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Analysis of ctDNA Biomarker in Breast Cancer

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Abstract

Breast cancer is the second most common cause of cancer-related mortality among women. Despite all the improvements so far, especially, failure to fully treat advanced breast cancer could be attributed to late diagnosis of disease and also the application of biopsy techniques that require surgical procedures for diagnostic purposes that lead to great difficulty to the patient. For these reasons, clinically important new biomarkers, which can be obtained easily and cheaply with reliable, minimal and non-invasive techniques and can be used in the early diagnosis, treatment and follow-up of the patient are of great importance. In this line, recent studies have focused on one of the most significant components of liquid biopsy technique known as circulating tumor DNA (ctDNA). ctDNA has the potential to be used as biomarker that enables the detection of genetic alterations in cancer cells in real-time for target treatment of breast cancer. In this review, the potential role of ctDNA in the diagnosis, prognosis, and the management of breast cancer patients will be summarized including general information on breast cancer diagnosis, cancer & liquid biopsy and importance of ctDNA together with recent researches evaluated on this timely topic.

Keywords: Breast cancer; ctDNA; Liquid biopsy; Targeted therapy

Introduction

Breast cancer is known to be the most common type of cancer among women worldwide. According to the data published by GLOBOCAN 2018, it is estimated that in 2018 there will be approximately 2.1 million (11.6%) new cases and 626 thousand (6.6%) deaths from breast cancer[1]. In spite of all the improvements to date, the fact that metastatic disease is not fully cured yet causes breast cancer to be the second highest mortality cancer among women.In this regard, many years have been studied to find prognostic and predictive markers that can be used in diagnosis and treatment. However, due to the advanced stage of breast cancer, drug resistance, metastasis and risk of recurrence, it has been a difficult disease to be treated.The application of biopsy techniques that require interventional or surgical procedures for diagnostic purposes together with the treatment also lead to great difficulty to the patient.Therefore, the detection of new biomarkers, the use of the correct drug dose and the application of non-invasive early screening techniques are of great importance in order to treat the disease more quickly and easily without the occurrence of undesirable symptoms. Correspondingly, the statistical data obtained show that more effective screening, diagnostic and treatment tools are needed for breast cancer.The main starting point of this advantage is the technology that enables the detection of genetic changes that allow effective treatment according to the biological course of each tumor and the tracking of the associated motions.In this review, as a new perspective, the potential of ctDNA by obtaining information about tumor genotype characteristics inscreening, diagnosis and treatment strategies of breast cancer researchers will be summarized in general view point.

Breast Cancer Classification and Diagnosis

Because breast cancer is a heterogeneous disease, the best classification of specific subtypes of disease for better treatment results is of great importance. In this line there are several classification methodologies for breast cancer. Accelerated in parallel with technological developments and published its first data in October 2012, “The Cancer Genome Atlas (TCGA)”-Breast Cancer Genome, analyzed primary breast cancers by genomic

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DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays. The project's data along with the results of other previous studies, has shed light on the detailed understanding of the molecular characteristics associated with the biology of breast cancer[2]. The study demonstrated that "the biological finding of the four main breast cancer subtypes caused by different subsets of genetic and epigenetic abnormalities have raised the hypothesis that much of the clinically observable plasticity and heterogeneity occurs within, and not across, these major biological subtypes of breast cancer".Thus, it was concluded that the patients who were previously planned to be treated in the same groups may have tumors of different genotypic characteristics and therefore different responses to the same treatment could be obtained.TCGA project data have also rationalized the treatment options specific to the tumor genotype[3]. As it is understood from the rationality of the radical change in cancer staging protocols updated in the internationally accepted consensus "American Joint Committee on Cancer (AJCC)" 2017, the transition to individualized specific treatments based on tumor genetics is inevitable[4].

Although, there are many subtype classifications of breast cancer, clinically, the disease is categorized into three basic therapeutic groups with four major molecular subtypes.

Table 1: Breast cancer's major molecular subtypes

Major molecular subtypes of breast cancer					
Subtype	ER	PR	HER2	Ki67	Incidance
Luminal A	+	+/-	-	Low	% 30-70
Luminal B	+	+/-	+/-	High	% 10-20
HER2-enriched	-	-	+	High	% 5-15
Triple-negative	-	-	-	High	% 15-20

The estrogen receptor (ER) positive group is the most numerous and diverse, with two subtypes Luminal A and Luminal B. Luminal A subtype is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative, and has low levels of the protein Ki-67, which helps control how fast cancer cells grow. Luminal A cancers are low-grade, tend to grow slowly and have the best prognosis. Luminal B subtype is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), and either HER2 positive or HER2 negative with high levels of Ki-67. Luminal B cancers generally grow slightly faster than luminal A cancers and their prognosis is slightly worse. As the ER positive group is a hormone positive all the patients receive endocrine therapy and undertake several genomic tests to assist in predicting outcomes for ER1 positivity. HER2-enriched subtype (also called ERBB2) is hormone-receptor negative (estrogen-receptor and progesterone-receptor negative) and HER2 positive. HER2-enriched cancers tend to grow faster than luminal cancers and can have a worse prognosis, but they are often clinically successfully treated because of effective therapeutic targeting of HER2 protein, which has led to intense efforts to characterize other DNA copy number aberrations[2,5,6].Triple-negative/basal-like subtype is hormone-receptor negative (TNBCs, lacking expression of ER, progesterone receptor (PR) and HER2) and is one of the most aggressive subtypes. This type of cancer is more common in women with germline BRCA1 gene mutations and because of hormone negativity patients receive only chemotherapy treatment options. However, because chemotherapy treatment options may lead to toxic effects and resistance problems, several research centers are working on toilluminate the mechanism of this mysterious subtype and find the best curative treatment strategy of TNCB as early as possible[2,5-7].

As understood, breast cancer is a highly heterogeneous disease, comprised of distinct biological subtypes which present a varied spectrum of clinical, pathologic and molecular characteristics with different prognostic and therapeutic implications[8]. So the studies on the genotyping of breast cancer are very important for breast cancer treatment decisions and prognosis prediction. Among the subtypes mentioned above triple-negative breast cancer (TNBC) is one of the most aggressive type and unfortunately still is more likely to be identified at advanced stages. However, advanced stages of disease and the application of biopsy techniques that require interventional or surgical procedures for diagnostic and treatment purposes lead to great difficulty to the patient with TNCB subtype[7]. Another, important point is that even the targeted treatments have markedly modified the treatment of breast cancer over the past 10 years and the detection of molecular genetic profiles is the most currently used method for categorizing tumors for clinical decisions because of tumor heterogeneity, clonal evolution and selection almost all of the tumors acquire resistance to systemic treatment. Moreover, during the treatment and follow-up of the disease, tumor tissues provide only a snapshot and are often difficult to obtain because invasive biopsies may need to be repeated and these could lead to many complications like bleeding or infections[9]. Thus, in order to overcome these problems and to identify these genetic abnormalities in an early stage of disease (especially TNBC), advanced technology is needed for rapid, cost-effective, and non-invasive identification of clinically important biomarkers at various time points during the course of disease. Hopefully, in the last 30 years, it has been observed that cancer cells may also be present in the blood circulation system depending on factors such as type, prevalence and location of cancer and the value of these circulating cancer cells in terms of diagnosis and treatment monitoring has been discussed. More recently, as a result of the better detection of the genetic profiles of cancer cells, liquid biopsy methods, which mean the detection of molecular fingerprints in blood and body fluids, have begun to develop rapidly. In this way, more easily accessible blood samples can be used instead of tissue. This method also demonstrates that it will facilitate the diagnosis, treatmentand follow-up of breast cancer[10,11].

Liquid biopsy can be defined as a test on blood samples to detect circulating tumor cells or DNA fragments of tumor cells[12]. One of the special points of the liquid biopsy technique is that it is a non-invasive method performed with a small amount of blood sample taken from the patient's blood, unlike tissue biopsy requiring a surgical procedure[10].

Cancer and Liquid Biopsy

One of the distinguishing features of cancer is the change in the genetic material (genome) in the cells manifested by specific mutations[13-16]. Cancers can be diagnosed and identified by taking tissue samples by tumor biopsy, which requires a surgical procedure.Tumor biopsies provide healthy and rich information when planning treatment.The benefits of biopsies include the

ability to provide information about the type, aggressiveness, spreading pathway, immunological and molecular characterization of the samples beyond the diagnosis of cancer.However, biopsies have limitations.First, as a tumor grows, it can change over time, spread (metastasis), and is exposed to cancer prevention drugs.Tumor biopsies taken when the disease is first diagnosed may not reflect the later state of cancer.Second, repeated biopsies to obtain current information about cancer can cause potential complications such as pain, infection, and bleeding.Third, cancer cells that spread to different parts of the body may be different from those in the region where they begin.Therefore, it is not possible for a tumor biopsy from a part of the body to adequately represent cancer in the body.Accordingly, liquid biopsies may be more appropriate for observing cancer over time.Because it is less expensive and less invasive, it is easy to repeat and can be considered as an alternative or auxiliary method[9-11,17].

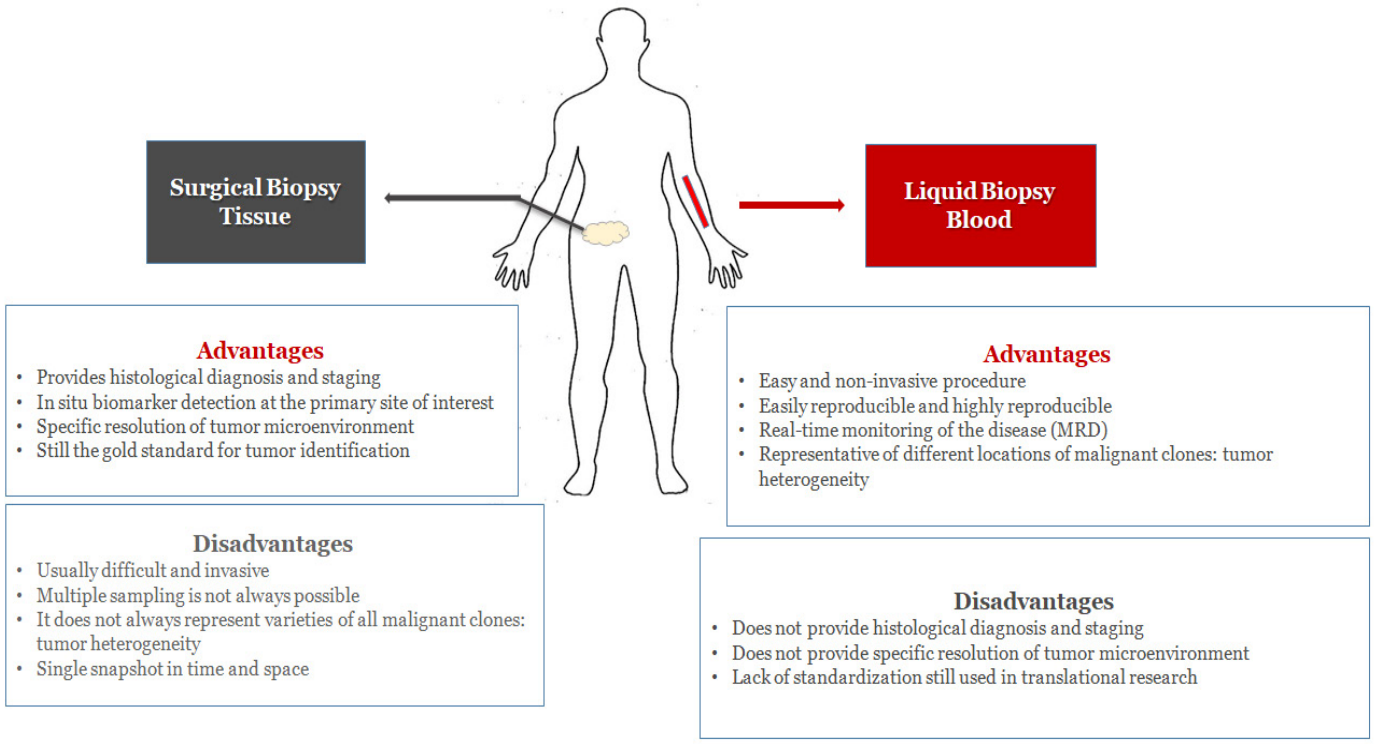

Figure 1: Tissue Biopsy & Liquid Biopsy

Blood may contain three types of cancer-related components.These include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and extracellular vesicles (EVs) (exosomes, miRNA, etc.) in blood[17,18]. As tumors grow in volume and increase in number, such components are released into the bloodstream by apoptosis or necrosis.In this case, since these analytes come directly from cancerous cells, circulating blood can be used and clinically important genetic biomarkers can be identified and analyzed in detail in molecular analyzes.Moreover, since genetic material from all disease sites is freely available in the circulation, blood sampling can provide a real-time, representative image of cancer developing in the body.This technique involves the analysis of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) exosomes, and miRNA biomarkers. While CTCs and ctDNA found in the blood have the potential to provide information corresponding to possible therapeutic targets and resistance mechanisms, EVs in many body fluids (including urine, saliva, breast milk, cerebrospinal fluid, etc.), including blood, are genetic messengers and it is believed to be an alternative mode of cancer progression[17-19]. In this review, we will focus on ctDNA among the components of liquid biopsy.

Discovery and Importance of Circulating Tumor DNA (ctDNA)

DNA is continuously released in the circulating system as fragments by apoptosis and necrosis of both cancerous and non-cancerous cells in our body and circulates freely. When released independently of the originating cell, it is typically referred to as cfDNA (cell-free DNA); but when secreted by cancer cells, it is often referred to as ctDNA (circulating tumor DNA)[17,18]. Although the mechanism of ctDNA release into the blood is not fully understood, it is believed to have significant potential as tumor biomarkers. Molecular properties of ctDNA include mutations, CNVs, SNVs, methylation changes, or tumor-associated integrated viral sequences[20]. Extensive interest in ctDNA research began in 1948 when Mandel and Metais identified the presence of freely circulating DNA outside the cell[21] (Figure 2). However, the release of ctDNA by tumor cells was first described in 1973 by Kunkel when a decrease in ctDNA levels was observed in patients receiving chemotherapy[22]. Over the years, many studies have shown higher ctDNA levels in cancer patients than in healthy individuals, especially if taken close to a sample tumor site or at advanced stages of cancer[23-25].

The clinical potential of ctDNA was first determined by prenatal studies on women with male fetuses containing Y chromosomal DNA from cfDNA in their plasma[26]. This study initiated the use of blood tests and other studies to identify fetal gender and any chromosomal abnormalities during pregnancy[27,28]. Subsequently, another study by using noninvasive prenatal testing (NIPT)

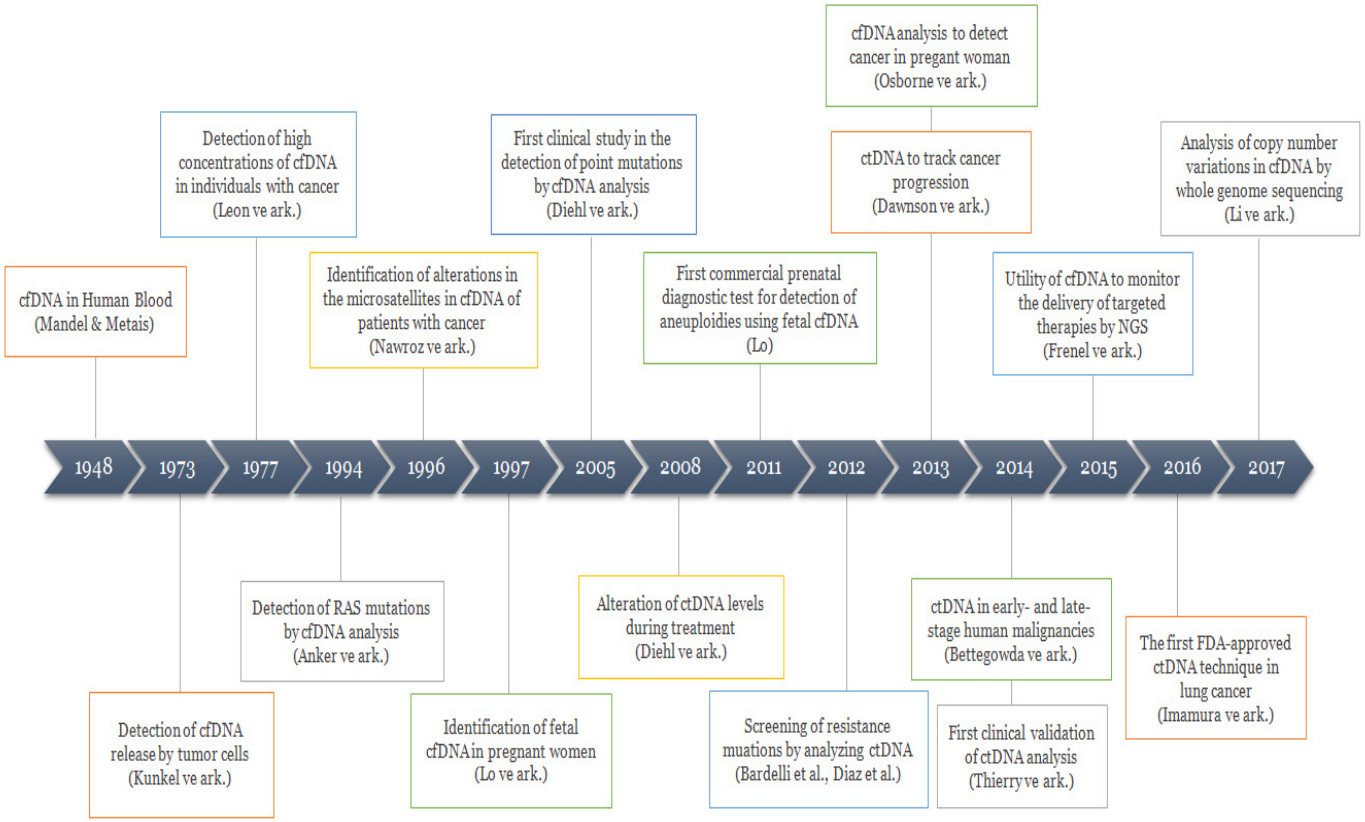

Figure 2: ctDNA Timeline and Important Dates [Modified from references 9 and 17].

for fetal aneuploidy by scanning cell-free fetal DNA (cffDNA) in maternal plasma incidentally observed 3 aberrant genome representation (GR) profiles which could not be attributed to the maternal or fetal genomic constitution. They found that these genetic aberrations were related to maternal cancer. The study concluded that that plasma DNA profiling allows for presymptomatic detection of tumors in pregnant women[29]. Further studies have shown a quantitative relationship between ctDNA levels and tumor burden. It is also believed that ctDNA contains significant cancer-related mutations[30-36].

The study also developed ctDNA analysis studies in clinical oncology to detect resistance mutations and other genotyping studies[37-40]. Furthermore, the potential of ctDNA to perform better than broadly studied biomarkers such as CTCs as a diagnostic tool has led to a number of studies on the use of ctDNA as a diagnostic tool.Increased levels of both CTC and ctDNA showed a poor prognosis in patients, while ctDNA showed a marked increase in sensitivity to CTC in determining tumor burden[27]. The viability of cfDNA in the blood has varying circulating half-lives ranging from 15 minutes to 2.5 hours, leading to the belief that ctDNA can be used as a real-time biomarker for cancer diagnosis[32]. As with any biomarker, the first step is to isolate ctDNA before any analysis is performed. In particular for ctDNA, the amounts present per ml are minute. This was due to the fact that in healthy individuals and cancer patients, the concentrations of cfDNA were between 1 to 100 ng per ml of plasma (with an average of 30 ng/mL), and 1 to 1000 ng per plasma (with an average of 180 ng/mL), respectively, depending on the plasma type. As a reference, a patient with 100g tumor burden releases 3.3% ctDNA into the circulation[30,41,42]. Recent advances in high throughput sequencing and complex computational methods have improved the ability to detect and characterize ctDNA[43]. In addition, recent advanced techniques have been able to identify single point mutations and track multiple genes related to increased sensitivity[44]. To this date, Roche's ctDNA-based detection of epidermal growth factor receptor mutations, a complementary diagnosis for erlotinib in lung cancer patients (NSCLC), is the first liquid biopsy to receive approval by the FDA in 2016[45]. Besides lung cancer, there is an urgent need to develop high sensitivity platforms towards the ctDNA-based detection techniques for breast cancer diagnostic purposes. Taken together, all these complementary diagnostic liquid biopsies can significantly improve the applicability of targeted therapy for patients in whom tumors are difficult to access safely in advanced stages of disease.

Utility of ctDNA in Various Stages of Breast Cancer Treatment Strategy

Determining the tumor-specific molecular profile is crucial for the success of targeted therapy and management of disease in breast cancer.The molecular profile of each tumor may be different, even if it is in the same patient, and the molecular motion of each tumor over time may change.This result, which is defined as intratumoral heterogeneity, is actually the clonal development of the tumor and it is essential to know and follow this dynamic molecular change and to observe the success of the treatment.Since long-term clonal evolution plays a major role in the success of treatment and should be monitored in real-time, ctDNA analysis can be an important potential biopsy source.It is important to follow up the residual disease with treatment plan updates according to the active follow-up of the genetic changes that the tumor will show and treatment response in the patient

under treatment and follow-up.

In this direction, several studies have shown that ctDNA liquid biopsies have different applications. ctDNA can be used to provide information about the tumor condition and tumor dynamics of cancer. Tumor condition information could be provided by ctDNA by evaluation of specific mutations when tissue biopsy is absent, insufficient and inappropriate to select the best treatment strategy for breast cancer patient. On the other side, tumor dynamics information include the monitoring of mutation load/level which would help in evaluation of treatment response, detection of resistance mutations and determination of recurrence risk after treatment. In fact application of ctDNA could be divided in two critical periods: Pre-treatment and post-treatment sections. Hence, being a crucial potential diagnostic, prognostic and predictive biomarker for breast cancer detection in an early stages and monitor the disease during or after treatment in real-time. Consequently, because ctDNA has a potential surrogate role for the entire tumor genome, the use of ctDNA as a liquid biopsy may provide the urgent need of the genetic follow-up data in the breast cancer treatment strategy by helping in selection of best applicable treatment that will reduce the risk of recurrence or progression, define resistance mechanism,and evaluate treatment response[17,46,47].

ctDNA Analysis in Advanced Stages of Breast Cancer

The first efforts toward the clinical utilization of cfDNA and ctDNA were focused on simple quantitative assessment of DNA concentrations present in blood circulation system. Several reported studies have shown that there exist significant differences in the amount of plasma DNA isolated from healthy individuals and cancer patients[48-51]. However, even ctDNA quantity can confirm the presence of cancer or disease-free status and relapse after curative surgery, several studies show that the amount of ctDNA quantified could be a useful diagnostic tool when used together with the evaluation of tumor-specific mutation status. So analysis of genetic alterations seems more promising in cfDNA research[43]. Recently, studies that analyzed ctDNA concentrations and especially most common tumor-specific mutations in breast cancer (PIK3CA, TP53, HER2 and AKT1) demonstrated that a higher amount of tumor-specific fragments and increased number of circulating tumor cells were related to biphasic size distributions of plasma DNA fragments[11,46]. A study in large cohort of breast cancer patients (n = 383) and a set of healthy individuals (n = 100) evaluated the integrity of cfDNA and demonstrated a hierarchical decrease in cfDNA integrity and an increase in cfDNA level from healthy controls to patients with localized diseases to metastatic breast cancer patients[52]. Moreover, the quantification of ctDNA together with the detection of mutated oncogenic main hotspots in advanced stages of breast cancer might tremendously impact on clinical management[11]. In a recent study oncogenic driver mutations were screened for in 587 postmenopausal women with HR+/HER2-locally advanced or metastatic breast cancer that progressed on/after AI (aromatase inhibitor) therapy to measure current PIK3CA mutation status. PIK3CA is the most recurrently mutated gene in breast cancer, and has been found to be important in several cancer types. So in this line the study aimed to show the sensitivity, reliability and applicability of ctDNA in proving the best choice for the treatment selection and understand the PFS (probability of progression-free survival) of patients treated by combined buparlisib and fulvestrant or monotherapy. Results of the study concluded that patients with tumor harboring PIK3CA muations detected in ctDNA performed poorly on fulvestrant monotherapy, demonstrating prolonged PFS for combined buparlisib and fulvestran. 3-8 month mPFS improvement was supported by higher response rates (18.4% vs. 3.5%) in this endocrine-resistant HR+/HER2-advanced breast cancer patient population. By achieving a clinically meaningful PFS improvement, study suggests that assessment of PIK3CA mutations in ctDNA may help select patients who benefit from adding a PIK3CA inhibitor to endocrine therapy and ctDNA obtained from blood samples has emerged as sensitive, reliable, and non-invasive way to measure current PIK3CA mutation status[53]. In this line, a very important new reference on this subject as the approval by FDA of companion diagnosis tool on ctDNA for the use of alpelisib, a targeted drug for PIK3CA mutated metastatic luminal breast cancers was already published under the AACR (American Association Cancer Research) General Session Abstracts by D Juric and et al[54]. The study concluded that, alpelisib in combination with fulvestrant showed consistent clinically meaningful treatment benefit for pts (Impact of treatment sequence in patients) with ctDNA PIK-3CA mutant status[54]. Another studies also mentioned that the management of advanced breast cancer require the monitoring of the tumor burden to determine the response to therapy, and improved clinically significant biomarkers are needed to follow up[55-60]. For this purpose, the study in 30 women with metastatic breast cancer who were receiving systemic therapy, identified somatic genomic alternations and designed personalized assays to quantify ctDNA in total of 141 serially collected plasma specimens[39]. The study identified an inverse relationship between quantification of ctDNA (indicated in copies/ml in plasma) and overall survival, with increasing levels significantly associated with inferior overall survival ($P < 0.001$). The prognostic discrimination power of ctDNA was greatest if the patients with levels more than 2000 copies/ml were uniformly found to have the worst prognosis[39]. Likely, another study, showed that the evaluation of ctDNA tumor fraction was feasible for nearly all 164 TNBC patients included in the analysis, and tumor fraction $\geq 10\%$ was associated with significantly worse survival in this large metastatic TNBC cohort. Moreover, specific somatic copy number alterations (SCNAs) were enriched and prognostic in metastatic TNBC, with implications for metastasis, resistance, and novel therapeutic approachesia[55].

Another study also not only looked at response to therapy but also monitored resistance mechanism of disease. Study demonstrated that activating mutations in the estrogen receptor 1 (ESR1) gene are acquired under treatment in approximately 20% of breast cancer patients and drive resistance to anti-hormonal therapy. Because such mutations could be predictive of endocrine resistance, the study analyzed serial plasma ctDNA samples from 48 ER+ mBC patients receiving antiestrogenic therapy. Results of the study showed that in four patients, an ESR1 resistance mutation was detected in cfDNA under therapy[56]. Similarly, several studies have demonstrated detection of ESR1 and other mutations associated with resistance to endocrine treatment or antic CDK agents[57-60]. Although, several comparable data were also reported by others, there exist an urgent need of large patient numbers and analysis strategies to evaluate whether and how the early detection of advanced breast cancer might improve patient outcome.

ctDNA Analysis in Early Stages of Breast Cancer

As aboveobserved, a majority of recent studies have focused on the clinical utility of ctDNA as a surrogate marker in the detection of only metastatic cancers, while there have been a few studies that have looked at the early stages[11,17,38,47,61-70]. In one of such few studies, matched tumor-plasma specimens were collected from 180 patients spanned across seven various types of tissue types with 20% early stage (up to Stage IIB classified as early), 73% advanced stage (Stage IIB and above) and 11% unknown stageto detect the mutation status using ctDNA for prognosis, treatment decisions and disease monitoring. Among 180 patients of different types of cancers, 42 (23.33%) were breast cancer patients. Hence, the data obtained from the study, has provided useful insights into the ctDNA levels across different stages and tissue of origin, where the tumor-plasma concordance was shown to be highly variable since it depended on the tissue of origin, tumor size, stage, lymph node metastasis, grade, time of sample collection, and even the platform used for detection[61]. Therefore, the reported concordance of 82% and 32% was observed in advanced (Stage IIB and above) and early (Stage I to Stage IIA) stage samples, respectively. Interestingly, patients' survival outcomes were shown to be correlated to the baseline ctDNA levels (presurgical/at-biopsy ctDNA levels). Indeed, baseline ctDNA stratified patients into three classes: a. high ctDNA indicate poor survival outcome; b. undetectable ctDNA show good outcome and, c. low ctDNA whose outcome was ambiguous. Study's results demonstrated that ctDNA and baseline ctDNA levels could be used as a powerful toolsto tract tumor-specific mutations and stratify patients into prognostic groups and thus being helpful in therapy decisions and patient management in a large number of cancers across a variety of stages[61]. Another study by Garcia-Murillas et al. assessed whether analysis of circulating tumor DNA (ctDNA) in plasma could be used to monitor for minimal residual disease (MRD) in early breast cancer patients. Their study included a prospective cohort of 55 early breast cancer patients receiving neoadjuvant chemotherapy, where the study demonstrated that the mutation tracking in ctDNA predicts relapse in early breast cancer by also defining the genetic events of MRD[62]. Another study in 38 nonmetastatic triple-negative breast cancer (TNBC) patients, investigated whether TP53 mutations detected in ctDNA can reflect the tumor response to neoadjuvan chemotherapy (NCT) and detect minimal residual disease (MRD) after surgery. As TP53 mutations are universal across cancer types and frequent tumor protein p53 (TP53) inactivating gene mutations are present in TNBC, the detection and quantification of ctDNA is a very promising tool that can assess tumor burden, treatment response and MRD[63]. The study collected the plasma samples at up to 4 time points: a baseline (before start of NCT); before the second cycle of chemotherapy (2-3 weeks after first cycle); at the last cycle of NCT (before before breast cancer surgery); and 2-10 weeks after surgery. Before NCT no correlation was observed between cfcDNA and ctDNA concentrations. However, it was found that ctDNA levels were significantly associated with clinical tumor size ($P = 0.004$), tumor stage ($P = 0.003$) and correlated with high proliferation rate, assessed either by mitotic index ($P = 0.003$) or tumor grade ($P = 0.003$). 2-3 weeks after first cycle of chemotherapy and during NCT cfcDNA concentrations increased significantly, while those of ctDNA decreased. After surgery there was not any detection of ctDNA levels. Thus, in order to detect the minimal residual disease, the study population was followed up to 2 years. Study showed that after 2 years, the few patients with remaining ctDNA after NCT were more likely to present with later metastatic relapse and associated with shorter disease-free survival ($P < 0.001$) and overall survival ($P = 0.006$)[63]. Besides TP53 detection, because there are a few reported studies on clinical implication of PIK3CA on TNBC, the prognostic role of PIK3CA mutations of cfDNA in early-stage of 49 TNBC patients was also investigated. This study results have shown that a total of 12 of 49 patients had PIK3CA mutations of cfDNA and in a median follow up of 54.4 months, the presence of PIK3CA mutations of cfDNA had significant impacts on relapse-free survival (RFS; $P = 0.0072$) and breast cancer-specific survival (BCSS; $P = 0.016$). Hence, the presence of PIK3CA major mutations of cfDNA could be a discriminatory predictor of RFS and BCSS early-stage TNBC patients[64]. Further recently studies have shown that besides the use of ctDNA to track tumor-specific mutations, ctDNA can be also useful to detect tumor-specific chromosomal rearrangements present in ctDNA that may be interrogated in blood plasma. The study evaluated serial monitoring of ctDNA in 20 patients diagnosed with primary breast cancer for detection of occult metastatic disease. After the sequencing of primary tumor and quantification of tumor-specific rearrangements in plasma specimens, obtained data has shown that ctDNA monitoring is highly accurate for postsurgical discrimination between patients with (93%) and without (100%) eventual clinically detected recurrence. ctDNA-based detection preceded clinical detection of metastasis in 86% of patients with an average lead time of 11 months (range 0-37 months), whereas patients with long-term disease-free survival had undetectable ctDNA at any time-point after surgery. The presence and quantity of ctDNA was predictive of poorsurvival in this cohort group[65]. Several studies mentioned above have shown good results of ctDNA on early-stages or advanced stages of breast cancer detection together with the real-time monitoring of disease response therapy, recurrence risk or overall survival rates, especially in known oncogenic driver mutations[66-68]. However, additional clinical studies in large cohort group are needed to compare the diagnostic, prognostic and predictive value of ctDNA detection of unknown oncogenic drivers and level alterations before, during and after therapy, all of which previously have been demonstrated to have a significant impact on early or advanced breast cancer treatment strategy.

Conclusion

Information from ctDNA analysis can help the physician choose the best treatment for a particular stage of metastatic disease. Blood-based monitoring may also indicate the need to switch to a different treatment regimen before changes appear on an imaging examination.In early stages of breast cancer and other cancer types, periodic checking of blood for cancer signals after treatment may identify patients at risk of recurrence.Some research suggests that ctDNA analysis can detect when cancer occurs before long after tumor reoccurrence.Early intervention can improve survival. Furthermore, one of the most widespread and significant applications of ctDNA is the monitoring of response treatment and thus determination of resistance mechanism and new treatments strategies.

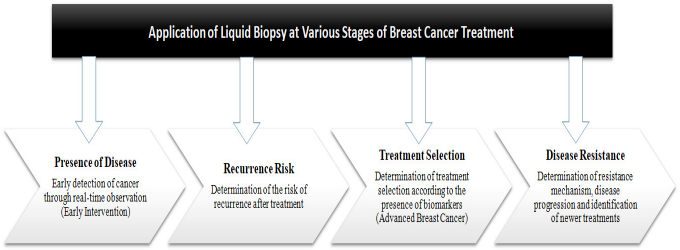

Figure 3: Advantages of application of liquid biopsy technique in breast cancer treatment strategy[69].

In the light of all this information, although liquid biopsy and ctDNA analysis is still under investigation, it promises to detect, improve and prevent response to cancer treatment.The concept of fluid biopsy can complement the personalized medical approach and offers an innovative way of patient selection in clinical trials; wherein the genome analysis supports the patient's suitability for targeted therapy.

However, despite the apparent advantages of the new diagnostic methods, including liquid biopsy, it is necessary to see that there are still problems in widespread use and the replacement of traditional methods.One of them is that the information that every cancer detected is biologically progressive and threatens the health of the patient is not correct. Some cancers of breast and prostate origin, in particular, may remain lifelong. Today, our knowledge of which cancer will progress and which will remain local remains unclear.However, it is clear that a diagnosis that is false or meaningless for the patient's life will bring physical, economic and psychological burdens to the patient. Secondly, although it was a desirable case, early diagnosis was not sufficient in all cancers. It is also known that normal DNA particles circulate in human blood. In diseases such as myocardial infarction associated with tissue damage, even in pregnancy, DNA can be detected in the blood.Therefore, it is essential that the DNA in the blood be cancer-specific, to determine which site reflects cancer if possible, and to understand which biological characteristics of the sequences are indicative of cancer[70].

New methods such as liquid biopsy together with analysis of ctDNA in breast cancer as a diagnostic and prognostic biomarker[71] and the like are becoming economically widespread today.Together with the traditional methods, they are expected to be used as techniques that provide cheap, effortless and new information for patients, with their clinical benefits in diagnosis and treatment.

Conflict of interest: The author claims no conflict of interest with regard to this manuscript.

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