S, et al. Secreted protein markers in oral squamous cell carcinoma (OSCC). Clin Prot. 2022;19(1):4. https://doi.org/10.1186/s12014-022-09341-5.
36. Tosoian JJ, Zhang Y, Xiao L, Xie C, Samora NL, Niknafs YS, et al. Development and validation of an 18-gene urine test for high-grade prostate cancer. JAMA Oncol. 2024;10(6):726-736. https://doi.org/10.1001/jamaoncol.2024.0455.
37. Li P, Liu S, Du L, Mohseni G, Zhang Y, Wang C. Liquid biopsies based on DNA methylation as biomarkers for the detection and prognosis of lung cancer. Clin Epigen. 2022;14(1):118. https://doi.org/10.1186/s13148-022-01337-0.
38. Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: Current technology and clinical applications. J Hematol Oncol. 2022;15(1):131. https://doi.org/10.1186/s13045-022-01351-y.
39. Buzas EI. The roles of extracellular vesicles in the immune system. Nature Rev Immunol. 2023;23(4):236-50. https://doi.org/10.1038/s41577-022-00763-8.
40. Halder R, Veeravelli S, Cheng C, Estrada-Mendizabal RJ, Recio-Boiles A. Health disparities in presentation, treatment, genomic testing, and outcomes of pancreatic cancer in Hispanic and non-Hispanic patients. J Rac Ethn Health Disparit. 2023;10(6):3131-9. https://doi.org/10.1007/s40615-022-01486-1.
41. Hinzman CP, Singh B, Bansal S, Li Y, Iliuk A, Girgis M, et al. A multi-omics approach identifies pancreatic cancer cell extracellular vesicles as mediators of the unfolded protein response in normal pancreatic epithelial cells. J Extracell Vesicl. 2022;11(6):e12232. https://doi.org/10.1002/jev2.12232.
42. Kim J, Kim M, Kim Y, Moon S, Lee S, Lee H, et al. Abstract 2436: In-depth analysis of microRNA signature in breast cancer derived extracellular vesicles: A potential biomarker repository for breast cancer diagnosis. Cancer Res. 2024;84(6):2436. https://doi.org/10.1158/1538-7445.AM2024-2436.
43. Nakamura K, Zhu Z, Roy S, Jun E, Han H, Munoz RM, et al. An exosome-based transcriptomic signature for noninvasive, early detection of patients with pancreatic ductal adenocarcinoma: A multicenter cohort study. Gastroenterology. 2022;163(5):1252-66. https://doi.org/10.1053/j.gastro.2022.06.090.
44. Cardinali B, Tasso R, Piccioli P, Ciferri MC, Quarto R, Del Mastro L. Circulating miRNAs in breast cancer diagnosis and prognosis. Cancers. 2022;14(9):2317. https://doi.org/10.3390/cancers14092317.
45. Kim M, Kim J, Kim Y. Abstract 1843: Integrating machine learning with microfluidic technologies for proteomic profiling of extracellular vesicles in triple-negative breast cancer. Canc Res. 2024;84(6):1843. https://doi.org/10.1158/1538-7445.AM2024-1843.
46. Guo W, Zhang C, Wang X, Dou D, Chen D, Li J. Resolving the difference between left-sided and right-sided colorectal cancer by single-cell sequencing. JCI Insight. 2022;7(1):e152616. https://doi.org/10.1172/jci.insight.152616.
47. Dieudé M, Hébert M.-J. Extracellular vesicles beyond biomarkers: Effectors of antibody-mediated rejection. Am J Transplant. 2022;22(9):2131-2. https://doi.org/10.1111/ajt.17133.
48. Ryu HS, Kim HJ, Ji WB, Kim BC, Kim JH, Moon SK, et al. Colon cancer: the 2023 Korean clinical practice guidelines for diagnosis and treatment. Ann Coloproctol. 2024;40(2):89-113. https://doi.org/10.3393/ac.2024.00059.0008.
49. Dorayappan KD, Wagner V, Park D, Newcomer MM, Lightfoot MD, Kalaiyarasan D, et al. ISG15 mediates the function of extracellular vesicles in promoting ovarian cancer

progression and metastasis. J Extracell Biol. 2024;3(2):e92. https://doi.org/10.1002/jex2.92.
50. Winn-Deen ES, Bortolin LT, Gusenleitner D, Biette KM, Copeland K, Gentry-Maharaj A, et al. Improving specificity for ovarian cancer screening using a novel extracellular vesicle-based blood test: Performance in a training and verification cohort. J Mol Diagn. 2024;26(12):1129-48. https://doi.org/10.1016/j.jmoldx.2024.09.001.
51. Oshi M, Murthy V, Takahashi H, Huyser M, Okano M, Tokumaru Y, et al. Urine as a source of liquid biopsy for cancer. Cancers. 2021;13(11):2652. https://doi.org/10.3390/cancers13112652.
52. Zhao Y, Tang J, Jiang K, Liu SY, Aicher A, Heeschen C. Liquid biopsy in pancreatic cancer – Current perspective and future outlook. Biochim Biophys Acta. Rev Canc. 2023;1878(3):188868. https://doi.org/10.1016/j.bbcan.2023.188868.
53. Aleksiev V, Markov D, Yavorov B, Bechev K, Markov G, Shterev F, et al. Enhanced diagnostic approaches for malignant pleural effusions: An extensive biochemical and statistical analysis. Folia Med. 2025;67(2):e145825. https://doi.org/10.3897/folmed.67.e145825
54. Liu Q, Zheng J, Xie A, Chen M, Gong RY, Sheng Y, et al. Exosome, a rising biomarkers in liquid biopsy: Advances of label-free and label strategy for diagnosis of cancer. Crit Rev Anal Chem. 2024;1-12. https://doi.org/10.1080/10408347.2024.2339961
55. Magbanua MJ, Hendrix LH, Hyslop T, Barry WT, Winer EP, Hudis C, et al. Serial analysis of circulating tumor cells in metastatic breast cancer receiving first-line chemotherapy. J Natl Cancer Inst. 2021;113(4):443-52. https://doi.org/10.1093/jnci/djaa113.
56. Herasymenko O, Herasymenko Y. Ultrastructural features of primary cancer and its metastases. Gac Med Caracas. 2024;132(2):440-53. https://doi.org/10.47307/gmc.2024.132.2.15
57. Spiliotaki M, Neophytou CM, Vogazianos P, Stylianou I, Gregoriou G, Constantinou AI, et al. Dynamic monitoring of PD-L1 and Ki67 in circulating tumor cells of metastatic non-small cell lung cancer patients treated with pembrolizumab. Mol Oncol. 2023;17(5):792-809. https://doi.org/10.1002/1878-0261.13317.
58. Su X, Shan Z, Duan S. Harnessing extracellular vesicles using liquid biopsy for cancer diagnosis and monitoring: highlights from AACR Annual Meeting. J Hematol Oncol. 2024;17(1):55. https://doi.org/10.1186/s13045-024-01577-y.
59. Kashanskiĭ SV, Zhetpisbaev BA, Il'derbaev OZ, Ermenbaĭ OT. Mesothelioma in the Republic of Kazakhstan: a review. Gig Sanitariia. 2008;(5):13-7.
60. Amir M, Djaharuddin I, Saputri SA, Qanitha A. Chemotherapy-Induced Cardiotoxicity in Lung Cancer Patients: A Systematic Review of Case Reports. Gac Med Caracas. 2024;132(3):772-84. https://doi.org/10.47307/GMC.2024.132.3.19
61. Gale D, Heider K, Ruiz-Valdepenas A, Hackinger S, Perry M, Marsico G, et al. Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer. Ann Oncol. 2022;33(5):500-10. https://doi.org/10.1016/j.annonc.2022.02.007.
62. Montayeva NS, Kushaliyev KZ, Grabarevic Z. Early symptoms of malignancy in the appearance of melanoma in dogs. Biol Med. 2016;8(1).
63. Chen S, Zhou Z, Li Y, Du Y, Chen G. Application of single-cell sequencing to the research of tumor microenvironment. Front Immunol. 2023;14:1285540. https://doi.org/10.3389/fimmu.2023.1285540