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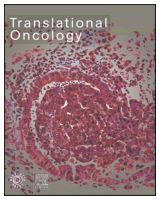

Liquid Biopsies: Emerging role and clinical applications in solid tumours

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ABSTRACT

Late detection and lack of precision diagnostics are the major challenges in cancer prevention and management. Biomarker discovery in specific cancers, especially at the pre-invasive stage, is vital for early diagnosis, positive treatment response, and good disease prognosis. Traditional diagnostic measures require invasive procedures such as tissue excision using a needle, an endoscope, and/or surgical resection which can be unsafe, expensive, and painful. Additionally, the presence of comorbid conditions in individuals might render them ineligible for undertaking a tissue biopsy, and in some cases, it is difficult to access tumours depending on the site of occurrence. In this context, liquid biopsies are being explored for their clinical significance in solid malignancies management. These non-invasive or minimally invasive methods are being developed primarily for identification of biomarkers for early diagnosis and targeted therapeutics. In this review, we have summarised the use and importance of liquid biopsy as significant tool in diagnosis, prognosis prediction, and therapeutic development. We have also discussed the challenges that are encountered and future perspective.

Introduction

Technological advancements have led to significant improvement in areas of cancer diagnostics and therapeutics. Newer and highly sensitive and specific tools for tumour profiling and management are being commercialised or under development. However, the conventional biopsy methods available for tumour profiling are invasive, and also pose challenges in specific cases where tissue accessibility is a concern and also in patients with comorbidity. In this context, developing non-invasive liquid biopsy methods for early cancer detection has been the prime focus of researchers [1].

"Liquid biopsy" (LB) refers to a method for detecting the presence of specific molecular markers of a disease in liquid samples including blood, saliva, urine, and other minimally invasive biological samples [2]. LB is emerging as a major realm in the precision oncology approach [3]. LB has been established as an innovative diagnostic measure by analysing the Cell-free DNA (cfDNA), exosomes, circulating tumour cells (CTCs), proteins/ peptides, and circulating tumour DNA (ctDNA) [4] in biological fluids. A clear and comprehensive picture of these multiple components and molecules in the body fluids in specific cancers will lead to early and improved cancer detection, better cancer management, predicting prognosis, and effective monitoring of therapeutic response [5] (Fig. 1).

As of now, few LB based methods for cancer detection are available commercially. In lung and colorectal cancer (CRC), US-FDA has approved ctDNA-based diagnostic markers [6] and the CELLSEARCH® CTCs as a metastatic diagnostics tool for colon, breast, and prostate cancer (PC) [7,8]. Despite their clinical significance, utility of these methods is limited, as they are not globally recognized and are not part of a standard cancer diagnosis as well. Solid tumours are quite complex in their origin, either due to genetic aberrations or environmental insults [9]. LBs have been very advantageous in the detection and profiling of solid tumours in order to get an overall tumour progression scenario [7, 10]. In this review, we have described the different types of liquid

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biopsies components and their clinical utility with respect to diagnostic, prognostic, and possible therapeutic uses in solid tumours.

Components of liquid biopsies and their use in solid tumours

Circulating tumour cells (CTC)

CTCs get detached from the site of primary tumour and disseminate into the bloodstream of cancer patients at different stages of cancer progression [11]. These CTCs can be easily isolated from human biological fluids (serum and plasma) and can be used for cancer diagnosis. Sample collection of bodily fluids offers twin advantages of being easily available and patient-friendly than conventional tissue biopsies. More importantly, it can detect tumour cells in the body fluid at an early stage, which allows for effective cancer management and therapeutic response [12,13]. Another significant advantage of these CTCs-based biomarkers in cancer detection is the sensitivity and specificity as chances of false positives are minimal [14].

CTCs were discovered by Thomas Ashworth. Detection and profiling of tumour through CTCs is more feasible than conventional biopsies and has led to the development of novel strategies not only in diagnosis, but also in the monitoring of metastasis and tumour recurrence of solid tumours [15]. Isolation of CTCs from from the primary solid tumours has been established and validated. Firstly, CTCs are collected and enriched based on their physical and biological characteristics. This is followed by detection of CTCs based on their size and electrical properties [13]. CELLSEARCH® was the first FDA-approved method that allowed scientists to enrich and culture CTCs from the peripheral blood of patients [15].

Several studies have reported the clinical significance of CTCs in detecting locally advanced cervical cancer [16,17]. Wen et al. [18] were also able to detect cervical cancer in serum samples of the patients using CTCs along with the Squamous cell carcinoma antigen (SCC-Ag), resulting in the development of an efficient predictive model for cervical cancer progression as compared to a single biomarker alone. Further, the significance of CTCs in Head and Neck cancer has been highlighted where a strong correlation between early clinical outcome and the presence of CTCs in patient samples has been observed, leading to a good prognosis [19].

Furthermore, a study has also compared CTC-based CELLSEARCH® and AdnaTest® tests to determine specificity and sensitivity variations, and observed that the combination of both the detection techniques was highly significant [20]. Because of the infrequent presence of CTCs in blood and their low number (very few in comparison to other blood cells), the development of more reliable CTCs detection approaches for diagnostics and therapeutic applications are under process (Table 1).

Circulating tumour DNA (ctDNA)

ctDNA-based liquid biopsy is an another tool for molecular diagnosis and surveillance of cancer [21]. Once released from tumour cells, ctDNA harboring original tumour mutations enters the bloodstream. Analysis of this ctDNA from blood samples has paved the way for determining the patients' genetic landscape, helping not only in tumour detection but also in saving time and cost as required for a genetic testing [22,23].

Several studies have established ctDNA as a diagnostic marker for molecular profiling of tumour e.g., in non-small cell lung cancer (NSCLC) and CRC: epidermal growth factor receptor (EGFR) and Vi-Ki-ras2 kirsten rat sarcoma viral oncogene homolog (KRAS) mutation, respectively [24,25]. Another study has shown its role in metastatic breast cancer for early diagnosis of disease and concluded that ctDNA might serve as a highly sensitive biomarker [26]. A study from John Hopkins screened 640 patient samples through the Digital Droplet PCR technique and found 48-73% of patients with colorectal, gastrointestinal, pancreatic, and breast cancer showing the presence of ctDNA [27]. Comparative analysis of BRCA1/2 and DNA repair gene mutation via liquid biopsy using ctDNA and tissue biopsy methods revealed concordance in the genetic landscape [28]. Preclinical and clinical research on ctDNA has shown its role in monitoring treatment response in real-time, selective treatment methods, and feasibility [29].

Cell-free DNA (cfDNA)

In 1948, Mandel and Metais discovered cfDNA, which has been observed to play a vital role in disease diagnosis. Apart from blood, cfDNA is also found in other biological fluids like saliva, urine, and cerebrospinal fluid [30,31]. It is released from cells mostly by apoptosis, necrosis, and active secretion [32]. Previous studies have shown cfDNA

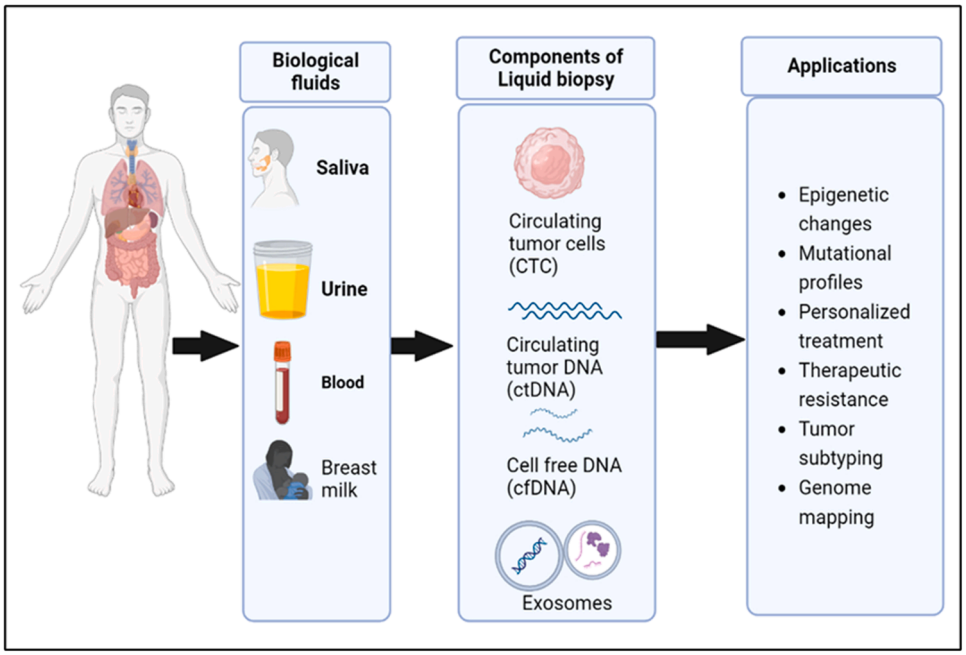

Fig. 1. An overview of liquid biopsies and their applications. (Created with BioRender.com)

Table 1
List of clinical trials conducted in liquid biopsies

S. No.	Study title	Cancer	NCT number	Location
1.	Study of Metabolites Markers in Adjuvant Breast Cancer	Breast cancer	NCT03367208	• Centre Antoine Lacassagne, Nice, Cedex 2, France
2.	Characterization & Comparison of Drugable Mutations in Primary and Metastatic Tumors, CTCs and cfDNA in MBCpatients	Metastatic Breast cancer	NCT02626039	
3.	Harvest of CTCs From MBC Patients Using the Parsortix™ PC1 System	Breast cancer	NCT03427450	• University of Southern California • Los Angeles, California, United States • Northwestern University • Chicago, Illinois, United States • University of Rochester Medical Center Wilmot Cancer Institute • Rochester, New York, United States • UT MD Anderson Cancer Center • Houston, Texas, United States
4.	Determination of RAS Mutation Status in Liquid Biopsies in Subjects With RAS Wild-type.PERSEIDA Study	Colorectal cancer	NCT03957564	• Research Site • Almeria, Andalucía, Spain • Research Site • Granada, Andalucía, Spain • Research Site • Sevilla, Andalucía, Spain
5.	Concordance of Inivata Liquid Biopsy with Standard Tissue Biopsy	Non-Small cell lung cancer	NCT03116633	• Mercy Hospital Cancer Center/Clinical Research • Fort Smith, Arkansas, United States, 72903 • Oklahoma City, Oklahoma, United States, 73120
6.	Concordance of Inivata Liquid Biopsy with Standard of Care Tissue Testing	Non-Small cell lung cancer	NCT02906852	• Facey Medical Foundation • Mission Hills, California, United States • University of Colorado Cancer Center • Aurora, Colorado, United States • Eastern CT Hematology Oncology • Norwich, Connecticut, United States
7.	TAGRISSO (Osimertinib) in NSCLC Patients in Whom T790 Mutations Are Detected by Liquid Biopsy	Non-Small cell lung cancer	NCT03394118	• Asan Medical Center Seoul, Korea, Republic
8.	Therapeutic resistance and Clonal Evolution Assessed with Liquid Biopsy of NSCLC Patients in China	• Lung Neoplasms • Lung Cancer, Non-small Cell • Adenocarcinoma of Lung • Squamous Cell Lung Cancer	NCT03059641	• The First Affiliated Hospital of Guangzhou Medical University • Guangzhou, Guangdong, China • First Hospital of Jilin University • Changchun, China • Sir Run Run Shaw Hospital, Zhejiang University School of medicine • Hangzhou, China
9.	Clinical Application of Liquid Biopsy for Precise Diagnosis and Prognosis in Lymphoma	Lymphomas	NCT04062877	• Institute of Hematology Southeast UniversitY • Department of Hematology Zhongda Hospital Southeast University Medical School, NanJing, Jiangsu, China
10.	Study to Detect Biomarker Gradients in Coronary Arteries Using the Liquid Biopsy System	Coronary Heart Disease	NCT02119767	• Papworth Hospital NHS Foundation Trust • Papworth Everard, Cambridgeshire, United Kingdom
11.	A Study to Evaluate the Value of Circulating Tumour DNA in Follow-up of Patients with an Advanced Gastroenteropancreatic or Lung Neuroendocrine Tumour Under Everolimus +- SSA Treatment	Neuroendocrine Tumors	NCT05255133	• Z Rivierenland • Bornem, Antwerp, Belgium • AZ Klina • Brasschaat, Antwerp, Belgium • AZ Monica • Deurne, Antwerp, Belgium
12.	The Correlation of PD-L1 Expression in Non-small Lung Cancer Tissue and Peripheral Blood T Cell and Serum.	Non-small Cell Lung Cancer	NCT03073902	• Xinqiao Hospital of Chongqing Chongqing, China
13.	Circulating Tumor DNA as Liquid Biopsy in Patients with Stage IV Solid Tumors, a Feasibility Study at MUSC HCC	Adenocarcinoma of the Colon Adenocarcinoma of the Rectum	NCT03302325	• Medical University of South Carolina • Charleston, South Carolina, United States
14.	Detection of Circulating Tumor DNA Through Liquid Biopsies in Ovarian Cancer Patients and Evaluation of Prognostic and Predictive Values of Circulating Tumor DNA Assay.	Ovarian cancer	NCT05504174	• Yonsei University Health System, Severance Hospital Seoul, Korea, Republic of
15.	Liquid Biopsy Using Methylation Sequencing for Lung Cancer	• Lung cancer	NCT04253509	• Samsung Medical Center, Seoul, Korea, Republic of
16.	Olmutinib Trial in T790M (+) NSCLC Patients Detected by Liquid Biopsy Using BALF Extracellular Vesicular DNA	• Non-Small Cell Lung Cancer	NCT03228277	• Konkuk University Medical Center Seoul, Korea, Republic of
17.	Collection of Blood, Urine, and Stool to Monitor Metastatic Colorectal Cancers	Metastatic Colorectal cancer	NCT03563651	• USC / Norris Comprehensive Cancer Center, Los Angeles, California, United States
18.	Study of Circulating Tumor DNA (ctDNA) Kinetics in Immuno-oncology (IO-KIN)	• Head and Neck Cancer	NCT04606940	• Princess Margaret Cancer Centre, Toronto, Ontario, Canada
19.	A Comprehensive Evaluation of Circulating Tumor DNA and Circulating Tumor Cells as a Predictive Marker in Lung Cancer	• Lung cancer	NCT04254497	

(*continued on next page*)

Table 1 (*continued*)

S. No.	Study title	Cancer	NCT number	Location
20.	AI-EMERGE: Development and Validation of a Multi-analyte, Blood-based Colorectal Cancer Screening Test	• Colorectal cancer • Colon cancer • Rectum cancer	NCT03688906	• Clinical Research Associates • Huntsville, Alabama, United States • Del Sol Research Management • Chandler, Arizona, United States • Del Sol Research Management, • Tucson, Arizona, United States
21.	Noninvasive vs. Invasive Lung Evaluation	• Non-Small Cell Lung Cancer	NCT03615443	
22.	Mutational Landscape in Hepatocellular Carcinoma	• Hepatocellular carcinoma • Cirrhosis	NCT03071458	• INSERM • Paris, France
23.	Use of Exome Sequence Analysis and Circulating Tumour in Assessing Tumour Heterogeneity in BRAF Mutant Melanoma	• Melanoma	NCT02251314	• Princess Margaret Cancer Centre, Toronto, Ontario, Canada
24.	Characterization & Comparison of Drugable Mutations in Primary and Metastatic Tumors, CTCs and cfDNA in MBCpatients	• Metastatic Breast cancer	NCT02626039	• Hospital General Universitario Gregorio Marañón, Madrid, Spain
25.	Harvest of CTCs From MBC Patients Using the Parsortix™ PC1 System	• Metastatic Breast cancer	NCT03427450	• University of Southern California, Los Angeles, California, United States • Northwestern University, Chicago, Illinois, United States • University of Rochester Medical Center Wilmot Cancer Institute, Rochester, New York, United States • UT MD Anderson Cancer Center, Houston, Texas, United States
26.	Multicenter Validation of the Sensitivity of Theranostic ALK Rearrangement Detection by FISH Analysis and Prevalence of Escaping Mutations in Circulating Tumor Cells for the Non-invasive Management of Lung Cancer Patients	• Lung Neoplasms	NCT02372448	• Centre François Baclesse, Caen, France • Grenoble university hospital, Grenoble, France • Marseille University Hospital, Marseille, France
27.	Circulating Tumor Cells and Tumor DNA in HCC and NET	• Hepatocellular carcinoma • Neuroendocrine Tumors	NCT02973204	• Department of Hepatology and Gastroenterology, Aarhus, Aarhus C, Denmark
28.	Circulating Tumour Cells in Somatuline Autogel Treated NeuroEndocrine Tumours Patients	• Neuroendocrine Tumours	NCT02075606	• Basingstoke & North Hampshire Hospital, Basingstoke, United Kingdom • Queen Elizabeth Hospital, Birmingham, United Kingdom • University Hospital Wales, Cardiff, United Kingdom
29.	Diagnostic Accuracy of Circulating Tumor Cells (CTCs) and Onco-exosome Quantification in the Diagnosis of Pancreatic Cancer - PANC-CTC	• Pancreatic Ductal Adenocarcinoma (PDAC)	NCT03032913	• Chu de Bordeaux, Bordeaux, France
30.	Liquid Biopsy in Lung Cancer	• Lung cancer • Circulating Tumor Cell	NCT03479099	• Samsung Medical Center, Seoul, Korea, Republic of

(Source: https://clinicaltrials.gov)

as an active biomarker for early cancer detection through digital polymerase chain reaction (dPCR), quantitative PCR (qPCR), and next-generation sequencing (NGS) based detection techniques [33].

A breast cancer study revealed the methylation dynamics of cfDNA during breast cancer progression [34]. An analysis of the genomic characterization of metastatic triple negative breast cancer (TNBC) showed that cfDNA is linked with poor survival in metastatic TNBC cohort [35]. cfDNA estimation and detection is favoured among liquid biopsies because it helps in monitoring the patient's real-time treatment outcome or response towards the targeted therapy [36]. However, cfDNA-based tools need to be validated on a large scale and in different tumours to increase its sensitivity for early diagnosis with high precision.

Exosomes

Exosomes are one of the emerging areas of biomarkers that are biologically active at various stages and also regulate multiple cell-cell interactions in carcinogenesis [37]. Exosomes are small cell-derived nanovesicles that help move the cargo from donor to the receiver cell. Exosomes derived from tumour serves as crucial biomarkers for early cancer detection and prognosis [38]. Exosomes are promising among the several liquid biopsy components that can be assessed due to their existence across all human body fluids and their possibilities for multi-component analysis. The concentration or amount of analytes in membrane-enclosed vesicles might provide greater precision and sensitivity than traditional liquid biopsies [39].

Several studies have shown a potential role of exosomes as diagnostics tools in solid tumours such as NSCLC [40], ovarian cancer [41], breast cancer [42], prostate cancer [43], hepatocellular carcinoma [44], CRC [45]. In a study on PC, exosomes in the blood and urine samples of invasive and metastatic cancer patients showed presence of PC-specific components that increase the detection specificity and sensitivity [46]. In a study on breast cancer, alterations in morphology and cargo of exosomes loaded with molecules like micro-RNAs and long noncoding RNAs were observed to be responsible for the activation of multiple aberrant signalling pathways [47]. Another study on TNBC breast cancer revealed the presence of specific miRNAs like miR246, 134, and 503 thought to be playing a significant role in tumour metastasis, and can be used as a metastatic biomarker [48]. However, cancer detection via exosomes also has its limitations, as some miRNAs encaged in these exosomes have been observed to be common in different cancers which make it difficult to differentiate among specific cancers. Nevertheless, exosomes need to be explored further for identification and development of specific biomarkers for different cancers [47].

Emerging clinical applications of liquid biopsy

Liquid biopsies have multiple benefits and have huge potential in improving multiple facets in cancer management, and tumour characterization, alongwith its implications in diagnosis, prognosis, treatment decisions, and long-term tracking. There are several aspects of cancer management where these liquid biopsies can be utilized, as discussed below in Table 2.

Diagnosis and tumour profiling

As discussed previously, the widely studied analytes of liquid biopsy are cfDNA, ctDNA, exoDNA and CTCs. It has been shown that quantity and quality [49,50] of cfDNA can differentiate between healthy persons and cancer patients. The total amount of cfDNA is usually found to be higher in cancer patients and it increases with disease progression and metastasis [51,52]. ctDNA is another tool which tells about the tumour genetic landscape including copy number variations (CNVs), methylation marks, insertions/ deletions, single nucleotide variants (SNVs) etc. These alterations revealed by LB using ctDNA are quite consistent with the conventional genetic testing through tissue biopsy in several solid tumours like lung [52], breast, colorectal, pancreatic, liver, esophageal, gastric, and ovarian cancers [50]. FDA has also approved tests for genetic testing for ovary, breast, lung and prostate cancer. EGFR Mutation Test v2 is currently being used for the NSCLC patients and is very beneficial for those who face difficulties in providing tumour biopsy samples [53]. Another approved test, Epi proColon® is commercially available which screens CRC via SEPTIN9 gene methylation [54].

Exosomes are released from the cells including tumour cells as membrane enclosed bodies, and reflects a more precise picture than ctDNA [55]. Contrary to exosomes, other membrane-derived vesicles originate from internal compartments known as tumour-associated microparticles (taMPs) that express markers on their cell surface based on their origin. The expression of cancer-specific markers such as CD147 in taMPs and epithelial cell adhesion molecules (EpCAM) are few examples that might be utilised for diagnosis. Further, double-positive taMPs are known to be linked strongly with CRC.

Lastly, CTCs from primary and metastatic tumour sites [55] appear to be augmented during metastasis and staging [56], indicating their diagnostic and prognostic significance. Although, the majority of systems used for CTC isolation, together with the CellSearch platform, are exclusively based on the identification of biomarkers such as EpCAM, and cytokeratins which are present on the cell surface, but an important subpopulation of CTCs may not be detected by these due to the absence of markers on cells that have undergone epithelial-to-mesenchymal transition (EMT) [57–59]. Efforts are currently being undertaken to combine the isolation/separation of such subsets of cells, e.g., by multiparametric analysis [59,60].

Table 2
List of some liquid biopsy based available tests approved by FDA / CE-IVD

S. no.	Test name (Manufacturer and approval)	Biomarker	Cancer Type	References
1.	CellSearch® (Menarini Silocon Biosystems) FDA /CE-IVD	CTC with CD45-, EpCAM+ and (CK8, 18 and/or 19)	• Metastatic Breast, • Colorectal • Prostate	[20]
2.	Cobas® EGFR mutation test V2 (Roche) FDA/CE-IVD	EGFR	NSCLC	[66]
3.	Therascreen (Qiagen) FDA /CE-IVD	PIK3CA KRAS BRAF EGFR FGFR	Breast CRC CRC NSCLC Urothelial	[67] [68] [68] [69] [70]
4.	UroVysion (Abbott) FDA /CE-IVD	Aneuploidy 3,7,17 and loss of 9p21 (p16)	Bladder (patients with haematuria)	[71]
5.	Epi proColon® (Epigenomics AG) FDA /CE-IVD	SEPT9 methylation	CRC	[72]
6.	Xpert® Bladder Cancer Detection Xpert® Bladder Cancer Monitor (Cepheid) CE-IVD	UPK1B, IGF2, CRH, ANXA10, ABL1	Bladder (patients with haematuria) NMIBC	[73]
7.	Uromonitor (Uromonitor) CE-IVD	FGFR3 and TERT PCR	NMIBC (patients with haematuria)	[74]
8.	OncoBEAM (Sysmex) CE-IVD	KRAS & NRAS	mCRC	[75]
9.	Idylla (Biocartis) CE-IVD	KRAS NRAS, BRAF	mCRC	[76]
10.	HCCBlood Test (Epigenomics AG) CE-IVD	SEPT9 methylation	HCC (patients with liver cirrhosis)	[77]
11.	COLOGUARD (ExactSciences) CE-IVD	BMP3 & NDRG4 methylation, KRAS, ACTB Haemoglobin	CRC or advanced adenoma	[78]
12.	IntPlex® (DiaDx) CE-IVD	BRAF	mCRC	[79]
13.	Target Selector™ (Biocept) CE-IVD	EGFR	NSCLC	[80]
14.	Epicheck (Nucleix) CE-IVD	15 DNA methylation markers	Bladder	[81]

(FDA: Food & Drug Administration; CE-IVD: In vitro Diagnostic device certification; CDx: Companion diagnostic; mCRC: metastatic colorectal carcinoma; CTC: Circulating tumor cells; NMIBC: Non-muscle invasive bladder cancer; NSCLC: Non-small cell lung carcinoma; HCC: Hepatocellular carcinoma;)

Treatment decision

US-FDA has approved six NGS-based tests for the treatment decision. A US-based company Foundation Medicine has three approvals: FoundationOne CDxTM, FoundationFocusTM CDxBRCA, and FoundationFocusTM CDxBRCA LOH. Among those three, the initial two tests detect somatic, and germline BRCA1/ BRCA2 mutations in ovarian cancer and FoundationFocus™ CDxBRCA LOH is being used for genomic loss of heterogenicity. FoundationOne CDxTM is approved for making treatment decisions in breast, colorectal melanoma, ovary, and NSCLC by identifying the insertions, substitutions, copy number changes, and deletions in 324 genes (FDA PMA P170019). Apart from this, FDA has authorised Illumina's PraxisTM Extended RAS panel to detect NRAS and KRAS mutations in CRC patients receiving Panitumumab therapy. In addition, ThermoFisher's Oncomine™ Dx Target Test is being used for detecting alterations among 23 genes for NSCLC, and MyChoice HRD CDx, (Myriad Genetic Laboratories) (FDA PMA P190014) for mutations in BRCA1 and BRCA2. All of these tests have been authorised for tissue samples. Although a similar test might be used to know the genetic landscape derived from ctDNA, validation studies must be conducted prior to clinical deployment [60].

Although, LB is a highly efficient and non-invasive method of testing compared to tumour tissue biopsy, it still needs to overcome the treatment decision issue, which is the prerequisite for large scale clinical trials demonstrating that using LB in treatment decisions can lead to better disease outcomes. Currently DYNAMIC-III clinical trial is going on to assess whether ctDNA-based chemotherapy decision is more effective than standard care treatment in stage III CRC patients. More clinical trial-based studies on LB are required concerning treatment decisions [60].

Follow-up of Cancer Patients

Apart from the above-mentioned uses, liquid biopsies are also used in monitoring cancer patients' disease progression and therapy response. Their non-invasive or less invasive character makes them an attractive option for disease monitoring for a longer period [49]. Contrarily, getting invasive tissue samples are one of the biggest challenges, and more significantly, these biopsies frequently overlook modifications observed in places apart from the primary tumour site that might impact and alter the therapeutic implications [49]. In addition, the imaging modalities frequently employed to monitor cancer patients undergoing therapy have numerous drawbacks. For instance, computed tomography (CT) scans has many disadvantages like radiation exposure to patients, expensive, less sensitive when detecting minor lesions, and delivering negligible information regarding genetic changes generated by treatment [61,62].

In addition, continuous collection of biofluids permits disease monitoring, therapy response evaluation, and detection of resistance mechanisms. Notably, because of the limited half-life of cfDNA, liquid biopsies permit the real-time monitoring of cancer patients. The utilisation of ctDNA analysis is currently being done to discover mutations in EGFR, ERBB2, PIK3CA, and RAS genes in NSCLC, CRC, and gastric cancer [63–65]. In addition, dynamics of ctDNA modifications can be monitored which can help in predicting response to treatment as well as clinical outcome and permitting prompt reorientation of treatment regimens. In fact, a decrease in ctDNA after therapy has been linked to a minimal risk of tumour progression and longer survival, whereas high levels of ctDNA have been linked to progression, relapse, and poor survival [60,66].

Compared to ctDNA, CTCs can better predict therapeutic response in comparison to imaging modalities. Identification of CTCc have also been done utilising a panel of genes which was instrumental in differentiating patients with metastatic colorectal cancer (mCRC) who showed no response to therapy, despite CT scans failing to detect them. Besides this, CTC analysis could also detect non-responders with decreased overall survival (OS) and progression-free survival (PFS) [50].

Limitations

Liquid biopsies have emerged as a highly promising tool in cancer diagnosis and monitoring. However, certain challenges related to sensitivity and specificity remains. The amount of ctDNA released into the bloodstream may vary, and tumours with low shedding of ctDNA may go undetected which leads to false-negatives or undefined results [67]. Besides, cancer with a highly complex pathophysiology, LBs may not be able to capture entirely a tumour's genetic or molecular landscape due to the limited amount of ctDNA present in the bloodstream or the release of of DNA from specific regions of the tumour. As a result, specific genetic alterations or mutations present in the tumour might be missed [67]. Further, it does not help localize the disease and does not identify the metastasized sites during tumour progression. Visual inspection is mandatory to detect solid tumours at higher stages, which is one of the major limitations of LB. Similarly, it is also difficult to detect particular cancers in the case of multiple cancers through liquid biopsy. In addition, isolating circulating tumour components is a challenge and requires advanced technologies. Lab to Lab variations, lack of standardized methods and quality control measures are significant issues that remain to be addressed.

Although, LBs hold promise as a non-invasive alternative to traditional tissue biopsies, their clinical utility is currently limited. Liquid biopsies are mainly used for specific cancer types and specific clinical scenarios, such as monitoring treatment response, detecting minimal residual disease, or identifying resistance mutations. Their utility as a primary diagnostic tool for cancer is still being explored [68]. Cost is another major limitation. LBs can be more expensive than traditional tissue biopsies, which limits their accessibility in specific healthcare settings or for patients without adequate insurance coverage [69]. Despite these limitations, LBs have shown great potential in certain applications and continue to be an active area of research and development in the field of cancer diagnostics and monitoring.

Conclusion and future prospective

Circulatory molecular markers are increasingly being researched for their use in precision diagnostics and targeted therapeutics in several diseases and disorders. Some of these markers carry specific disease signatures, and are termed as biomarkers. In this context, LBs present with vast benefits, such as being non-invasive, reproducible, rapid, and real-time. Although, it cannot be considered a replacement for tissue biopsy, but it has been shown to be an excellent alternative that needs to be tapped well. A comprehensive molecular understanding, development of innovative point of care strategies for isolation and precise detection of circulatory tumour components will definitely help in improving accessibility and affordability of LBs in the clinical settings.

Besides, advances in nanobiotechnologies including nanodiagnostics and nanomedicine will further help in tapping the full potential of LBs in solid tumours. As of now, detection of solid tumours using LBs along with visual inspection can help to precisely identify the severity of tumour progression and metastasis for better management. The area of liquid biopsy research has vast possibilities for cancer management, but it needs to be validated in such a manner so it can be used as a gold standard for early detection, disease progression, and treatment outcomes.

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Ethical statement

Not applicable.

Conflicts of Interest

Authors declare no conflict of interest.

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CRediT authorship contribution statement

Sandeep Sisodiya: Writing – original draft, Data curation, Writing – review & editing. **Vishakha Kasherwal:** Writing – original draft, Data curation, Writing – review & editing. **Asiya Khan:** Writing – review & editing. **Bishnudeo Roy:** Writing – review & editing. **Anjana Goel:** Writing – review & editing. **Sandeep Kumar:** Writing – review & editing. **Nazneen Arif:** Writing – review & editing. **Pranay Tanwar:** Writing – review & editing. **Showket Hussain:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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