REVIEW
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Diseases & Research

Significance of Liquid Biopsy Containing Circulating Tumor DNA (ctDNA) Analysis for Evaluation and Management of Metastatic Cancer

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Abstract: According to the GLOBOCAN 2020 data from WHO, nearly 10 million deaths took place due to cancer thus, cancer became a leading cause of death all over the world. The most common cancer are breast cancer, lung cancer, colon cancer, prostate cancer, and pancreatic cancer. In the last few decades, Tissue Biopsy was considered the major approach to finding a confirmed diagnosis of particular cancer but due to the invasive process, it has become difficult for the clinician to ask for frequent tissue biopsies for the monitoring targeted treatment of the patients and thus clinicians generally assume the potency of the drugs without doing any biopsy analysis and thus there is an increase in the mortality of cancer patients. However, the liquid biopsy technique has changed this protocol of such treatment and greatly revolutionized the field of Clinical Oncology. Liquid biopsy ensures better-targeted treatment and many times help to give a complete cure. Liquid Biopsy is a non-invasive standard procedure to identify and prognose the tumor markers from circulating blood samples. It also offers the ease of sampling continuous screening, monitoring devices, therapeutic regimens, etc. Liquid Biopsy is composed of several components such as Circulating Tumor Cells (CTCs), free Circulating Tumor DNA (ctDNA), exosomes, vesicles, and platelets. The liquid biopsy includes body fluids such as blood, saliva, plasma, cerebrospinal fluid, and urine. Circulating Tumor DNA (ctDNA) in the blood sample of metastatic cancer patients has become the most critical technology to evaluate the progression of metastasis and give a precise treatment for many cancers. ctDNA analysis provides insight into the molecular profiling and biological characteristics of several tumors. This review is mainly aimed at highlighting the important technological development in ctDNA analysis and also to provide the significance of it in monitoring metastatic cancers and providing the best treatment to cure cancer.

Keywords: Tissue biopsy; Liquid biopsy; Heterogeneity; Cell-free nucleic acids; Metastatic cancer.

Introduction

Cancer is a significant threat to the world right now and needs more attention toward diagnosis prognosis and management. To improve prognosis and management several efforts are being made by scientists to use tissue biopsy histopathology as a gold standard for precise diagnosis and therapy of cancer patients. However, in recent years this tissue biopsy technology is overruled by Liquid biopsy because tissue biopsy is an invasive process, and difficult to take frequent biopsies for evaluation of metastatic stages during the treatment. Several studies conducted, it has been shown that tissue biopsies do not reflect tumor dynamics[1]. However, the vast amount of data produced from the efforts reveals the heterogeneous nature of tumors where neoadjuvant tumor treatment has reduced the size of the tumor to an undetectable level leaving no issues for further investigations[2]. Similarly, a single biopsy sample taken for this study can not represent the heterogeneity of the tumor. Therefore, cancer research has shifted its focus towards conventional procedures of analysis of ctDNA from liquid biopsy where the samples do not have such limitations as a tissue biopsy repeated sampling can be taken during treatment as well as it matches with the genetic makeup of patients to determine the malignancy due to gene mutations[3].

Liquid Biopsy is a technique that refers to the rapid sampling and analysis of non-solid tumor tissues. It is a non-invasive approach that allows real-time monitoring of cancerous progression[4]. Liquid Biopsy is considered a beneficial approach for establishing efficient methods of tumor monitoring that are proven to be significant for effective therapeutics and cancer mortality reduction[5]. This approach is used to study tumor dynamics such as tumor heterogeneity, gene rearrangement, gene mutations, chromosomal aberrations, etc. Due to the emerging tumor-specific molecules, and tumor heterogeneity. liquid biopsy is the major thrust area of scientific evaluation and molecular profiling of cancer tissues[6]. It exists at multiple levels within different tumor regions and metastasis (Fig.1).

Component of liquid biopsy

Liquid biopsy contains Circulating tumor nucleic acids (ctDNA), Circulating Tumor Cells (CTC), nucleic acid-containing vesicles

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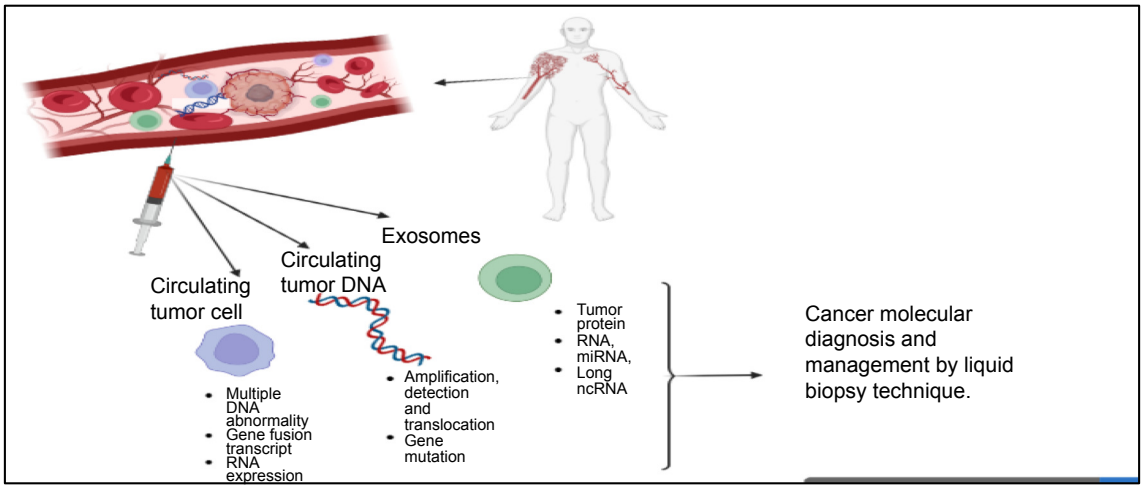

Fig. 1. Liquid Biopsy components include Circulating Tumor Cells (CTCs), Circulating Tumor DNA (ctDNA) and nucleic acids with or without vesicles (Exosomes) can be used in multiple ways to look for appropriate diagnosis and therapeutics of cancer.

(exosomes), and proteins obtained from body fluid like blood, plasma, cerebrospinal fluids, saliva, etc[7,8]. Among all liquid biopsy sources, plasma is the most common non-invasive source which can be easily collected without much pain to the patient and also easily analyzed for specific biomarkers required for targeted therapy. Ideally, these biomarkers have the strong potential to correlate directly with clinical treatments. These biomarkers are validated in clinical trials to improve treatment and therapeutic efficacy. Many more advantages posed by these biomarkers are non-invasive and can provide information on heterogeneous tumor origin, specific mutations, and alterations. In recent years, the liquid biopsy approach is likely to be cost-effective and rapid for the detection, prognosis, and diagnosis of malignant tumors[9]. Among all the biomarkers, ctDNA is the critical tumor-free DNA that has gained interest in tumor profiling and investigation[10]. whereas CTCs are small in number and large in size therefore there is a major difficulty in isolating the same from liquid biopsy[11]. There are many methods of isolation of CTCs from liquid biopsy including the Ficoll Gradient method, ISET size-based separation method and Cell Search Kit FDA-approved method, and Microfluidic method[12,13]. Exosomes are a subset of 100 nm extracellular vesicles surrounded by a lipid bilayer membrane and secreted from almost all of the cells[14]. Exosomes participate in cancer progression and metastasis by transferring bioactive molecules between cancer and various cells in the local and distant microenvironments. There are many methods of isolation of exosomes including Differential Ultracentrifugation (dUC), Ultrafiltration(UF), Poly-Ethylene Glycol(PEG) Based Precipitation, Immunoaffinity Capture, Microfluidics and Size-exclusion Chromatography(SEC)[15,16]. A blood-based liquid biopsy consists of the isolation and analysis of tumor-derived or tumor-associated components that circulate in the bloodstream (Fig. 2).

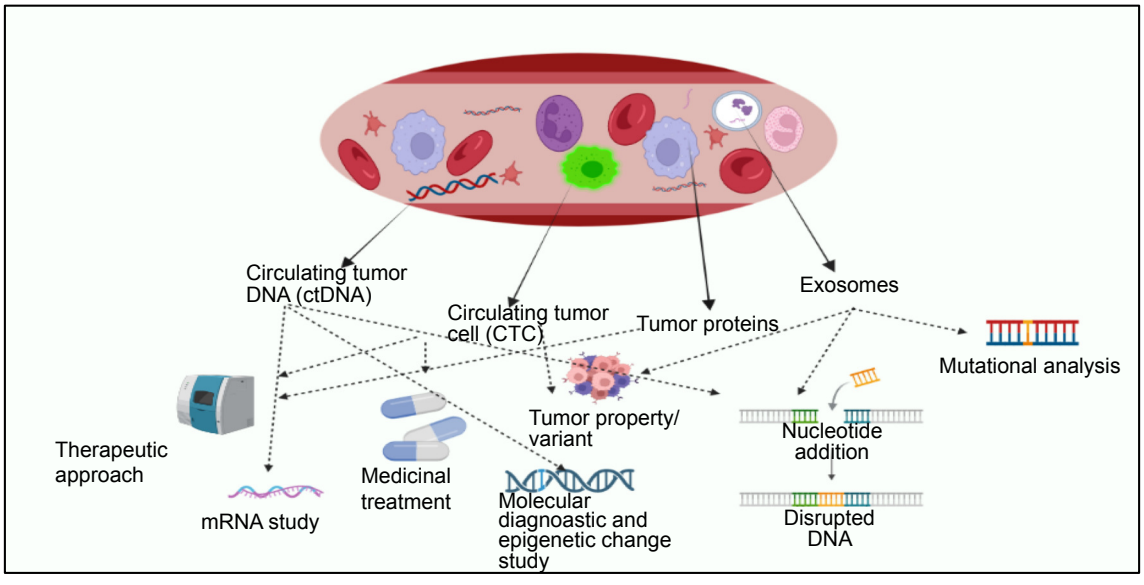

Fig. 2. Various components of liquid biopsies and their respective applications in cancer diagnosis and therapy.

Circulating tumor DNA (ctDNA) analysis from liquid biopsy

ctDNA represents the in-vivo counterpart of the original tumor as it shows tumor-specific mutations and alterations found in serum or plasma in very low amounts of total cell-free DNA. Accordingly, assumptions state that ctDNA sheds into the bloodstream from tumor cells through apoptosis, necrosis, autophagy, and other physiologic processes. Two mechanisms behind the release of ctDNA are illustrated as active and passive i.e directly from apoptotic or necrotic bodies or indirectly by engulfed necrotic cells from macrophages[17]. Although due to the incomplete information regarding mechanisms but hypothesis states that cancer cells release nucleic acids to transform the targeted recipient cells at distant locations[18]. Due to their origin from tumor cells, they depict associated genomic variations. It is thought that they make up less than 1% of total circulating nucleic acids[19,20]. It can be identified easily from other biomarkers in terms of its size, and genetic abnormalities in comparison to normal cells. Jiang et.al 2015 have shown in their study that chromosomal arm size and length variation could also contribute to the differences between the genomic and tumoral DNA where genomic chromosomal arms show a large contribution in molecular processes and tumoral DNA on amplification shows less contribution. They also showed that with 12 HCC patients, the size of ctDNA in these patients were ranging from 150-180 bp. They further stated that in these HCC patients, they observed a prominent peak of ctDNA at 166 bp[21,22]. There are two mechanisms of ctDNA release into the blood. First, due to enzymatic digestion of DNA fragments during apoptosis where the ctDNA is found to be associated with the nucleosomes that range to 166-1000 bp while, larger fragment length starts at 320 bp. The second mechanism states the active secretions such as exosomes and mtDNA egestion[23,24]. In general, these fragments are shorter and found to vary across histologic tumor types at some stages of cancer[25]. Due to the presence in low amounts and sizes such as in breast cancer, short fragments are more frequently found at later metastatic stages while some larger fragments are found in early stages of cancer that are easy to identify and detect for mutations[26]. Due to the rapid clearance from blood, it can be a major detection analyte for oncology. Circulating tumor cells (CTC) in the bloodstream originate from the original tumor site and travel throughout the vasculature to cause metastasis[27]. ctDNA isolation methods and molecular profiling are critical procedures to perform. Furthermore, these procedures assist in monitoring tumor progression and treatments. The genetic variations posed by ctDNA may be accessed by molecular approaches such as Digital Polymerase Chain Reaction (dPCR), and Next Genome Sequencing (NGS). Clinical development of ctDNA genotyping created several challenges for scientists to develop new technologies to combat the issues of monitoring and analysis. The clinical utility helped in the invention of ddPCR, NGS, and WGS-like techniques to detect tumor heterogeneity and clonal evolution, monitoring certain types of cancers such as oesophageal cancer, Pancreatic cancer, Lung cancer, and colorectal cancer. Several challenges and future aspects associated with the validity of methods, and utility have been described to show the significance and sensitivity of this liquid biopsy technique.

Strategies and methods for isolation of ct DNA from liquid biopsy

Shedding of ctDNA into the vasculature depends on its size as well as the tumor location and therefore, there are differences in the levels of ct DNA among cancer patients. The concentration of cell-free nucleic acids in healthy individuals is ranging from 0-100 ng/mL whereas, in the cancer patient, this can be 1000 ng/mL[28,29]. This creates a growing demand for standard isolation procedures to produce novel, fast, and efficient cancer therapies. The wide availability and comparison of protocols reveal the latest approaches that can replace the older ones. To extract circulating DNA from a patient's blood tremendous efforts were made by scientists to achieve minimal handling, and reduce laboratory errors by creating standard and conventional protocols[30]. For the entire workflow stages sample preparation and DNA isolation are the critical steps. To extract DNA from blood plasma should be processed from cfDNA blood tubes. To prevent clotting issues EDTA, heparin, and citrate are used which also increases the DNA contamination risk in lysed cells[31]. Salt precipitation methods are preferred over the use of column-based methods in which centrifugation steps are considered as a rate-limiting step that increases genomic DNA contamination[32]. Therefore, to minimize the risks of contamination methods based on Capillary electrophoresis are used to elevate the estimation of fragment sizes due to sensitizing activities of PCR, and NGS technologies[25]. Liquid biopsy components consist of PCR inhibitors which may hamper NGS sequencing thus to conflict with the issue samples should pass through the NGS quality control[33]. After the preanalytical methodology, several analytical studies have been done to obtain the samples. However, several conventional and standard methods include in-silica, magnetic bead-based, and phenol-chloroform assays. Among all assays, magnetic bead-based assays showed the highest yield and efficiency compared to others[30]. Disadvantages associated with the silica-based method are low yield and fragment partial loss due to column-based extraction. However, an alternative approach to this is an enriched functionalized magnetic bead[30] to compare the extraction methods and enhance the purity of DNA samples. Other than these methods, high throughput Microfluidic chips method is developed for the extraction of cfDNA such as Pressure and Immiscibility-Based EXtraction (PIBEX) centrifugation method for cfDNA analysis with a silica membrane under vacuum pressure using an immiscible liquid[34]. The use of microfluidic chips reduced the contamination risks, where multiple processing steps were replaced by chip and manual handling required for these experiments[35]. These experiments result in advanced approaches toward cell-free nucleic acid extraction procedure, however, researchers suggest that the use of the magnetic bead method improved the ctDNA fragment size, yield, and reduced laboratory errors. Other than these techniques some more procedures are still in demand such as PHASIFY and PHASIFY enriched[36] methods that are proven to achieve high recovery and purity of tumor nucleic acids.

Molecular profiling of circulating tumour DNA (ctDNA)

After the isolation of ctDNA from the patient's blood, molecular

profiling needs to be done to quantify the samples and their efficiency in the treatment of cancer. The techniques mentioned above are compatible with the targeted and untargeted approaches of PCR[37]. There are several Targeted and Untargeted approaches used to detect genome alterations in malignant tumors. One of the Targeted approaches includes specific gene mutations in specific genome regions in the given neoplasia. However, untargeted approaches such as WGS (whole genome sequencing), and WES (Whole Exome Analysis) does not require any prior knowledge of molecular alteration for the detection of de novo ctDNA mutations and somatic copy number variations (CNVs)[38] (Table 1). Both of these approaches have advantages and limitations as shown in Fig. 3. The Targeted approaches include Polymerase chain reaction-based technology which sequences hundreds of genes present in ctDNA of cancer patients. To cover clinically relevant mutations relatively low throughput, and high sensitivity can be achieved by deep sequencing of the specific regions[39]. Target enrichment is done to maximize the amplification of target gene fragments by multiplex or Hybridization capture strategies. This targeted approach is most suited for clinical diagnosis due to the high sensitivity, and specificity whereas, in the case of an untargeted approach, no enrichment step was performed while sequencing the whole genome to discover new genomic aberrations related to the patient's prognosis and diagnosis[40,41].

Table 1. Methods of ctDNA analysis.

Methods	Approach	Purpose	Advantage	Limitations
Next Generation Sequencing based Targeted ctDNA approach	1. PCR-based (ddPCR, BEAMing) Beads, Emulsion, Amplification, Magnetic). (TAm seq, CAPP seq, Safe, seq ampliseq, PARE Whole exome analysis (WEA)	Detection of somatic Point mutations of a defined unknown sequence Detection of a predefined targeted and deep seq Detection of specific chromosomal rearrangement Analysis of coding DNA regions	• Sensibility 0.001% • Small and multiple samples were analyzed. • Rapid, cheap • Highly Sensitive <0.01%-2% • Large known single genome with deep analysis • Rapid	• The novel target needs to be known • Generic technique • Less comprehensive • Need customized assay • Expensive • Less sensitive • Expensive • Comprised deep sequence analysis
Untargeted approach	Whole Genome Analysis	Analysis of complete DNA sequence	Highly sensitive	Low detection limits

PCR: Polymerase chain Reaction, NGS: Next Generation Sequencing, ddPCR: Droplet digital PCR, BEAMing: Bead, Emulsion, Amplification, Magnetic, TAM: Tagged Amplification sequence, CAPP: Cancer Personalised Profiling Deep Sequence, Safe-seqs: Safe sequence system, PARE: Personalised Analysis of Rearranged Ends, WGS: Whole Genome Sequencing, WES: Whole Exome Analysis.

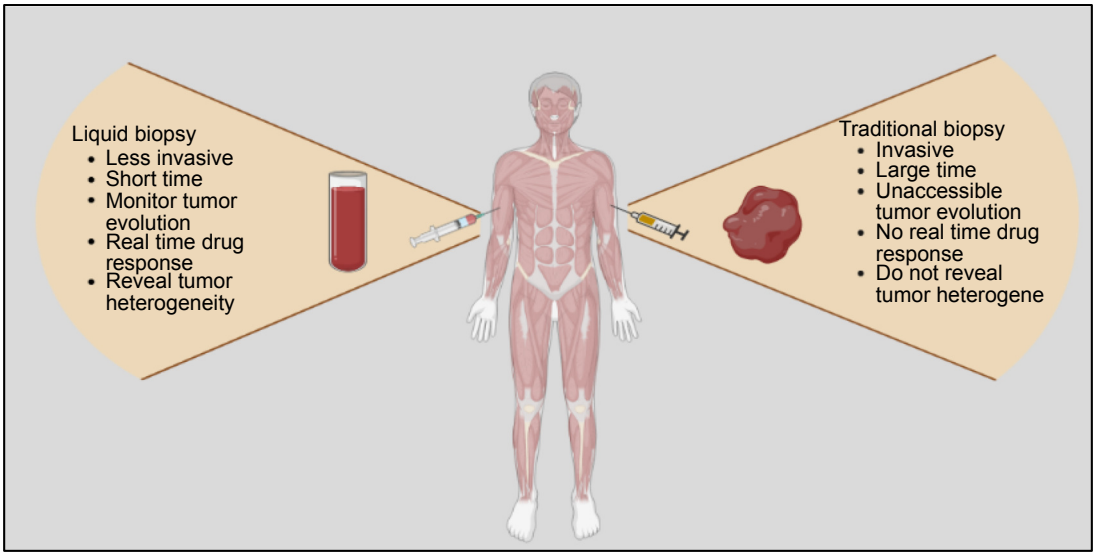

Fig. 3. Comparative approach of Tissue Biopsy versus Liquid Biopsy techniques.

Polymerase Chain Reaction (PCR) based analysis of ctDNA

A novel approach was used to identify the mutations in unknown regions of the gene segments. PCR-based methods involve dPCR, ddPCR, BEAMing, etc. The functionality of the technique includes partitioning and individual testing of the target sequence within the separate individual compartments[42]. These compartments and sequence analysis make this technique a sensitive one. Droplet digital PCR was invented and considered a novel approach to Next Generation Sequencing. At present, ddPCR is one of the most powerful and accurate methods of quantification and

identification of circulating nucleic acids in body fluids[43]. In cancer, ddPCR represents high sensitivity ranging from 0.001% to 0.1%. BEAMing (Bead, Emulsion, Amplification, Magnetic) on the other hand, is the modification of emulsion PCR where in a single tube different samples are amplified in emulsion droplets which are bounded with primers and recovered with the help of magnetic fields[44]. Assays such as Personalized analysis of rearranged ends (PARE)have shown significant results of low levels of ctDNA in patients with Colorectal cancer (CRC) and Breast cancers[45]. Other somatic point mutation detecting techniques include NGS-based approaches such as Tagged amplicon sequencing (TAMseq) and CAPP sequencing.

Next Generation Sequencing (NGS) platform for ctDNA analysis

Next Generation Sequencing involves a variety of tests that detect and sequence large genomes by parallel sequencing numerous DNA fragments and comparing these fragments with the human reference genome to find variations, and differences in bases, exons, genes, translocations, and inversions[46]. Various NGS sequencing platforms are introduced including Ion Torrent, PacBio, Illumina Seq, Tagged Amplicon sequencing (TAM-seq), Cancer personalized deep sequencing (CAPP-seq), safe sequencing system (Safe-seq), Amplicon sequencing[47]. Illumina sequencing includes typical handling and synthesis of the samples as to cluster the sample template strand and it needs a fluorescent tag of dNTPs so that the original template sequence can be recovered by using fluorescent signals[48]. Nanopore sequencing is an advanced technology that has been used. Nanopore sequencers include feeds of Nucleic acid samples through nanopores embedded membrane which creates disturbances in electrical signals by the base pair and these disturbances are measured to check for the mutations in each base[49]. TAM-seq works on parallel amplification of multiple targets within the entire gene of interest. It is a two-way process that involves amplification by adding specific primers in multiplex PCR that are further followed by a microfluidic system to amplify target regions from first-step amplicons[50]. CAPP-Seq is a capture-based technique that uses Biotinylated DNA oligonucleotides to target mutated regions in the cancer of interest[51]. In Safe-seq there is an addition of a unique identifier to each template and after the amplification takes place to get a direct sequence of that particular genome[52].

Clinical application of ctDNA in cancer therapeutics.

The effective therapeutics for cancer lies in an accurate diagnosis to guide treatment and monitoring of the disease. To study tumor-specific mutation in ctDNA the need for quantification is more critical[53]. Because of the selective locations of tumor cells such as the lung, pancreas, colorectum, breast, brain, head, neck, urogenital, etc. in the body, it is difficult to monitor them with precision. The use of ddPCR, and NGS sequencing techniques make it easier to detect the mutations or aberrations in genes, and checkpoints of several pathways. In tumors such as glioma(brain) or Non-small cell lung carcinoma[54], there are some common mutations identified genes in ctDNA of patients including KRAS, and TP53 without a PTEN loss, leading to increased PD-L1 expressions and mutational burden, thus increasing response to Immune checkpoint inhibitors (ICI) due to which ctDNA serve as a predictive biomarker[55]. Lung cancer possesses some mutations that also show precisions up to 90% which are somewhat resistant to therapy and can be quantified by deep sequencing[56]. Glioma cell results in 33-73% mutations at the repetitions match with alterations in the biopsy. In Cerebrospinal fluid, ctDNA facilitates mutations such as IDH1, IDH2, ATRX, TP53, H3F3, and HIST1H3B for molecular diagnostics in diffuse gliomas in a non-invasive manner hence, these potential alterations are easily detected from Cell-free DNA analysis. However, in the breast cancer patient, there were high levels of mutated PIK3CA found in ctDNA responsible for short progression-free and overall survival as compared to patients with low or no detectable amounts of mutated PIK3CA in its ctDNA[57]. Likewise, the Most potent application as a predictive biomarker for early detection of metastasis and addressing the tumor heterogeneity in colorectal cancer that showed resistance to epidermal growth factor receptors due to the KRAS mutations detection[58]. Furthermore, In gastrointestinal tumors, Thymidine Kinase (TK) inhibitors have shown decreased sensitivity to the hotspot mutations in genes such as KIT (S821F) and PDGFRA in the ctDNA of these patients[59]. Methylation patterns on ctDNA can be used as biomarkers to detect the epigenetic deregulation of these genes. In renal cancer, the hypermethylation of the promoter region of RASSF1A, FHIT, and APC was shown to be useful early-stage diagnostic markers. Whereas, detection of hypermethylation of the MLH1 gene promoter in ctDNA could be used as a predictive biomarker for acquired resistance in ovarian cancer resulting in poor overall and progression-free survival[60]. Similarly, the identification of methylation of the ESR1 promoter gene in ctDNA of metastatic breast cancer patients was found to be associated with a lack of response to everolimus/ exemestane therapy. The analysis of single nucleotide variants in KRAS, NRAS, PIK3CA, BRAF, and EGFR using cfDNA has been shown to have >80% concordance as compared to tumor tissue of colorectal, lung, and breast cancer patients[61]. In virally mediated cancers of Head and neck squamous cell carcinoma circulating viral DNA could be accessed to get the early detection and residual disease[62].

Clinical utility and challenges of ctDNA

ctDNA is a potent biomarker studied to date due to the tumor dynamics it shows on its surface as it has more potential to get studied in further studies. Studies revealed its holistic nature in characterizing and monitoring tumors[63]. The undetected mutation which was difficult to detect with conventional tissue biopsy gets an enhanced view by ctDNA from Liquid Biopsy. Due to the rapid clearance of circulating cell-free nucleic acids from the blood, it was difficult to predict the exact treatments and therapies but ctDNA cleared the issues with liquid biopsy[64]. ctDNA genome sequencing assists in detecting copy alterations of genes in pancreatic cancers, colorectal cancer, breast cancer, gastroesophageal cancer, and many more which reveals its content-changing nature every time (Table 2). Clonal variations of these cancers enabled molecular profiling with a real-time monitoring system against any blockage in vasculature such as in colorectal cancer, and EGFR blockade was profiled[65]. Tumor subtypes can also be studied by conducting genotyping assays such as B-cell Lymph-

oma. Several markers which show wide mutations can be revealed by plasma-seq analysis. In glioma cells ctDNA shows 73% efficacy, using qPCR assays cfDNA samples showed 65% yield whereas, in lung cancer precision yield follows 97% by running on[66]. The established mean recovery of cfDNA was 82%. Precisions of Multiple Allele Frequency or Variable allele frequency (MAF, VAF) may be computed by using ratios of wild-type droplets to mutant ones with a limit of around 0.001%[17] The technical challenges in ctDNA isolation and analysis are blood draw issues due to coagulation property and due to the ctDNA shedding may give false negative results hampering the protocols, and thus its use in clinical decision makings is yet to be considered due to the rapid mutations in biomarkers[67]. Apart from all the implications mentioned above, an epigenetic alteration that is found in tumor-derived nucleic acids also provides a view of gene detection and analysis[68]. At last, due to the reasons of lack of sensitivity, and precisions of identification of tumor genes, Liquid Biopsy is gaining much weight[69] (Table 3).

Table 2. Application of ctDNA in cancer diagnosis and therapy.

LB analyte	Tumor type	Technology Used	Sensitivity/Detection limit	Ref
ctDNA	BC, PCa, CRC	Droplet digital PCR	MAF detection < 0.1%	[38,39]
		BEAMing	MAF detection ~ 0.02%	[3,39−41]
	CRC, BC	PARE	ctDNA detection < 0.001%	[42,43]
	OVC, BC	TAm-Seq/eTAm-Seq	MAF detection ~ 2% MAF detection ~ 0.25%	[39,44]
	NSCLC	CAPP-Seq	MAF detection ~ 0.02%	[39,45]
	BC	cMethDNA	—	[46]
	HEPC	MCTA-Seq	—	[47]

Table 3. Summary and positive outcomes from the use of ctDNA analysis in metastatic cancer.

Major studies	Method utilized	Positive outcomes	Ref
Genetic mutation Screening/Genotyping on (NSCLC, Breast, Gastric, Head, neck brain, and colorectal cancer)	Next-generation sequencing BEAMing, TAM-sequencing, CAPP-sequencing	• Highly sensitive and specific. • Early detection. • Target mutating regions specifically with 96% specificity. • 100% results in stage II-V and 50% of stage I Non-small cell lung carcinoma (NSCLC).	[10]
	Immunotherapy	100% survival rate after ctDNA clearance with drugs and mAb in Non-small cell lung carcinoma.	[52]
	Exome-wide analysis (drug resistance therapy)	• Mutant gene treatment with drug complements to ctDNA Analysis and disease-free survival. • Activated PIK3CA with Paclitaxel. • Truncated RB1 with cisplatin • Truncated MED1 with tamoxifen, • Splicing GAS6, EFGR with gefitinib.	[48]
	Cell-free DNA methylation immunoprecipitation- sequencing (DNA methylomes)	Cost-effective DNA methylation assays developed to vanish cancerous cells in early cell tumors.	[46]

Conclusion and future direction

In recent years, liquid Biopsy containing ctDNA analysis is considered a major advanced technique to identify gene mutations in particular tissue types with 70−75% accuracy. The high-throughput, sensitive, cheap, efficient profiling procedures by Next Generation Sequencing (NGS), Polymerase Chain Reaction modification (PCR) make it easier to handle than other tissue biopsy techniques. Diverse features allow this technique to perform much better in the future to transform the oncology field. In an opinion from the review studies, blood biomarkers can assist in revealing much more information than the present. The precise services of liquid biopsy containing ctDNA can be enhanced by more practicing methods and procedures to get accurate data. The widely adopted routine clinical trials need to get advanced with price reductions. The review was conducted to show the minimally invasive procedures of liquid biopsy technology to detect and find alternatives to cancer studies and molecular diagnosis. Liquid biopsy containing ctDNA has the potential to derive heterogeneous tumor mutations, and early-stage cancer detection, till it has reduced the burden but due to the lack of certain standardized procedures, and isolation protocols, deep sequencing of multiple

tumors needs more attention to clear the goal of cancer treatment. This technique revolutionizes the field of oncology and can take much more advancements in the field of molecular medicine.

Abbreviations

BC, Breast Cancer; BEAMing, Bead, Emulsion, Amplification, Magnetic; CAPP, Cancer Personalised Profiling; Deep Sequence, DeepSec; Safe-SeqS, Safe sequence system; cfDNA, Cell-free DNA; CNVs, Copy Number Variations; CRC, Colorectal cancer; CTC, Circulating tumor cell; ctDNA, Circulating-tumor DNA; ddPCR, Droplet digital Polymerase chain reaction; dPCR, digital polymerase chain reaction; HCC, Hepatocellular cancer; MAF, Multiple Allele Frequency; NGS, Next-generation sequencing; OVC, Ovarian Cancer; PARE, Personalised Analysis of Re-arranged Ends; PC, Pancreatic cancer; PCR, Polymerase chain reaction; qPCR, Quantitative PCR; TAM, Tagged Amplification sequence; TK, Tyrosine Kinase; VAF, Variable Allele Frequency; WGS, Whole-genome sequencing.

Conflict of interest

There is no conflict of interest in the manuscript. I hereby permit the use of figures and tables attached to the manuscript.

Authors' contributions

PP suggested topic for review, supervised and made necessary corrections in the manuscript, whenever required. MG wrote manuscript as per instructions by PP and made necessary corrections whenever required. PP & MG have equal contribution in this manuscript.

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