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

Fatemeh Khatami¹ · Seyed Mohammad Tavangar¹,²

Received: 17 November 2017 / Accepted: 17 January 2018 / Published online: 26 March 2018
© Springer International Publishing AG 2018

Abstract
The heterogeneity of tumor is considered as a major difficulty to victorious personalized cancer medicine. There is an extremenedeed 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, clinicians are looking 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].
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 “ctDNA” or "circulating tumour DNA" or “ctDNA” or "cell free DNA" or “CTC” or "circulating tumor DNA" and “personalized medicine” or “Precision 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 ctDNA presence in the plasma was first accounted in 1948 by Mandel and Metais [29], it was just recent years that tumor-derived ctDNA was revealed that cancer patients had greater levels of plasma ctDNA 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 associated with 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 straighthrough thegreater levels of ctDNA [49].

Long before personalized 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 management approaches. 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-stage breast 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].
Several preceding 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, so according 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 (ESRI) 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 ESRI mutations can be strongly recognized with ctDNA analysis, and predict [63, 64]. ESRI 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 (PIK3CA) 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 project 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 theEGFR 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-Seq and 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 (GI) tract, 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 diseases has 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 ctDNA 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/PPARγ [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 confirmed superior 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 non-invasive 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 ctDNA to optimize finding of the molecular diversity of ovarian cancer [146].
| 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        | • 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. |
|                            |               |      | Ovarian Cancer           |                                                                                                                                       |
|                            |               |      | Colorectal Cancer        |                                                                                                                                       |
|                            |               |      | Bladder Cancer           |                                                                                                                                       |
|                            |               |      | Gastro esophageal        |                                                                                                                                       |
|                            |               |      | Breast Cancer            |                                                                                                                                       |
|                            |               |      | Melanoma                 |                                                                                                                                       |
|                            |               |      | Hepatocellular Carcinoma |                                                                                                                                       |
|                            |               |      | Head and neck Cancers    |                                                                                                                                       |
| 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            | • Exome-wide analysis of ctDNA could complement current invasive biopsy approaches to identify mutations associated with acquired drug resistance in advanced cancers |
|                            |               |      | Ovarian Cancer           |                                                                                                                                       |
|                            |               |      | Lung Cancer              |                                                                                                                                       |
| Nicholas C. Turner [62]    | United Kingdom| 2016 | Breast Cancer            | • Exome-wide analysis of ctDNA could complement current invasive biopsy approaches to identify mutations associated with acquired drug resistance in advanced cancers |
|                            |               |      | Lung Cancer              |                                                                                                                                       |
| Richard D. Baird [65]      | USA           | 2016 | Breast cancer            | • Exome-wide analysis of ctDNA could complement current invasive biopsy approaches to identify mutations associated with acquired drug resistance in advanced cancers |
| Cristofanilli M [147]     | United Kingdom| 2016 | Metastatic Breast cancer | • Circulating tumor DNA is an informative, inherently specific, and highly sensitive biomarker of metastatic breast cancer. |
| A Bosch [67]               | USA           | 2015 | 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. |
| Cloud P. Paweletz [87]    | USA           | 2016 | Lung Cancer              | • Increased ER transcriptional activity may be a reactive mechanism that limits the activity of PI3K inhibitors and that combined PDK and ER inhibition is a rational approach to target these tumors. |
| Snadar 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]         | USA           | 2016 | Lung Cancer              | • Exome-wide analysis of ctDNA could complement current invasive biopsy approaches to identify mutations associated with acquired drug resistance in advanced cancers |
| D.J. Merriott [91]        | USA           | 2017 | Lung cancer              | • Analysis of ctDNA potentially allows early identification of NSCLC patients who will have DCB from ICI-animals. |
| Spindler KL [111]         | Denmark       | 2012 | Metastatic Colorectal Cancer (mCRC) | • Analysis of ctDNA potentially allows early identification of NSCLC patients who will have DCB from ICI-animals. |

Table 1: The summary of studies related to ctDNA and personalized cancer management
Both ctDNA quantity and genetic hallmarks of ctDNA can be taken into consideration for personalized cancer management. 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.

Acknowledgements Special thanks to Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.

Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author.

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.

Funding This article was a part of a larger project which was granted by the National Institute for Medical Research Development (NIMAD, Grant number: 957222).

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; EGRF, Epidermal growth factor receptor; ESRI, 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, Medullary Thyroid Cancer; NSCLC, Non-Small Cell Lung Cancer Research; NGS, Next-generation sequencing; PFS, Progression free survival; P1K3C, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; P13K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; PDGFRA, Platelet-derived growth factor receptor alpha

References

1. Larijani B, Shirzad M, Mohagheghi M, Haghpanah V, Mosavi-Jarrah A, Tavangar S, et al. Epidemiologic analysis of the Tehran cancer institute data system registry (TCIDSR). Asian Pac J Cancer Prev. 2004;5(1):36–9.
26. Haghpanah V, Soliemanpour B, Heshmat R, Mosavi-Jarrah A, Tavangar S, Malekzadeh R, et al. Endocrine cancer in Iran: based on cancer registry system. Indian J Cancer. 2006;43(2):80–5.
3. Underhill HR, Kitzman JO, Hellwig S, Welker NC, Daza R, Baker DN, et al. Fragment length of circulating tumor DNA. PLoS Genet. 2016;12(7):e1006162.
4. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011;11(6):426–37.
5. Stroun M, Anker P. Nucleic acids spontaneously released by living frog auricles. Biochem J. 1972;128(3):100P.
6. Stroun M, Lyautey J, Lederrcy C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA: apoptosis and active DNA release. Clin Chim Acta. 2001;313(1):139–42.
7. Anker P, Stroun M, Maurice PA. Spontaneous release of DNA by human blood lymphocytes as shown in an in vitro system. Cancer Res. 1975;35(9):2375–82.
8. Rogers JC, Boldt D, Kornfeld S, Skinner SA, Valeri CR. Excretion of deoxyribonucleic acid by lymphocytes stimulated with phytohemagglutinin or antigen. Proc Natl Acad Sci. 1972;69(7):1685–9.
9. Diaz Jr LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014;32(6):579–86.
10. Hamakawa T, Kukita Y, Kurokawa Y, Miyazaki Y, Takahashi T, et al. Monitoring gastric cancer progression with circulating tumour DNA. Br J Cancer. 2015;112(2):352.
11. Tavangar SM, Larijani B, Mahta A, Hosseini SMA, Mehrazine M, Bandarian F. Craniopharyngioma: a clinicopathological study of 141 cases. Endocr Pathol. 2004;15(4):339–48.
12. Pantel K, Alix-Panabères C. Real-time liquid biopsy in cancer patients: fact or fiction? Cancer Res. 2013;73(21):6384–8.
13. Costs AA. Outcomes comparison of tissue and blood based biopsies for the purpose of biomarker testing. Value Health. 2016;19(3):A143–A4.
14. Crowley E, Di Nicolantonio F, Lopukis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol. 2013;10(8):472–84.
15. Fisher R, Pusztai L, Swanston C. Cancer heterogeneity: implications for targeted therapeutics. Br J Cancer. 2013;108(3):479.
16. X-x S, Yu Q. Intra-tumor heterogeneity of cancer cells and its implications for cancer treatment. Acta Pharmacol Sin. 2015;36(10):1219.
17. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2010;1805(1):105–17.
18. Murtaza M, Dawson S-J, Pogrebniak K, Rueda OM, Provenzano E, Grant J, et al. Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. Nat Commun. 2015;6:8760.
19. Malapelle U, de-Las-Casas CM, Rocco D, Garzon M, Pisapia P, Jordan-Ariza N, et al. Development of a gene panel for next-generation sequencing of clinically relevant mutations in cell-free DNA comprised an in vivo nucleosome footprint that informs its targetable regions. Oncotarget. 2015;6(10):71013.
20. Bennett CW, Berchem G, Kim YJ, El-Khoury V. Cell-free DNA. Next-generation sequencing in the service of personalized medicine for lung cancer. Oncotarget. 2016;7(43):71013.
21. Cani AK, Hovelson DH, Demirci H, Johnson MW, Tomlins SA, Rao RC. Next-generation sequencing of vitreoretinal lymphomas from small-volume intraocular liquid biopsies: new routes to targeted therapies. Oncotarget. 2017;8(5):7989–98.
22. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol. 2017;14(9):531–48.
23. Gerdes L, Iwobi A, Busch U, Pecoraro S. Optimization of digital droplet polymerase chain reaction for quantification of genetically modified organisms. Biomolecular detection and quantification. 2016;7:9–20.
24. Jovelet C, Madic J, Remon J, Honora B, Girard R, Rouleau E, et al. Crystal digital droplet PCR for detection and quantification of circulating EGFR sensitizing and resistance mutations in advanced non-small cell lung cancer. PLoS One. 2017;12(8):e0183319.
25. Schmitt MW, Kennedy SR, Salk JJ, Fox EJ, Hiatt JB, Loeb LA. Detection of ultra-rare mutations by next-generation sequencing. Proc Natl Acad Sci. 2012;109(3):14508–13.
26. Shu Y, Wu X, Tong X, Wang X, Chang Z, Mao Y, et al. Circulating tumor DNA mutation profiling by targeted next-generation sequencing provides guidance for personalized treatments in multiple Cancer types. Sci Rep. 2017;7:1:583. https://doi.org/10.1038/s41598-017-00520-1.
27. Bratman SV, Newman AM, Alizadeh AA, Diehn M. Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq. Taylor Francis; 2015.
28. Chaudhuri A, Lovejoy A, Chabon J, Newman A, Streh H, Say C, et al. CAPP-Seq circulating tumor DNA analysis for early detection of tumor progression after definitive radiation therapy for lung Cancer. International journal of radiation oncology biology. Physics. 2016;96(2):S41–52.
29. Mandel P. Les acides nucleiques du plasma sanguin chez l'homme. CR Acad Sci Paris. 1948;142:241–3.
30. Komatsu-tsubura KM, Sacher AG. Circulating Tumor DNA as a Liquid Biopsy: Current Clinical Applications and Future Directions. Oncology (Williston Park, NY). 2017;37(18).
31. Leon S, Shapiro B, Sklaroff D, Yaros M. Free DNA. In the serum of cancer patients and the effect of therapy. Cancer Res. 1977;37(3):646–50.
32. Stroun M, Anker P, Maurice PA, Lyautey J, Lederrcy C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology. 1989;46(5):318–22.
33. Jahr S, Hentze H, Engisch S, Hardt D, Fackelmoyer F, Hesch R-D, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 2001;61(4):1659–65.
34. Anker P, Stroun M, Maurice PA. Spontaneous extracellular synthesis of DNA released by human blood lymphocytes. Cancer Res. 1976;36(8):2832–9.
35. Dielh F, Li M, Dressman D, He Y, Shen D, Szabo S, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci U S A. 2005;102(45):16286–93.
36. Mouliere F, Rosenfeld N. Circulating tumor-derived DNA is shorter than somatic DNA in plasma. Proc Natl Acad Sci. 2015;112(11):3178–9.
37. Allis CD, Jenuwein T, Reinberg D. Epigenetics: CSHL Press; 2007.
38. Abbott DW, Ivanova VS, Wang X, Bonner WM, Ausió J. Characterization of the stability and folding of H2A. Z chromatin particles implications for transcriptional activation. J Biol Chem. 2001;276(45):41945–9.
39. Andersen J, Thaström A, Widom J. Spontaneous access of proteins to buried nucleosomal DNA target sites occurs via a mechanism that is distinct from nucleosome translocation. Mol Cell. 2002;22(20):7147–57.
40. Jansen A, Verstrenjen KJ. Nucleosome positioning in Saccharomyces cerevisiae. Microbiol Mol Biol Rev. 2011;75(2):301–20.
41. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. Cell. 2016;164(1):57–68.
42. genomics WDC. A nucleosome footprint reveals the source of cfDNA. Nat Rev Genet. 2016;17(3):125.
43. Ma X, Zhu L, Wu X, Bao H, Wang X, Chang Z, et al. Cell-free DNA provides a good representation of the tumor genome despite its biased fragmentation patterns. PLoS One. 2017;12(1):e0169231.

44. Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer—a survey. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2007;1775(1):181–232.

45. Yong E. Cancer biomarkers: written in blood. Nature. 2014;511:524–6.

46. Heidary M, Auer M, Ulz P, Heitzer E, Petru E, Gasch C, et al. The dynamic range of circulating tumor DNA in metastatic breast cancer. Breast Cancer Res. 2014;16(4):421.

47. Guo N, Lou F, Li J, Yang B, Chen W, et al. Circulating tumor DNA detection in lung cancer patients before and after surgery. Sci Rep. 2016;6

48. Wei Z, Wang W, Zitan Shu XZ, Zhang Y. Correlation between circulating tumor DNA levels and response to tyrosine kinase inhibitors (TKI) treatment in non-small cell lung cancer. Medical science monitor: international medical journal of experimental and clinical research. 2017;23:3627–34.

49. Sacher AG, Paweletz C, Dahlberg SE, Alden RS, O’Connell A, Feeney N, et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. JAMA oncology. 2016;2(8):1014–22.

50. af Härlström TM, Puhka M, Kallioniemi O. Circulating tumor DNA in early-stage breast cancer: personalized biomarkers for occult metastatic disease and risk of relapse? EMBO Molecular Medicine. 2015;7(8):994–5.

51. Khatami F, Aghayan HR, Sanaei M, Heshmat R, Tavangar SM, Larijani B. The potential of circulating tumor cells in personalized management of breast cancer: a systematic review. Acta Medica Iranica. 2017;55(3):175–93.

52. Olsson E, Winters C, George A, Chen Y, Howlin J, Tang MHE, et al. Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Molecular Medicine. 2015;7(8):1034–47.

53. Betegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early and late-stage human malignancies. Science translational medicine. 2014;6(224):224ra24-ra24.

54. Leary RJ, Kinde I, Diehl F, Schmidt K, Clouser C, Duncan C, et al. Development of personalized tumor biomarkers using massively parallel sequencing. Sci Transl Med 2010;2(20):20ra14-20ra14.

55. Dawson S-J, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin S, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med. 2013;368(13):1199–209.

56. Murtaza M, Dawson S-J, Tsui DW, Gale D, Forshew T, Piskorz AM, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature. 2013;497(7447):108.

57. Sidransky D. Nucleic acid-based methods for the detection of cancer. Science. 1997;278(5340):1054–8.

58. Heitzer E, Auer M, Ulz P, Geigl JB, Speicher MR. Circulating tumor cells and DNA as liquid biopsies. Genome medicine. 2013;5(8):73.

59. Heitzer E, Ulz P, Geigl JB. Circulating tumor DNA as a liquid biopsy for cancer. Clin Chem. 2015;61(1):112–23.

60. Mohammadi-asl I, Larijani B, Khorgami Z, Tavangar SM, Haghnpanah V, Khetrollab M, et al. Qualitative and quantitative promoter hypermethylation patterns of the P16, TSHR, RASSF1A and RARγ2 genes in papillary thyroid carcinoma. Med Oncol. 2011;28(4):1123–8.

61. Garcia-Murillas I, Beamey M, Epstein M, Howarth K, Lawson A, Hrebien S, et al. Abstract 2743: comparison of enhanced tagged-amplicon sequencing and digital PCR for circulating tumor DNA analysis in advanced breast cancer. Cancer Res. 2017;77(13 Supplement):2743.

62. Tzankou E, Markou A, Poliak E, Koysodontis G, Psyrrni A, Georgoulias V, et al. Abstract 1725: Detection of <em>ESR1</em>-D538G mutation in circulating tumor cells (CTCs) and paired circulating tumor DNA (ctDNA) samples of breast cancer patients. Cancer Research. 2017;77(13 Supplement):1725–

63. Schiavon G, Hrebien S, Garcia-Murillas I, Pearson A, Tarazona N, Lopez-Knowles E, et al. Abstract 926: ESR1 mutations evolve during the treatment of metastatic breast cancer, and detection in ctDNA predicts sensitivity to subsequent hormone therapy. Cancer Res. 2015;75(15 Supplement):926.

64. Turner NC, Jiang Y, O’Leary B, Hrebien S, Cristofanilli M, Andre F, et al. Efficacy of palbociclib plus fulvestrant (P+Pf) in patients (pts) with metastatic breast cancer (MBC) and ESR1 mutations (mus) in circulating tumor DNA (ctDNA). Journal of Clinical Oncology. 2016;34(15_suppl):512–

65. Baird RD, Rossum AV, Oliveira M, Beelen K, Garcia-Corbacho J, Mandjes IAM, et al. POSEIDON trial phase 1b results: Safety and preliminary efficacy of the isoform selective PI3K inhibitor taselisib (GDC-0032) combined with ta-moxifen in hormone receptor (HR) positive, HER2-negative metastatic breast cancer (MBC) patients (pts) - including reponsence monitoring by plasma circulating tumor (ct) DNA. Journal of Clinical Oncology. 2016;34(15_suppl):2520–

66. Baselga J, Im S-A, Iwata H, Clemons M, Ito Y, Awada A, et al. Abstract S6–01: <em>PIK3CA</em>-status in circulating tumor DNA (ctDNA) predicts efficacy of buparlisib (BUP) plus fulvestrant (FULV) in postmenopausal women with endocrine-resistant HR+/HER2− advanced breast cancer (BC): First results from the randomized, phase III BELLE-2 trial. Cancer Res 2016;76(4 supplement):S6–01–S6–

67. Bosch A, Li Z, Bergamaschi A, Ellis H, Toska E, Prat A, et al. PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor–positive breast cancer. Science translational medicine. 2015;7(283):283ra51-ra51.

68. Oshiro C, Kagara N, Naoyi Y, Shimoda M, Shimomura A, Maruyama N, et al. PIK3CA mutations in serum DNA are predictive of recurrence in primary breast cancer patients. Breast Cancer Res Treat. 2015;150(2):299–307.

69. Garcia-Murillas I, Schiavon G, Weigelt B, Ng C, Hrebien S, Cutts RJ, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015;7(302):302ra133-302ra133.

70. Ma C, Bose R, Gao F, Freedman R, Telli M, Kimmick G, et al. Abstract CT011: Circulating tumor DNA (ctDNA) sequencing for <em>HER2</em> screening and response monitoring to neratinib in metastatic breast cancer (MBC). Cancer Res 2017;77(13 Supplement):CT011-CT.

71. Qiu M, Wang J, Xu Y, Ding X, Li M, Jiang F, et al. Circulating tumor DNA is effective for the detection of EGFR mutation in non–small cell lung cancer: a meta-analysis. Cancer Epidemiology Biomarkers &amp; Prevention. 2014.

72. Goto T, Hirotu Y, Amemiya K, Nakagomi T, Shikata D, Yokoyama Y, et al. Distribution of circulating tumor DNA in lung cancer: analysis of the primary lung and bone marrow along with the pulmonary venous and peripheral blood. Oncotarget. 2017;8(35):59268–81.

73. Hirsch FR, Varella-Garcia M, Bunn Jr PA, Franklin WA, Dzidziuszko R, Thatcher N, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non–small-cell lung cancer. J Clin Oncol. 2006;24(31):5034–42.
reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun. 2016;7:11815.

91. Merriott DJ, Chaudhuri AA, Jin M, Chabon JJ, Newman A, Stehr H, et al. circulating tumor dna quantitation for early response assessment of immune checkpoint inhibitors for lung cancer. Int J Radiat Oncol Biol Phys. 99(2):S20–S1.

92. Khatami F, Larjani B, Heshmat R, Keshkhar A, Mohammadmola M, Teimoori-Toobabi L, et al. Meta-analysis of promoter methylation in eight tumor-suppressor genes and its association with the risk of thyroid cancer. PLoS One. 2017;12(9):e0184892.

93. Cai X, Gao Y, Shen H, Laird P, Fan, J-B, Xu W, et al. Non-invasive diagnosis of early-stage lung cancer via targeted high-throughput DNA methylation sequencing of circulating tumor DNA (ctDNA). AACR; 2017.

94. Pantel K, Alix-Panabieres C. Liquid biopsy in 2016: circulating tumour cells and cell-free DNA in gastrointestinal cancer. Nat Rev Gastroenterol Hepatol. 2017;14(2):73–4.

95. Yan W, Zhang A, Powell MJ. Genetic alteration and mutation profiling of circulating cell-free tumor DNA (ctDNA) for diagnosis and targeted therapy of gastrointestinal stromal tumors. Chinese journal of cancer. 2016;35(1):68.

96. Sotoudeh M, Derakhshian MH, Abedi-Ardakani B, Nouraei M, Yazdanbod A, Tavangar SM, et al. Critical role of helicobacter pylori in the pattern of gastritis and carditis in residents of an area with high prevalence of gastric cardia cancer. Dig Dis Sci. 2008;53(1):27–33.

97. Malekzadeh R, Sotoudeh M, Derakhshan M, Mikaely J, Yazdanbod A, Merat S, et al. Prevalence of gastric precancerous lesions in Ardabil, a high incidence province for gastric adenocarcinoma in the northwest of Iran. J Clin Pathol. 2004;57(1):37–42.

98. Szpechcinski A, Chorostowska-Wynimko J, Struniawski R, Kupis W, Rudzinski P, Langfort R, et al. Cell-free DNA levels in plasma of patients with non-small-cell lung cancer and inflammatory lung disease. Br J Cancer. 2015;113(3):476.

99. Shapiro B, Chakrabarty M, Cohn EM, Leon SA. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer. 1983;51(11):2116–20.

100. De Mattos-Arruda L, Olmos D, Tabernero J. Prognostic and predictive roles for circulating biomarkers in gastrointestinal cancer. Future Oncol. 2011;7(12):1385–97.

101. Howell JA, Khan SA, Knapp S, Thrurz MR, Sharma R. The clinical role of circulating free tumor DNA in gastrointestinal malignancy. Transl Res. 2017;183(Supplement C):137–54.

102. Nasers-Moghadam S, Malekzadeh R, Sotoudeh M, Tavangar M, Azimi K, Sohrabpour S, et al. Lower esophagus in dyspeptic Iranian patients: a prospective study. J Gastroenterol Hepatol. 2003;18(3):315–21.

103. Schwarzenbach H, Stoehlmacher J, Pantel K, Goekkurt E. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. Ann N Y Acad Sci. 2008;1137(1):190–6.

104. Boni L, Cassinotti E, Canziani M, Dionigi G, Rovera F, Dionigi R. Free circulating DNA as possible tumour marker in colorectal cancer. Surg Oncol. 2007;16:29–31.

105. Frattini M, Gallino G, Signoroni S, Balestra D, Battaglia L, Sozzi G, et al. Quantitative analysis of plasma DNA in colorectal cancer patients. Ann N Y Acad Sci. 2006;1075(1):185–90.

106. Frattini M, Gallino G, Signoroni S, Balestra D, Lusa L, Battaglia L, et al. Qualitative and quantitative characterization of plasma DNA identifies primary and recurrent colorectal cancer. Cancer Lett. 2008;263(2):170–81.

107. Danese E, Montagnana M, Minicozzi AM, De Matteis G, Scudo G, Salvagno GL, et al. Real-time polymerase chain reaction quantification of free DNA in serum of patients with polyps and colorectal cancers. Clin Chem Lab Med. 2010;48(11):1665–8.

108. Hedtke M, Haselmann V, Brechtl V, Duda A, Neumaier M. Use of liquid profiling/liquid biopsy to detect Ras mutations in ctDNA of...
patients with metastatic colorectal cancer (mCRC). Clinical Chemistry and Laboratory Medicine. 2016;54(10):e441.

109. Tavangar SM, Sharifizad A, Soroush AR. Her-2/neu overexpression correlates with more advanced disease in Iranian colorectal cancer patients. Med Sci Monit. 2005;11(3):CR123–CR6.

110. Pereira AA, Morelli MP, Overman M, Kee B, Fogelman D, Vilar E, et al. Clinical utility of circulating cell-free DNA in advanced colorectal cancer. PLoS One. 2017;12(8):e0183949.

111. Spinelli K-LG, Pallisgaard N, Vogelius I, Jakobsen A. Quantitative cell free DNA. KRAS and Braf mutations in plasma from patients with metastatic colorectal cancer during treatment with cetuximab and irinotecan. Clinical Cancer Research. 2012;clinCan.rrs.2011;0564.

112. Reintert T, Schüler LV, Thomsen R, Tobiasen H, Vang S, Nordenstöfl I, et al. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. Gut. 2016;65(4):625–34.

113. Lipson EJ, Veleculescu VE, Pritchard TS, Sause M, Pardoll DM, Topalian SL, et al. Circulating tumour DNA analysis as a real-time monitoring tool in melanoma patients undergoing treatment with immune checkpoint blockade. Journal for immunotherapy of cancer. 2014;2(1):42.

114. Chen W, Zheng R, Zhang S, Zhao P, Li G, Wu L, et al. The incidences and mortalities of major cancers in China, 2009. Chinese journal of cancer. 2013;32(3):106.

115. Bass AJ, Thorsson V, Shmulevich I, Reynolds SM, Miller M, Bernard B, et al. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202.

116. Zhang W. TCGA divides gastric cancer into four molecular subtypes: implications for individualized therapeutics. Chinese journal of gastroenterology. 2014;33(10):469.

117. Nishida T, Biological HS. Clinical review of stromal tumors in the gastrointestinal stromal tumors: origin and molecular oncology. Nat Rev Cancer. 2011;11(12):865.

118. Nannini M, Astolfi A, Urbini A, Santini D, Jardin F, van Nhieu JT, et al. Copy-neutral loss of heterozygosity and chromosome gains and losses are frequent in gastrointestinal stromal tumours: an integrative analysis of gene expression profiling and high-resolution genomic copy number. Lab Invest. 2010;90(9):1285–94.

119. Nannini M, Astolfi A, Urbini M, Indio V, Santini D, Heinrich MC, et al. Integrated genomic study of quadruple-WT GIST (KIT/PDGFRα/SDH/RAS pathway wild-type GIST). BMC Cancer. 2014;14(1):685.

120. Gronchi A. Risk stratification models and mutational analysis: keys to optimising adjuvant therapy in patients with gastrointestinal stromal tumour. Eur J Cancer. 2013;49(4):884–92.

121. Şengur MA, Özdemir NY, Akinci MB, Ucuncu D, Zengin N, Aksoy S. Is exon mutation analysis needed for adjuvant treatment of gastrointestinal stromal tumor? World J Gastroenterol: WJG. 2013;19(1):144–6.

122. Wada N, Kurokawa Y, Takahashi T, Hamakawa T, Hirota S, Naka T, et al. Detecting secondary C-KIT mutations in the peripheral blood of patients with imatinib-resistant gastrointestinal stromal tumour. Oncology. 2016;90(2):112–7.

123. Boonstra PA, At E, Tibbesma M, Mathijsen RH, Arafat F, Fy C, et al. Abstract 4951: dynamics of KIT exon 11 mutations in cell free plasma DNA of patients treated for advanced gastrointestinal stromal tumours: results from the Dutch GIST bio-databank. Cancer Res. 2017;77(13 Supplement):4951.
144. Harris FR, Kovtun IV, Smadbeck J, Multinu F, Jatoi A, Kosari F, et al. Quantification of somatic chromosomal rearrangements in circulating cell-free DNA from ovarian cancers. Sci Rep. 2016;6:29831.

145. Sarmadi S, Izadi-Mood N, Sotoudeh K, Tavangar SM. Altered PTEN expression; a diagnostic marker for differentiating normal, hyperplastic and neoplastic endometrium. Diagn Pathol. 2009;4(1):41.

146. Arend RC, Londono AI, Alvarez RD, Huh WK, Bevis KS, Leath CA, et al., editors. Circulating cell-free DNA: The future of personalized medicine in ovarian cancer management. Journal of Clinical Oncology; 2016: AMER SOC CLINICAL ONCOLOGY 2318 MILL ROAD, STE 800, ALEXANDRIA, VA 22314 USA.

147. Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. The Lancet Oncology. 2016;17(4):425–39.