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REVIEW ARTICLE

Circulating tumor DNA (ctDNA) in the era of personalized cancer therapy

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Abstract

The heterogeneity of tumor is considered as a major difficulty to victorious personalized cancer medicine. There is an extremeneed of consistent response evaluation for in vivo tumor heterogeneity anditscoupledconflict mechanisms. In this occasion researchers will be able to keep pace withpredictive, preventive, personalized, and Participatory (P4) medicine for cancer managements. In fact tumor heterogeneity is a central part of cancer evolution,soin order to progress in understanding of the dynamics within a tumor some diagnostic apparatus should be improved. Latest molecular techniques like Next generation Sequencing (NGS) and ultra-deep sequencing could disclose some clones within a liquid tumor biopsy which mainly responsible of treatment resistance. Circulating tumor DNA (ctDNA) as a main component of liquid biopsy is agifted biomarker for cancer mutation tracking as well as profiling. Personalized medicine facilitate learning regarding to genetic pools of tumor and their possible respond to treatment which could be much easier by using of ctDNA.With this information, cliniciansarelooking forward to find the best strategies for prevention, screening, and treatment in the way of precision medicine. Currently, numerous clinical efficacy of such informative improved treatment are in hand. Here we represent the review of plasma-derived ctDNA studies use in personalized cancer managements.

Keywords Circulating tumor DNA (ctDNA) · Personalized medicine · Cancer

Introduction

Cancer is one of the problematic issue of human health and the second main reason of death all over the word [1, 2]. Circulating tumor DNAs (ctDNA) are short tumor-derived fragments of DNA (≅166 base pairs) which are not associated with cells and freely are circulating in serum and plasma [3]. The precise mechanism of ctDNA release has not been cleared yet, but they are some suggesting role for tissue necrosis and apoptosis as well as dynamic secretion from tumor cells [4–8]. In the honor of ctDNA it can be said easily that it is a real time representative of tumor, so it can be checked for genetic and epigenetic changes of tumor in order to define the accurate treatment plan as well as monitoring the tumor progression during the therapy [9–11]. In reality using of ctDNA as a diagnostic or prognostic tool outweighs the other common biopsy methods like tissue biopsy [12, 13]. The ctDNA collection characteristics as a non-invasive biopsy method in addition to several sampling at different time after treatment will be possible and consequently keeping an eye on tumor progression and response to treatment will be much feasible [14].

One of the problematic issues of cancer therapy is drug-resistant tumors due to intra- and inter-tumor heterogeneity [15, 16]. Unfortunately even a minor genetic clone within the tumor if carries a drug-resistant mutation can be developed after treatment [17]. ctDNA is a repeatable non-invasive biopsy method and contrary to tissue biopsy as a *'snapshot'*, ctDNA is a '*screenshot*' of the primary and metastatic tumor [18]. At the cutting-edge of targeted treatment approach, sequencing of ctDNA can be really informative for finding genetic hotspots of targeted tumor [19]. This is mainly significant for informing treatment specially when mutations are critical as drug targets [20, 21].

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Consequently in each patient, personalizing targeted analysis of ctDNA can be promising by incorporating the liquid biopsies and common tissue biopsies [22].

Targeted approaches have the benefit of amplifying ctDNA in the course of polymerase chain reactions (PCR) or digital PCR (dPCR) [23]. It is above all essential because there are quite small amount of ctDNA circulating in the blood [23]. For that reason, amplification of interested region can considerably recover the weak points of ctDNA detection methods [24]. Unfortunately, PCR a an amplification tool can launch some known errors which will pass to the sequencing step [25].

The latest advances in whole genome and targeted next generation sequencing (NGS) techniques are breakthroughs for detection of genetic abnormalities of a patient's tumor [26]. Moreover the Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) is an insightful and sensitive method in order to quantify DNA in cancer because It measures ctDNA which is originated from tumor cells into the bloodstream [27]. This method can be widespread for any cancer type and is able to identify one molecule of mutant DNA in ten thousands molecules of normal DNA [28].

In the current review we are presenting the importance and value of ctDNA in the place of precision cancer medicine in both era of cancer diagnosis and cancer prognosis. The study was based on searching the PUBMED, Scopus, Web of Science, and EMBASE from 1990 to 2017.The search syntax were "cancer" or "neoplasm" or "tumor" and "cfDNA" or "circulationg tumor DNA" or "ctDNA" or "cell free DNA" or "CTC" or" circulating tumor DNA" and "personalized medicine" or "Precision medicine" or "P4 medicine" and "treatments" or "therapy" or "Diagnosis". All final selected articles should be written in English (19 articles).

ctDNA as a liquid biopsy component

In spite of the fact that the cfDNA presence in the plasma was first accounted in 1948 by Mandel and Metais [29], it was just recent years that tumor-derived cfDNA was revealed that cancer patients had greater levels of plasma cfDNA than normal controls [30–32]. Actually the exact biological mechanism by which DNA is releasing into the peripheral blood has not been well understood; nonetheless, it is thinking to come about through multiple mechanisms, including extracellular vesicle secretion, tumor cell apoptosis, and necrosis [6, 33, 34]. The fact that dissimilar to genomic DNA, ctDNA is extremely fragmented around nucleosomes (approximately 150 base pairs in length) (Fig. 1), supports the hypothesis that ctDNA originates through cell necrosis or apoptosis [35, 36]. The DNA of eukaryotic cells is coiling around histone protein complexes, shaping nucleosomes as the basic form of chromatin [37]. Judge against to the naked DNA, DNA of nucleosome is fewer reachable to the transcription factors and regulatory elements [38, 39]. The precise physical situations of nucleosomes can affect vital process of cells including replication, DNA repair, and transcription [40]. Actually, depending on the cell type, nucleosome positioning is completely different so ctDNA deep sequencing, isolated from circulating blood plasma haven path of transcription factors [41]. It could be said that ctDNA nucleosome positioning is directly connected to the nuclear architecture and gene expression profile so it could be the exact representative of the origin of tumor [41–43].

More than the origin of tumor the concentration of ctDNA can be an informative substances. Peripheral ctDNA isasmall proportion of DNA in bloodsteam, fewer than 100 ng/mL [44] and no more than a fraction of this whole ctDNA (< 1% of total ctDNA) is in certainty tumor-derived [45]. The quantityof released DNA into the peripheral blood is in coincide with the concentration of evident ctDNA sincectDNA shedding to the bloodis associatedwith the cell death,cell division rate, and tumor vascularization [30]. As a result the degree of metastatic tumor is joined to thevolume of ctDNA [33, 46–48]. By way of illustration, the direct existence of metastasis to the liver or bone has been straightattachedto thegreater levels of ctDNA [49].

Long beforepersonalized medicine, patients had the identical treatment, but next off it became clear that dependent to genetic profile of patients, certain treatments are much better for some patients than for others. This explained the dissimilar responses to cancer managementapproaches. Nowadays, personalized cancer treatment is an active branch of the treatment plan or even an essential part of a clinical trial. A few, but not all, of the cancers where targeted treatments are used consist of; breast cancer (BC), Lung cancer (LC), Gastrointestinal (GI) Cancer, and endocrine related tumors.

Breast Cancer

There is a long-standing hope of precision medicine to find a genetic markers to guess reaction of a solid tumor to treatment, approximate patient prognosis and early prediction of tumor relapse [50]. Primary research were mainly paying attention to circulating tumor protein biomarkers in the glycosylated form, while it is now speedily altered to novel prospect like circulating tumor cells (CTCs), extracellular vesicles (exosomes), micro-RNAs and circulating tumor DNA (ctDNA) [50, 51]. In early-stagebreast cancer there are some appreciated indications for ctDNA quantifications [52]. In fact raised plasma ctDNA levels using specific digital droplet PCR (dd-PCR) assays in plasma samplesheaded clinical detection of tumor recurrence in patients [52].

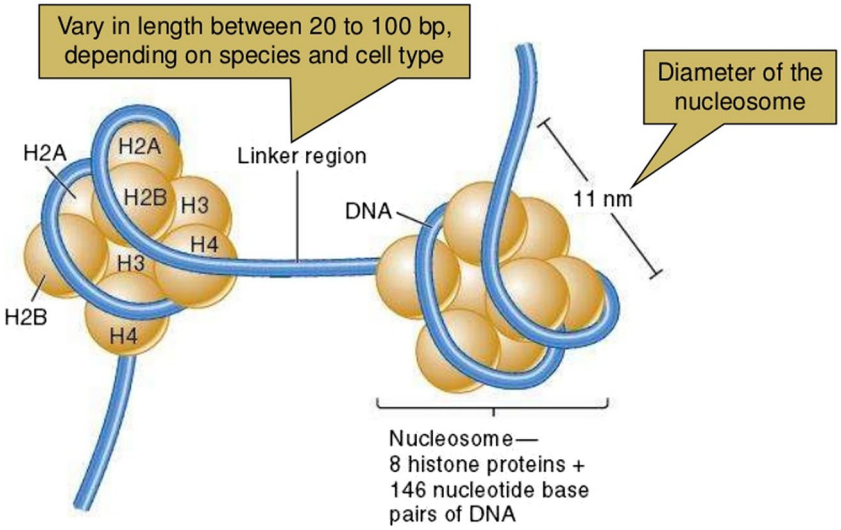

Fig. 1 The "beads-on-a-string" structure taken from slide share (http://slideplayer.com/slide/5675348/). It is composed of eight specific histones come together (octamer). The linker DNA length is about 150 base pairs which are next to the ctDNA length

Severalpreceding studies had focused on metastatic disease, in order to state the ctDNA amount and response to surgery, treatments or as a measurement tool of overall survival [53], such as colorectal [54], breast [55], ovarian and lung cancer [56]. The justification of this is that released tumor cells are typically phagocytosed by macrophages which engulf necrotic cells that release digested DNA fragments into the cell environment with a half-life in the circulation ranging from some minutes to several hours [57, 58]. Through tumor growth and turnover both wild-type and tumor-derived ctDNA can be shed into the blood, soaccording to the state and size of the tumor, the percentage of ctDNA that originates from tumor cells fluctuates [59]. It was shown by Sarah-Jane Dawson et al. that circulating tumor DNA was a permanent time-dependent inconsistent in the way that its levels were a signature of substandard overall survival of breast cancer patients [55]. The quantity of ctDNA was predictive of poor survival and ctDNA evaluating has worth as a observing component for early metastasis detection, therapy adjustment, and to support in overtreatment avoidance in the way of precision medicine [52].

More than ctDNA quantity the genetic and epigenetic alterations of ctDNA can be used for breast cancer personalized therapy. The level of plasma samples mutations imitate the clonal hierarchy concluded from sequencing of tumor biopsies [18]. The evaluation of biopsy and plasma samples in one metastatic breast cancer patient displays that ctDNA form a concurrent sampling of multifocal clonal evolution [18, 60]. Hopefully a study confirmed that ctDNA analysis via eTAm-Seq and digital PCR have high clinical validity in mutation detection [61]. Detection of Estrogen receptor alpha (*ESR1*) D538G mutation in circulating tumor cells (CTCs) and ctDNA can be used in for assessing response to endocrine therapies in breast cancer [62]. For resistance to subsequent aromatase inhibitor therapy *ESR1* mutations can be strongly recognized with ctDNA analysis, and predict [63, 64]. *ESR1* mutations are infrequently developed during adjuvant aromatase inhibitor (AI) therapy, but are frequently designated by therapy for metastatic disease, supporting that the mechanisms of resistance to targeted therapy possibly will be considerably dissimilar between the treatment of micrometastatic and overt metastatic cancer [63]. Monitoring of ctDNA is extremely essential for preliminary security and efficacy checking of *HER2*-negative metastatic breast cancer treatment with*Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)* inhibitor Taselisib (GDC-0032) together with Tamoxifen in hormone receptor (HR) positive [65]. During a phase III clinical trial in postmenopausal women with endocrine-resistant *HR+/HER2–* advanced breast cancer, it was shown that checking the *Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3C)* of ctDNA can guesses efficacy of Buparlisib (BUP) plus fulvestrant (FULV) [66].

In estrogen receptor (ER)–positive breast cancer, mutations of *Phosphatidylinositol-4, 5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA)* are common genomic alterations and a self-governing analytical feature in breast cancer patients [67, 68]. Analysis of ctDNA in plasma could be used for minimal residual disease (MRD) monitoring in breast cancer [69]. It was verified that mutation tracking of ctDNA through sequencing could outline the genetic events of MRD in order to projected the genetic background of the subsequent metastatic relapse extra precisely than sequencing of the primary tumor [69]. Following adjuvant therapeutic interventions possibly will be personalized with the genetic profile existing in the MRD, a therapeutic

approach that could solve the problem of intra-tumor genetic heterogeneity [69, 70].

Lung cancer

The breathtaking advances in lung cancer therapy is the application of personalized chemotherapy planning according to the individual's genetic profile [71]. It has been suggested that ctDNA "spill over" into an immediate outflow tract pulmonary venous blood (Pul.V) and peripheral blood (Peri.B), and after scattering to the whole body [72]. Thus, it can be inferred that ctDNA reflects the cancer progression and could function as a prognostic marker. It has been accepted that epidermal growth factor receptor *EGFR* mutation status is a delicate biomarker for the *epidermal growth factor receptor tyrosine kinase* inhibitors (*EGFR-TKIs*) therapy [73, 74]. In fact, patients with the L858R point mutation in exon 21 or deletion mutation in exon 19 display respectable response to *EGFR-TKIs* [74]. The problematic issue is that after chemotherapy *EGFR* mutation status might exchange from positive to negative [75]. For that reason, tracking the*EGFR* mutations is important to control an applicable treatment approach, mainly designed for the supervision of *EGFR-TKIs* to identify acquired resistance at early time [9, 76–78[. Indeed, ctDNA can be a potential source of tumor DNA alteration pursuing for the documentation of tumor-associated genetic changes in order to real-time tumor monitoring [4, 53, 78, 79]. For Non-Small Cell Lung Cancer Research (NSCLC), numerous clinical centers have investigated the diagnostic precision of ctDNA for *EGFR* mutation detection [80–83]. In 2016 the U.S. Food and Drug Administration (FDA) agreed to the *EGFR* Mutation Test v2, a blood-based companion diagnostic for the cancer drug Tarceva (Erlotinib) [84].

More than *EGFR* some other genetic and epigenetic changes has been considered for personalized lung cancer target therapy. By way of illustration, the existence of *Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS*) mutations in plasma might be an indicator of deprived prognosis and may also embrace predictive value [85, 86]. The clinical trial phase I, combination study of a kinase inhibitors in patients with *RAS* mutated cancers, indicated that increasing dose levels resulted in more consistent decreases in *KRAS* mutation in ctDNA, so the potential value of serial plasma ddPCR as a pharmacodynamic (PD) biomarker in early phase clinical trials was marked [87]. A promising step on the way to precision medicine is that genomic analysis of lung-tumor growth has been practiced to make personalized blood tests that allow successful clinical observing for early signs of cancer relapse [88]. Multiplex ctDNA Gene analysis in lung cancer revealed promising treatment options to guide clinicians to choice the accurate therapy plan for the right person [89].

Additional analysis of ctDNA through CAPP-Seqand resistance mechanisms in NSCLC patients cured with Rociletinib highlighted frequent intra-patient heterogeneity [90]. In fact, Met Proto-Oncogene copy number increasing involves in resistance recurrently [90]. Those results emphasized the position of tumor heterogeneity in NSCLC and the utility of ctDNA-based resistance mechanism calculation [90, 91]. Apart from genetic mutation some epigenetic changes of ctDNA can be recruited for prognosis and diagnosis [92]. By far the most important epigenetic alteration is DNA methylation that occurs by adding the methyl (CH3) group to DNA, in that way often modifying the function of the genes and affecting gene expression without changing the DNA sequences. Very recently the improvement of a highly sensitive blood-based non-invasive diagnostic assay for documentation of primary lung cancer stages, which can aid clinical decisions for patients with a CT scan positive for lung nodules, has been suggested [93]. This method can similarly be stretched to non-invasive early screening for various cancer types [93].

Gastrointestinal Cancer

Regarding to the liquid biopsy components it can be said easily that in patients with cancer of the gastrointestinal cancer (GI), major advances have been completed in the use of circulating tumor cells (CTCs) and ctDNAs for monitoring tumor evolution [94]. This is principally right in the case that in the peripheral blood circulation of GI cancers patients, the mutant form of "driver" genes and "drug-resistant" alleles of tumor are represented in the circulating cell-free tumor DNA (cfDNA) [95–97]. The discriminative accuracy of ctDNA the amount for diagnosis of gastrointestinal cancer contrast to the benign inflammatory diseaseshas been distinguished [98–102].In order to prove the comprehensive diagnostic value of ctDNA through diverse gastrointestinal tumor types, ctDNA of 640 patients evaluated by Bettegowda et al. [53]. The NGS method used to find out target mutations of tumor tissue, and then by using RT-PCR quantified in ctDNA [53]. Moreover, complete cfDNA and tumor-specific ctDNA have been exposed in several researches to be higher in patients with colorectal cancers (CRC) compared with healthy controls [103–107]. Use of *RAS* mutations in cfDNA of patients with metastatic colorectal cancer brought a promising personalized dashboard for this cancer [108, 109]. Clinical utility of ctDNA sequencing in advanced CRC can provide appropriate information

on potential mutations, in that way to ease clinical trial enrollment and enlightening the supposed value of care [110]. The quantitative relationship of cfDNA with tumor specific mutations in plasma from metastatic colorectal cancer (mCRC) patients was related to the efficacy of third line treatment with cetuximab and irinotecan [111]. Checking the quantity of ctDNA levels within a post-surgery surveillance study by Reinert and colleagues in and five no relapsing and six relapsing patients with colorectal cancer showed that relapses could be detected months in advance compared to conventional follow-up [112]. Moreover, ctDNA analysis can be used for tumor burden and standard chemotherapy reaction estimation in patients with early-stage colorectal cancer [113]. Gastric cancer is a leading cause of cancer deaths in the world with highly heterogeneous etiology and clinical characteristics [114]. The Cancer Genome Atlas (TCGA) network shed light on the heterogeneity and possible targeted therapeutics for various subtypes of gastric cancer according to comprehensive genomic platforms [95, 115]. The most usual mesenchymal tumors of the gastrointestinal tract are gastrointestinal stromal tumors (GISTs) [116, 117]. GISTs are described by mutations in a *receptor tyrosine family* (mainly *KIT* gene) which are linked to the mast cell growth factor receptor or in the *platelet-derived growth factor receptor alpha(PDGFRA)* coding gene [118–121]. The relationship between tumor genotype and positive effect of adjuvant imatinib stated that GIST with a *KIT* exon 11-deletion beneficially respond to treatment, with a considerably extended progression free survival (PFS) compared with placebo [122–124]. It was shown that mutation detection in cfDNA of GIST patients with metastatic disease can be recruited for personalized usage of imatinib and monitoring of early treatment adaptations [125, 126]. A panel called ('SiRe') with 568 mutations in six genes *(EGFR, KRAS, NRAS, BRAF, cKIT* and *PDGFRα)* evaluated in different cancers including GIST and can be optimized for its precision medicine in the near future [19]. A comparison of cfDNA levels after the six months after surgery and at the time of recurrence were considered in 18 gastric cancer patients who did not receive adjuvant chemotherapy, indicated to the fact that this patients had high pre- and postoperative cfDNA [127].

Thyroid tumors

The increasing prevalence of thyroid nodules and tumors had been resulted in a higher demand for the accurate diagnosis of thyroid nodules, and the best treatment strategies for this aggressive disease. A usual diagnostic tool is fine needle aspiration (FNA) samples from thyroid nodules with a mutations profiles that typically includes *BRAF, RAS, RET/PTC*, and *PAX8/PPARg* [128–132]. Combining the use of these molecular markers of ctDNA and new high-throughput molecular techniques will improve significantly the accuracy of cancer diagnosis in thyroid nodules [133]. By way of illustration, Anaplastic Thyroid Carcinoma (ATC) is an aggressive type of thyroid cancers that requires rapid diagnosis and multimodality management approaches. At the MD Anderson Cancer Center of University of Texas the NGS platforms over 70 genes of 23 patients ctDNA suggested that both tumor-based and ctDNA examination in the setting of clinical-trial application is beneficial for ATC patients [134]. Further innovative, realistically designed therapeutic strategies are under active expansion both for patients with Differentiated Thyroid Tumors (DTC) and for patients with ATC, within several phase II and phase III randomized clinical trials currently continuing [135] (Table 1).

In advanced Medullary Thyroid Carcinoma (MTC), ctDNA *RET* M918 T mutations of circulating tumor DNA can be predictive for overall survival (OS) and could take part in a role in monitoring response to treatment [136]. Moreover, in thyroid tumors the published result related to a phase II clinical study in Philadelphia demonstrated treating metastatic thyroid cancer patients with the targeted therapy of Vemurafenib to launch the activity of Vemurafenib in the only patients with BRAFV600E-positive papillary thyroid [137]. In fact it was shown that Vemurafenib had antitumor activity in patients with progressive, BRAFV600E-positive papillary thyroid cancer refractory to radioactive iodine who had never been cured with a multi-kinase inhibitor [137, 138]. More than that it has been revealed that detectable levels of *BRAF(V600E)* ctDNA pre-operatively, thus *BRAF(V600E)* ctDNA can be a discriminative tool between benign and malignant thyroid nodules [139, 140].

In fact personalized ctDNA biomarkers dynamically can be a good predictor of treatment response and survival in a wide range of cancer types including gynecologic cancers [141, 142]. It was shown that ctDNA level increased in advanced stage of ovarian cancers compared to controls, so ctDNA quantity can be useful for noninvasive screening and this disease surveillance [143]. Although point mutations have been extensively studied, chromosomal rearrangements have confirmedsuperior tumor specificity [144, 145]. A panel of individualized junctions consequent from tumor DNA possibly will be an useful way to monitor cancer patients for relapse and therapeutic efficacy using ctDNA [144]. ctDNA as noninvasive biomarkers of gynecological cancers, ovarian, endometrial. For example ctDNA can detect more mutations than DNA extracted from solid tumor and when performing genetic profiling in order to precision medicine programs should consider cfDNA to optimize finding of the molecular diversity of ovarian cancer [146].

Table 1 The summary of studies related to ctDNA and personalized cancer management

Author	Country	Year	Type of Cancer	Result
Eleonor Olsson [52]	Sweden	2015	Breast Cancer	• ctDNA quantity was a prognostic tool of poor survival • ctDNA is a monitoring tool for early metastasis detection, therapy modification, and to aid in avoidance of overtreatment
Chetan Bettegowda [53]	USA	2014	Pancreatic Cancer Ovarian Cancer Colorectal Cancer Bladder Cancer Gastro esophageal Breast Cancer Melanoma Hepatocellular Carcinoma Head and neck Cancers	• ctDNA *KRAS* gene mutations as a broadly applicable, sensitive, and specific biomarker that can be used for a variety of clinical and research purposes in patients with multiple different types of cancer.
Sarah-Jane Dawson [55]	United Kingdom	2013	Breast cancer	• Circulating tumor DNA is an informative, inherently specific, and highly sensitive biomarker of metastatic breast cancer.
Muhammed Murtaza [56]	United Kingdom	2012	Breast Cancer Ovarian Cancer Lung Cancer	• Exome-wide analysis of ct DNA could complement current invasive biopsy approaches to identify mutations associated with acquired drug resistance in advanced cancers • Serial analysis of cancer genomes in plasma constitutes a new paradigm for the study of clonal evolution in human cancers.
Nicholas C. Turner [62]	United Kingdom	2016	Breast Cancer	• *ESR1* mus, detected in plasma ctDNA, were identified in a high percentage of pts. with HR+ MBC confirming an important role in endocrine-resistance • P + F treatment provided significant benefit for MBC pts. with and without *ESR1* mus
Richard D. Baird [65]	USA	2016	Breast cancer	• Taselisib in combination with tamoxifen is generally well tolerated. Preliminary evidence of anti-tumor activity was seen, in some patients preceded by a fall in plasma PIK3CA ctDNA levels. The recommended phase II dose of taselisib in combination with tamoxifen is 4 mg on a daily continuous schedule. Clinical trial information: NCT02285179.
Cristofanilli M [147]	United Kingdom	2016	Metastatic Breast cancer	• Fulvestrant plus palbociclib was associated with significant and consistent improvement in progression-free survival compared with fulvestrant plus placebo, the combination could be considered as a therapeutic option for patients with recurrent hormone-receptor-positive, HER2-negative metastatic breast cancer that has progressed on previous endocrine therapy.
A Bosch [67]	USA	2015	Breast Cancer	• Increased ER transcriptional activity may be a reactive mechanism that limits the activity of PI3K inhibitors and that combined PI3K and ER inhibition is a rational approach to target these tumors.
Cloud P. Paweletz [87]	USA	2016	Lung Cancer	• Increasing dose levels resulted in more consistent decreases in KRAS mutation in cfDNA, consistent with a dose-dependent pharmaco-dynamic effect. These results highlight the potential value of serial plasma ddPCR as a PD marker in early phase clinical trials.
Smadar Geva [89]	Israel	2016	Lung Cancer	• Liquid biopsy ctDNA testing revealed possible treatment options for more than two-thirds of patients analyzed, including FDA-approved drugs as well as eligibility for clinical trials and guide clinicians to select the *right therapy for the right patient.*
Jacob Chabon [90]	2016	USA.	Lung Cancer	• Underscore the importance of tumor heterogeneity in NSCLC and the utility of ctDNA-based resistance mechanism assessment
D.J. Merriott [91]	USA	2017	Lung cancer	• Analysis of ctDNA potentially allows early identification of NSCLC patients who will have DCB from ICIs • Response assessment by ctDNA may therefore be useful in clinical studies examining combinations of ICIs and radiotherapy
Spindler KL [111]	Denmark	2012	Metastatic Colorectal Cancer (mCRC)	• cfDNA alleles and KRAS and BRAF mutation alleles analysis in plasma is a viable alternative to tissue analysis

Table 1 (continued)

Author	Country	Year	Type of Cancer	Result
Evan J Lipson [113]	USA	2014	Melanoma	• Quantitative levels of cfDNA and pmKRAS are strongly correlated and hold promise of clinical application. • Levels of ctDNA correlated with clinical and radiologic outcomes, and, in one case, preceded eventual tumor regression. Further prospective analysis is required to assess the utility of ctDNA as an early biomarker of clinical outcomes in patients receiving immune checkpoint blocking drugs.
Weixin Yan [95]	USA	2016	Gastrointestinal stromal tumors (GISTs)	• Mutant tumor DNA derived "driver" and "drug-resistant" alleles that are present in the cfDNA could be widely applied for minimally invasive molecular testing in the therapeutic management of GISTs.
Wada N [124]	Japan	2016	Gastrointestinal stromal tumors (GISTs)	• Detection of secondary C-KIT mutations in ctDNA could be useful for the selection of targeted agents and prediction of antitumor effects.
PA Boonstra [125]	USA	2017	Gastrointestinal stromal tumors (GISTs)	• Mutation detection in cfDNA of GIST patients with metastatic disease is feasible, which may guide early treatment adaptations.
Marcia S Brose [137]	USA	2016	Thyroid Tumor	• Vemurafenib showed anti-tumor activity in patients with progressive, ctDNABRAFV 600E-positive papillary thyroid cancer refractory to radioactive iodine that had never been treated with a multi-kinase inhibitor
Elena Pereira [141]	USA	2015	Gynecologic Cancers	• ctDNA was an independent predictor of survival in patients with ovarian and endometrial cancers. Earlier recognition of disease persistence and/or recurrence and the ability to stratify into better and worse outcome groups through ctDNA surveillance may open the window for improved survival and quality and life in these cancers.

Conclusion

Both ctDNA quantity and genetic hallmarks of ctDNA can be taken into consideration for personalized cancer managements. There is a big hope that by utilizing of ctDNA mutation the problem of resistance to drug in some patients will be overcome specially in breast, lung and colorectal cancers.

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Authors' contributions Professor Seyed Mohammad Tavangar made substantial contributions to conception and design, supervision, acquisition of data, and interpretation of data. Mrs. Fatemeh Khatami had been involved in drafting the manuscript or revising it critically for important intellectual content.

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Compliance with ethical standards

Ethics approval and consent to participate This manuscript does not report on or involve the use of any animal or human data or tissue, so ethical approval is not applicable in this section.

Consent for publication This review article does not contain data from any individual person; consequently the consent for publication is "Not applicable" in this section.

Competing interests All authors declare that they have no competing interests" in this section.

Abbreviations *ATC,* Anaplastic Thyroid Cancer; *AI,* Aromatase inhibitor; *BC,* Breast Cancer; *CAPP-Seq,* Cancer Personalized Profiling by deep Sequencing; *CRC,* Colorectal cancers; *ctDNA,* Circulating Tumor DNA; *CTCs,* Circulating Tumor Cells; *ddPCR,* Droplet Digital PCR; *EGFR-TKIs,* Epidermal growth factor receptor tyrosine kinase inhibitors; *EGFR,* Epidermal growth factor receptor; *ESR1,* Estrogen receptor alpha; *FDA,* U.S. Food and Drug Administration; *HER2/neu,* Human epidermal growth factor receptor 2; *KRAS,* Kirsten Rat Sarcoma Viral Oncogene Homolog; *mCRC,* Metastatic colorectal cancer; *MRD,* Minimal residual disease; *MTC,* Medularlly Thyroid Cancer; *NSCLC,* Non-Small Cell Lung Cancer Research; *NGS,* Next-generation sequencing; *PFS,* Progression free survival; *PIK3C,* Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; *PI3K,* Phosphatidylinositol-4,5-bisphosphate 3-kinase; *PDGFRA,* Platelet-derived growth factor receptor alpha

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