Mutation Research-Reviews in Mutation Research 781 (2019) 100–129

Contents lists available at ScienceDirect

Mutation Research-Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/mutrev

Review

Diagnostic and prognostic impact of cell-free DNA in human cancers: Systematic review

Klara Cervena[a,b,*], Pavel Vodicka[a,b,c], Veronika Vymetalkova[a,b,c]

[a] Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Videnska 1083, 14220 Prague, Czech Republic
[b] Institute of Biology and Medical Genetics, First Faculty of Medicine, Albertov 4, Charles University, 12800 Prague, Czech Republic
[c] Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Alej Svobody 76, 32300 Pilsen, Czech Republic

ARTICLE INFO

Keywords:
Cell-free DNA
Circulating-tumor DNA
Liquid biopsy
Cancer
Biomarker

ABSTRACT

The development of minimally invasive and low-cost assay enabling early diagnosis, treatment response and prognosis in cancer patients may provide a promising alternative to tumor biopsy. Circulating cell-free DNA (cfDNA) is probably the most promising tool among all components of liquid biopsy. This review includes studies exploring cfDNA as the diagnostic, prognostic or predictive biomarker for all types of cancer. In this article, we systematically reviewed the relevant literature from PubMed about cfDNA. All articles presented higher cfDNA concentration in cancer patients when compared with patients with benign disease or healthy individuals. Most of the articles showed a connection between cfDNA and prognosis. The presence of high cfDNA level in serum or plasma was associated with worse overall patient's survival. This review supports the idea that the cfDNA analysis represents a promising research area and hopefully in the future, could be applied as a new biomarker for cancer detection, prognosis determination and prediction of the response to therapy.

1. Introduction

New non-invasive approaches that can supplement and improve on current strategies for cancer screening and management are urgently needed. A growing number of publications have given an evidence that components of tumors, which are shed into the circulation, can be detected in the form of liquid biopsies and can be used to detect cancer at early stages, to predict response to certain therapies and to detect cancer recurrence in a minimally invasive way. The analysis of circulating tumor DNA (ctDNA) in blood and other body fluids, has a great potential to improve different aspects of cancer management [1–3]. The cfDNA has potential to become "liquid biopsy". Liquid biopsy strives to analyze cfDNA or circulating tumor cells (CTCs) from the blood or other body fluid of cancer patients and provide relevant information about the tumor. Several studies showed that several pathologies may be associated with increased levels of cfDNA, like inflammation, tissue trauma, autoimmune diseases or cancer [4,5]. Regarding cancer and cfDNA, a number of studies confirmed that cancer patients have higher levels of cfDNA than patients with benign diseases or healthy individuals [6–9].

1.1. History

For the first time, cfDNA was discovered in the blood of healthy individuals by Mandel and Métais [10]. The first association between cfDNA and cancer was reported in 1977 by Leon and Shapiro in the serum of patients with various types of cancer, who had a higher level of cfDNA in comparison to healthy individuals. Patients with metastases displayed a higher level of cfDNA than those without. Authors noted that after radiation therapy cfDNA levels decreased while constant or increased levels of cfDNA were associated with worse survival or tumor recurrence. Authors hypothesized that cfDNA in the serum could enable evaluation of therapy response [11]. Following studies focused on the ability of cfDNA to characterize neoplastic features of tumor. Tumor-specific variations, like mutations in oncogenes and tumor suppressor genes [12], microsatellite instability (MSI) [13], DNA methylation [14], were identified and confirmed that cfDNA is released into the circulation from tumors.

1.2. Biological aspects

CfDNA occurs in serum, plasma and other body fluids like urine or saliva [15,16] (Fig. 1) but the mechanism of its release into the

* Corresponding author at: Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Videnska 1083, 14220 Prague, Czech Republic.
E-mail address: klara.cervena@iem.cas.cz (K. Cervena).

https://doi.org/10.1016/j.mrrev.2019.05.002
Received 15 February 2019; Received in revised form 3 May 2019; Accepted 7 May 2019
Available online 11 May 2019
1383-5742/

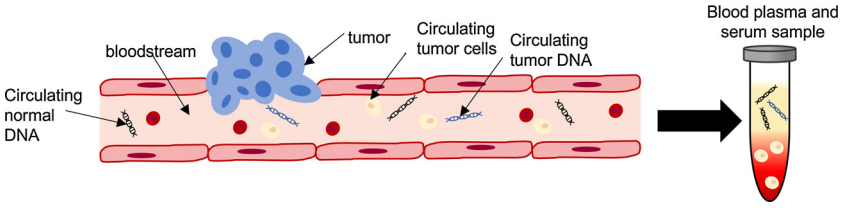

Fig. 1. Schema depicting principle of liquid biopsy.

bloodstream is not completely understood. In general, cfDNA may be derived from a primary tumor, metastatic lesions or CTCs [17]. There could be two scenarios of release – passive and active. The passive way is represented by necrotic and apoptotic cells, whereas active secretion can be mediated by nucleated cells such as lymphocytes. In general, the major sources of cfDNA are apoptotic and necrotic cells [18,19].

CfDNA is typically fragmented to 160–180 bp fragments, corresponding to nucleosome-protected DNA observed in apoptotic cells, whereas tumor cells undergo many different processes, like necrosis or autophagy, and they can release cfDNA fragments of different sizes cfDNA [18,20–22].

1.3. Technical aspects

According to the majority of the quantitative studies performed until the present, cfDNA is observed in healthy individuals at concentrations between 0 and 100 ng/ml of blood with an average of 30 ng/ml, whereas in cancer patients the concentration in plasma or in serum varies between 0 and 1000 ng/ml, with an average of 180 ng/ml [11,23,24].

Several studies aimed to compare the cfDNA levels in paired plasma and serum samples and observed significantly higher cfDNA concentrations in serum [25–27]. The higher serum cfDNA concentration is due to the clotting process of white blood cells in the collection tube leading to their lysis. Therefore, serum cfDNA is contaminated by germline DNA released from the lysed blood cells and ctDNA is diluted. The use of plasma for the isolation and analysis of cfDNA has several advantages over serum, such as significantly higher cfDNA concentrations; however, it also has a few disadvantages which decrease its utility. For example, red blood cell (RBC) lysis (hemolysis) can occur during improper specimen collection (storage for longer periods of time, > 6 h) or mechanical processing of blood which causes rupturing of RBCs and release of their contents (including DNA) into the surrounding fluid [28–31]. This increase in DNA contamination from nucleated cells can complicate the analysis of cfDNA and test results are less accurate. While some studies support the use of serum over plasma, many reports state that cfDNA analysis from the plasma fraction of blood drawn is preferred over serum [32,33].

1.4. Analytical methods for detecting CFDNA

The era of personalized cancer management has started emphasize a focus of the research on choosing targeted procedures based on patients' genetic profile to achieve the optimization of their clinical benefits. Personalized medicine requires comprehensive and precise knowledge of patient's genetic profile [34]. The "omics" sciences offer several high-throughput technologies for assaying biomarkers in body fluids, cells and tissues. These technologies are constantly being improved upon specific detection and measurements of low-abundant targets. The development of methods based on Next-Generation sequencing (NGS) represented substantial progress in revealing the cancer genome characteristics and thus facilitating personalized cancer patients' therapy thanks to its precision, sensitivity and high-throughput [35]. Since ctDNA reflects the entire tumor genome, it has become attractive because of its potential clinical utility as 'liquid biopsies'. Although the proportion of ctDNA in cell-free DNA (cfDNA) fraction is lower than 0.01% [36], it is possible to detect ctDNA even in asymptotic individuals with the use of highly sensitive, specific and reliable detection methods. CtDNA detection analysis covers from single mutations up to the entire genome. Methodological approaches for the ctDNA detection can be divided into two major types: 1) targeted methods to assay a few hot spot mutations with high sensitivity, such as hot spot mutations in *KRAS, BRAF* and *EGFR* genes, and 2) untargeted methods with high costs, which allow simultaneous sequencing of millions of DNA fragments without prior sequence information and require the quantity of ctDNA to be around 5%–10% of the total DNA fraction.

Table 1 summarized different methods for ctDNA detection supplemented with their main advantages and limitations.

Initially, the detection of a specific mutations in ctDNA relied upon real-time allele-specific PCR (RT-PCR) method [37]. Due to the limited sensitivity (0.1–1%) and specificity of RT-PCR approaches, patients in advanced stages of the disease, and thus with higher ctDNA levels in the plasma, were analyzed predominantly [38,39]. These limitations of RT-PCR have been overcome by recent development and optimization of digital PCR (dPCR) that is extensively used in biomarker analysis in body fluids [37]. In liquid biopsy samples, dPCR is capable to detect mutant alleles with a fractional abundance from 0.005 to 0.1% [40–42]. dPCR possess numerous advantages, such as the quantification of low copy number targets, detection and quantification of rare cancer mutations with no need for reference standards, detection of small fold changes, increased resistance to PCR inhibitors, and finally, it enables the detection of multiple mutations (multiplexing) from the same sample [43–45]. The main disadvantage of dPCR is the limited capacity to investigate different genomic alterations simultaneously [46]. Thus, without prior precise knowledge of the DNA rearrangements, translocations and/or mutations of interest, dPCR utility to detect cancer process in the human body and monitor tumor growth is limited [47].

During the last few years, the analytical sensitivity to detect genetic alterations in ctDNA has been significantly improved by the implementation of new high-throughput technologies. These include Beads-Emulsion-Amplification and Magnetics (BEAMing [48], Amplification Refractory Mutations System (ARMS, Spindler et al. [49], Surface-enhanced Raman scattering (SERS [50], and High-throughput multiplex ultrasensitive mutation detection (UltraSEEK [51],). The main disadvantage of PCR-based approaches is, however, the limited capacity to investigate larger numbers and different kinds of genomic alterations [47].

Massive parallel sequencing (MSP), also known as NGS, does not require prior knowledge of particular/specific genetic changes in the primary tumor. This method can detect unknown mutations in a specific gene or can be used for broader genomic analysis. NGS was recently applied for molecular characterization of cfDNA in cancer, and importantly, its clinical utility was also described in large clinical cohorts [52–56]. These untargeted approaches, although less analytically sensitive, represent important part in the exploration of tumor biology. Since plenty of genes and patients can be analyzed at the same time, it allows the management of the intra-tumor heterogeneity and tumor evolution in time. NGS approaches include Tagged-amplicon deep sequencing (TAm-Seq [57], Cancer Personalized Profiling by deep Sequencing (CAPP-Seq [58], Safe-sequencing system (Safe-SeqS, [52], Circulating Single-Molecule Amplification and Resequencing Technology (cSMART [59]).

The various modifications of NGS provide partial or complete

Table 1
A summary of ctDNA detection techniques.
(adopted from [19,246]).

Technique	Limit of detection	Type of detectable alteration	Advantages	Disadvantages
PCR-based approaches (RT-PCR, COLD-PCR, PNAs – LNA, ARMS, etc.)	0.1–1%	SNV, indels	Low cost Easy to perform	Low sensitivity Limited number of studied genes at a time Genes need to be pre-determined
Digital PCR-based ddPCR and BEAMing	0.05% or less	SNV, indels, CNV	High sensitivity and specificity Reasonable cost Easy to perform	Limited number of studied genes at a time Genes need to be pre-determined
NGS-based Deep sequencing, TAm-seq, Safe-SeqS, CAPP-Seq, cSMART, digital sequencing	0.01–2%	SNV, indels, CNV, rearrangements	Possibility to analyze more genes at a time	Wide range of sensitivity depending on NGS platform used (PCR amplicon strategies are more sensitive and less expensive than whole genome or exome sequencing) Higher cost
SERS-nanotags	0.01%	SNV	Reduced susceptibility to photobleaching Bandwidths are significantly narrower	Raman signal deterioration upon prolonged laser illumination
UltraSEEK	0.1%	SNV, indels	Low cost and low DNA input	Lower sensitivity

PCR – Polymerase chain reaction, RT-PCR – Real-time allele-specific PCR, COLD-PCR – Co-amplification at lower denaturation temperature-PCR, PNAs-LNA – Peptide nucleic acid-locked nucleic acid, ARMS – Amplification Refractory Mutations System, ddPCR – droplet digital PCR, BEAMing – Beads-Emulsion-Amplification and Magnetics, NGS - Next-Generation sequencing, TAm-Seq – Tagged-amplicon deep sequencing, Safe-SeqS – Safe-sequencing system, CAPP-Seq – CAncer Personalized Profiling by deep Sequencing, cSMART – Circulating single molecule amplification and re-sequencing technology, SERS – Surface-enhanced raman spectroscopy, UltraSEEK – High-throughput multiplex ultrasensitive mutation detection, SNV – Single nucleotide variation, CNV – Copy number variation.

information of genomic aberrations and are applicable to either the entire genome (whole genome sequencing - WGS), protein-coding genes (whole exome sequencing - WES), or custom panels. Among the main advantages of NGS approaches belongs the ability to detect the presence of mutations (both somatic and germline), copy number variants (CVNs), and other structural chromosomal variants, including translocations, transversions and inversions (Heitzer, Ulz, et al. 2013; Leary et al. 2012). Both WGS and WES require high costs, large sample amount and bioinformatics assistance. The high tumor heterogeneity results in many gene/genetic variants presented at low frequencies. Their identification requires a high read depth often not achieved by WGS due to the high cost of generating sequence. Depth of coverage is relevant for the detection of low-abundance mutations and important for liquid biopsy analysis. Custom panels tend to be cheaper and fast to run. However, they are limited in scope as they focus only on regions of interest and are thus unable to reveal all genetic alterations in the tumor. On the other hand, custom panels require higher sequencing depth than WGS or WES approaches. Many tumors harbor multiple chromosomal alterations that are unlikely presented in non-malignant cells. Leary et al. designed a WGS-based method called Personalized Analysis of Rearranged Ends (PARE) in order to identify translocations in solid tumors with application to plasma ctDNA [60]. Indeed, they detected several chromosomal copy number changes or rearrangements. Similarly, Comparative Genomic Hybridization array (aCGH) enabled the identification of tumor-specific copy number alterations in plasma cfDNA of cancer patients [62].

Dynamic changes in the levels of methylated DNA may be used to monitor treatment response, disease relapse, or minimal residual disease. There are also several methods for examining the methylation patterns in cfDNA. These include Methylation-specific PCR (MSP), Methylation CpG-island Amplification (MCA), Amplification of Intermethylated Sites (AIMS), Restriction landmark genomic scanning (RLGS), Bisulfite sequencing, Methylation-sensitive restriction endonuclease PCR (MSRE-PCR), Quantitative MSP (MethyLight), and High-Performance Liquid Chromatography HPLC. Various studies of methylation alterations in cfDNA have shown their high specificity (mostly higher than 90%) for various cancer types [63–65]. However, their sensitivity has been poor with small improvements in custom studies. For example, the sensitivity of MethyLight is 1 methylated copy of ctDNA in 10,000 non-methylated in comparison to 1 in 1000 for traditional MSP. Pyrosequencing, sequencing-by-synthesis system, has low analytical sensitivity around 5% which is unsuitable for liquid biopsy analysis [66].

The ratio of longer to shorter DNA fragments is known as cfDNA integrity number and is determined as the ratio of two qRT-PCR products of different lengths. Umetani et al. developed the method to measure the cfDNA integrity by qRT-PCR for ALU (the name ALU comes from the restriction endonuclease Alu I that cleaves it) repeats (247 bp ALU vs 115 bp ALU) [67]. The reason for choosing ALU sequences is that ALU repeats are the most abundant repeated sequences in the human genome [68]. However, Cheng and Madhavan used a different primer set for ALU sequences (260/111) and introduced LINE-1 (266/197) as another DNA repetitive element target [21,69].

2. Material and methods

In this systematic review, we summarized the latest findings on cfDNA in many cancers and showed the possibility of using cfDNA as a cancer biomarker. We searched the Pubmed database to obtain relevant literature from January 2018 to December 2018. The identified articles are from 1977 to 2018. The retrieving query formulation used for the search was a combination of keywords “cfDNA” or “ctDNA “or “liquid biopsy “with specific cancers like “breast cancer “or “colorectal cancer “or “ovarian cancer “etc. The language of the articles was limited to English. First, titles and abstracts were carefully screened for relevance and duplicates were removed. Second, full text was retrieved and

Table 2
– Overview of the studies investigating cfDNA in relation with leukemia's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[70]	10	–	UK	plasma	mutation	qPCR	*N-RAS*	diagnostic
[80]	45	–	USA	plasma	LOH	sequencing	*5q* *7q* *8* *17p* *20q*	diagnostic
[79]	25	–	Germany	serum	concentration	spectrophotometry		Diagnostic prognostic
[76]	142	41	Italy	plasma	concentration	qPCR	*β-globin*	diagnostic
[71]	20	20	Egypt	serum	Mutation concentration	qPCR	*TP53*	diagnostic
[78]	21	12	Germany	plasma	concentration	qPCR	*β-actin*	diagnostic
[247]	60	30	China	plasma	concentration DNA integrity	qPCR	*ACTB*	prognostic
[248]	49	61	UK	serum	concentration	ELISA		prognostic
[249]	5	–	Japan	plasma	methylation	pyrosequencing	*LINE-1*	diagnostic
[250]	66	100	China	plasma	concentration	qPCR		diagnostic
[77]	201	15	Italy	plasma	concentration	qPCR	*POLR2*	prognostic
[72]	100	–	China	plasma	mutation	qPCR	*NPM*	diagnostic
[251]	88	–	France	plasma	mutation	Ion torrent personal genome machine sequencing	*XPO1* *EZH2* *MYD88*	diagnostic
[73]	1	–	USA	cerebrospinal fluid	mutation	Droplet digital PCR	*MYD88*	diagnostic
[74]	45	–	Netherlands	cerebrospinal fluid plasma	mutation	Droplet digital PCR	*MYD88*	diagnostic
[75]	25	–	France	plasma	mutation	Ion torrent personal genome machine sequencing	*MYD88*	diagnostic

qPCR – quantitative PCR, ELISA – Enzyme-Linked ImmunoSorbent Assay.

assessed for inclusion into analysis. No limitations were applied in the search. The following data were extracted from studies in structured forms: number of patients, number of controls, country, source of cfDNA, type of abnormalities, methodology, targets, and clinical relevance. All the reference lists of the identified articles and relevant reviews were also manually screened.

3. Results

3.1. Hematological malignancies

There are only a few studies focused on cfDNA analysis in hematologic malignancies; the oldest originates from 1994, where the presence of *N-Ras* mutations (in codon 12 a 13) in blood cells, plasma and bone marrow (BM) of patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) was examined. The different *N-Ras* mutations were always present in the plasma cfDNA, while in the DNA of blood cells or BM were sometimes absent. This study suggested a high potential of plasma cfDNA for detection of myeloid disorders [70] (Table 2). Further, *TP53* (G249 T, G249A, T176C, C250 T and T238 G) and nucleophosmin mut.A (a duplication of the TCTG at positions 956–959) mutations were detected in cfDNA from patients with non-Hodgkin lymphoma (NHL) and AML [71,72].

Diagnosis of lymphomas in the central nervous system (CNS) is still complicated due to a risk of several complications in obtaining the brain biopsy. Markers in blood and cerebrospinal fluid could represent a potentially new tools for early diagnosis of lymphomas. In the study of Zorofchian et al., authors examined one patient with suspected CNS lymphoma for the possibility to detect *MYD88* mutation (L265 P and V217 F) in the cerebrospinal fluid (CSF). Authors detected *MYD88* L265 P mutation in both tumor tissue and cfDNA from CSF. Interestingly, both the tumor tissue and CSF cfDNA were negative for *MYD88* V217 F mutation. Zorofchian et al. thus concluded that analysis of CFS cfDNA could represent a minimally invasive diagnostic tool for CNS lymphomas [73]. The potential to detect *MYD88* mutation in CSF and as well as in plasma was proved by another studies [74,75].

Patients with lymphoma displayed higher levels of cfDNA in their plasma than healthy volunteers. Hohaus et al. observed the differences in cfDNA levels between the patients with diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL) and Hodgkin's lymphoma (HL), however these differences were not observed in patients with follicular lymphoma (FL). In addition, patients with > 60 years and with higher cancer stages had higher plasma cfDNA concentration [76]. Furthermore, enhanced cfDNA levels were also observed in children; cancer patients had higher plasma cfDNA levels [77,78].

Nucleosomal DNA (ncDNA), another source of cfDNA, could represent a valuable diagnostic for AML patients as changes of ncDNA correspond with a satisfactory response to the treatment [79].

The plasma may be used for the study of genomic abnormalities and could alternatively replace BM cells as a diagnostic marker in the future. Rogers et al. compared chromosomal abnormalities, like LOH and X-chromosome inactivation in BM cells and plasma cfDNA. Authors detected LOH in the plasma cfDNA in all patients with cytogenetically documented chromosomal abnormalities: BM cells showed LOH in 98% MDS patients and 70% AML patients. The authors suggested plasma as a stable source of tumor DNA for testing of genomic abnormalities and proved that plasma is enriched by tumor DNA [80].

In conclusion, these studies showed the cfDNA potential for the detection of mutation and chromosomal abnormalities. The level of plasma cfDNA might represent an important non-invasive diagnostic tool for patients with leukemia.

3.2. Solid tumors

3.2.1. Gastrointestinal malignancies

3.2.1.1. Hepatocellular carcinoma. Only few tumor biomarkers in hepatocellular carcinoma (HCC) diagnosis reached clinical practice, such as α-fetoprotein or des-gamma-carboxy prothrombin (DCP). However, these biomarkers lack sufficient sensitivity and specificity and often provide often false-positive results [81]. Therefore, there is currently a significant effort to detect biomarkers that can aid in HCC diagnosis.

Most studies related to the analysis of cfDNA and HCC were focused on its plasma concentration measurement in patients with benign liver

Table 3
Overview of the studies investigating cfDNA in relation with HCC's clinical outcome.

References	Patients	Controls	Origin of study	Source of cfDNA	Study focus	Methodology	Target	Clinicalrelevance
[91]	22 HCC 38 nHCC	10	Hong Kong	plasma serum	methylation	MS-PCR	*CDKN2A*	prognostic
[85]	25 HCC 35 nHCC	20	Hong Kong	plasma serum	methylation	MS-PCR	*p15*	diagnostic prognostic
[92]	29 HCC 15 nHCC	20	Hong Kong	plasma serum	methylation	MS-PCR	*CDKN2A*	diagnostic prognostic
[252]	186 HCC 98 nHCC	348	Gambia	plasma	mutation concentration	qPCR	*TP53*	diagnostic
[88]	40	10	Hong Kong	plasma	methylation	MS-PCR	*RASSF1A*	diagnostic prognostic
[84]	52 HCC 30 HCV patients	18	Japan	serum	concentration	qPCR	*β-globin* *GSTP1*	diagnostic
[87]	79 HCC 20 nHCC	20	China	plasma	concentration	UV transillumination		prognostic diagnostic
[253]	26 HCC 8 nHCC	12	China	serum	methylation	MS-PCR	*GSTP1*	diagnosis prognostic
[93]	85 HCC 73 nHCC	30	Thailand	serum	methylation	MS-PCR	*LINE-1*	prognostic
[254]	96 HCC	100	Japan	serum	concentration	qPCR	*GSTP1*	predictive diagnostic
[255]	50	50	Taiwan	serum	methylation	MS-PCR	*CDKN2A* *p15* *RASSF1A*	diagnostic
[89]	63 HCC 63 nHCC	50	Hong Kong	serum	methylation	MS-PCR	*RASSF1A*	diagnostic prognostic
[82]	76 HCC 110 nHCC	69	Egypt	serum	concentration mutation	qPCR	*TP53* *CTNNB1*	diagnostic prognostic
[256]	25 HCC 25 nHCC	15	Egypt	serum	DNA integrity	qPCR	Alu fragments	prognostic diagnostic
[83]	72 HCC 37 nHCC	41	China	plasma	concentration	qPCR	*ACTB*	diagnostic predictive
[90]	40 HCC 40 nHCC	20	Egypt	serum	methylation	MS-PCR	*RASSF1A*	diagnostic
[257]	39	45	China	serum	concentration	qPCR	α-fetal protein α-L-fucosidase	diagnostic
[258]	66 HCC 76 nHCC	–	Italy	plasma	concentration	qPCR	*hTERT*	prognostic diagnostic
[259]	43 HCC 24 nHCC	26	China	serum	methylation	MS-PCR	*TFPI2*	diagnostic predictive
[260]	66 HCC 43 nHCC	–	USA	serum	methylation	pyrosequencing	*INK4A*	diagnostic prognostic predictive
[261]	121 HCC 37 nHCC	31	China	serum	methylation	MS-PCR	*MT1M* *MT1G*	diagnostic
[262]	90 HCC 103 hepatitis patients	32	Hong Kong	plasma	DNA integrity	MSP	*HBB*	diagnostic
[263]	31 HCC 8 hepatitis patients	–	China	plasma	CNV	NGS	*ACTB* *β-tubulin*	diagnostic
[94]	69 HCC 15 nHCC	22	China	plasma	concentration DNA integrity	qPCR	*ALU* fragments	diagnostic
[86]	62 HCC 24 nHCC	–	China	plasma	concentration	Qubit		diagnostic

nHCC – non-hepatocellular carcinoma, MS-PCR- methylation specific PCR, qPCR – quantitative PCR, CNV – copy number variation, MSP – massive parallel sequencing, NGS – next generation sequencing.

diseases (hepatitis B or C) and patients with HCC. HCC patients displayed higher plasma or serum cfDNA concentration than patients with hepatitis B (HBV), C (HCV) or healthy individuals [82,83] (Table 3). These results were confirmed by others [84–86].

Relationships between the plasma cfDNA levels and clinicopathological features were analyzed in other studies: significantly higher plasma cfDNA levels were observed in HCC patients with larger tumor size (≥ 5 cm) or vascular invasion [83,84]. Ren et al. observed the association between overall survival (OS) and disease-free survival (DFS) in HCC patients stages I-IV and plasma cfDNA level; higher levels of cfDNA were associated with worse survival [87]. Huang et al. monitored changes in cfDNA plasma levels in stages I-IV HCC patients over time and observed that cfDNA concentration decreases after tumor removal [83].

Besides the cfDNA concentration in plasma or serum, researchers also studied the methylation status of cfDNA. DNA methylation may be involved in the inactivation of tumor suppressor genes or activation protooncogenes. Yeo et al. observed that the presence of *RASSF1A* promoter hypermethylation in plasma cfDNA correlated with tumor size in HCC patients [88]. Further, patients with hypermethylated *RASSF1A* in cfDNA at the time of diagnosis or 1 year after tumor resection showed shorter DFS [89]. Promoter methylation of *RASSF1A* was detected in cfDNA in the serum of 90% of HCC patients and only in 10% healthy individuals. The mean serum levels of methylated *RASSF1A* in HCC patients were significantly higher when compared to healthy subjects [90].

Apart from *RASSF1A*, the aberrant methylation of *CDKN2A* gene (encoding p16 protein) in the cfDNA was also analyzed. HCC patients (stages I-IV) displayed cfDNA *CDKN2A* methylation while patients with cirrhosis, hepatitis or healthy subjects did not [91]. Changes in cfDNA

levels in HCC patients were further monitored between the pre and postoperative samples. The average concentration of *CDKN2A* methylation in post-operative plasma was 12 times lower than that in pre-operative plasma samples. Methylation index (MI), calculated as IM = 100 × [copy number of methylated/(copy number of methylated + unmethylated gene)], pointed to a decreasing trend of methylation *CDKN2A* status after surgical resection. In plasma samples of healthy volunteers no methylation of *CDKN2A* was observed [92]. On the other hand, hypomethylation of long interspersed nuclear elements 1 (*LINE-1*) was recorded [93].

HCC patients showed lower cfDNA integrity (integrity was calculated as the ratio of qPCR results ALU247/ALU115) than patients with benign liver disease or healthy individuals. The integrity number increased after hepatectomy in cancer patients [94].

These studies showed that cfDNA concentration correlates with clinical pathological characteristics and that the most promising biomarker related with methylation could be represented by promoter hypermethylation of *RASSF1A*. In summary, we can conclude that cfDNA has the potential to be considered as a biomarker of diagnosis and prognosis in HCC.

3.2.1.2. Colorectal cancer. Despite significant improvement in treatment, colorectal cancer (CRC) is a still one of the leading causes of death worldwide. Survival and mortality in CRC are strongly influenced by the stage of the disease at the time of the diagnosis. Patients with early stage (stage I) have high 5-years survival rate (90%) and, on the contrary, the late diagnosis means less than 10% chance to survive 5-years [95,96]. Early detection of colonic/rectal malignancies can decrease the mortality of CRC.

A number of studies proved the diagnostic value of cfDNA in both plasma and serum of patients with CRC. Specifically, median concentration of cfDNA in serum was five times higher in serum, and 25–50 times higher in plasma or serum of healthy subjects [97–100] (Table 4). The concordance between the patient's survival and recurrence with cfDNA concentration was affirmed [101,102]. After primary tumor resection, the plasma cfDNA in CRC decreased, however in patients with CRC recurrence, cfDNA level dramatically increased, while in "disease-free" patients the decreasing tendency in plasma cfDNA levels continued [99,103]. The pre-operative measurement of cfDNA contributes to better estimation of prognosis, and the post-operative measurement could represent a promising tool for early detection of recurrence. Higher plasma cfDNA concentration in the pre-treatment phase significantly correlated with worse survival [100,104].

Several studies also compared the plasma cfDNA levels between rectal and colon cancer patients. Patients with colon cancer displayed higher cfDNA concentration than those with rectal cancer (RC) (colon: 500 ng/ml, rectum: 250 ng/ml in plasma) [105], while Cassinotti et al. observed higher cfDNA concentration in RC [103]. Preoperative chemoradiotherapy (CRT) is considered as the standard treatment for locally advanced RC. The changes in cfDNA levels were observed before, during and at the end of the preoperative CRT. In the group of patients responding to chemoradiotherapy (responders, stage I–II) the plasma cfDNA level decreased, while in the group of non-responders the level of plasma cfDNA showed increasing tendency [106]. The cfDNA integrity index was lower after CRT in responders when compared to non-responders; generally CRC patients had 10 times higher cfDNA integrity number than healthy subjects [107]. In responders decreasing the incidence of *KRAS* at codon 12 mutations in cfDNA was observed by Sun et al. [108]. Patients with locally advanced RC (stage III-IV) with a high baseline level of cfDNA showed shorter DFS [109].

Activated *KRAS* mutations are considered as predictors of poor response in metastatic CRC patients receiving anti-epidermal growth factor receptor (*EGFR*) antibody-based therapy. These patients are therefore routinely tested for *KRAS* mutations before receiving biological therapy [110,111]. Several studies tried to prove that *KRAS* mutation can be detected in cfDNA. Wang et al. detected *KRAS* mutations (in codon 12,13 and 61) in tumor tissue of patients with stage I-IV and evaluated the presence of these mutations in serum samples. Their results showed about 45% concordance between the CRC tumor tissues and cfDNA. *KRAS* mutations were not detected in cfDNA among healthy volunteers [12]. Higher detection concordance (86%) of *KRAS* mutations in codon 12 between tumor tissue and plasma was observed in the study of Anker et al. [112]. Even higher concordance (96%) between *KRAS* in codons 12 and 13 mutations in tumor tissue and plasma analysis was reported by Thierry et al. and the concordance reached 100% for *BRAF* (V600E) mutations [113].

Methylation status of candidate genes in cfDNA has been barely studied. Herbst et al., analyzed the aberrant methylation of ten markers (*ALX4*, *CACNAG1*, *CDH1*, *HLTF*, *HPP1*, *IGF2*, *MDR1*, *NEUROG1*, *RUNX3*, *SEPT9*, *SOCS1*, *TIMP3*, *Vimentin*) in the serum of CRC patients and healthy subjects by methylation-specific PCR (MS-PCR). Hypermethylation of *NEUROG1* gene was found in 63 of 95 cases (stages I, II, III, IV) [114]. The presence of methylation in *HLTF* and *HPP1* genes in cfDNA in patients with I-IV colorectal cancer was associated with a worse OS [115]. Promoter methylation of three genes (*HPP1, HLTF* and *hMLH1*) in plasma cfDNA positively significantly correlated with tumor size, and methylations of *HLTF* and *HPP1* genes were observed more frequently in metastatic CRC patients and in patients with higher tumor stages [116]. Methylated status of *APC* gene in cfDNA was associated with the higher stage and older age. Patients with methylated *APC* gene had hypermethylation in *RASSF1A* gene. Moreover, patients with unmethylated promoter of *APC* or *RASSF1A* genes showed better OS than patients with promoter hypermethylation [117]. The presence of high serum *TAC1* methylation levels 6–12 months after diagnosis was associated with earlier recurrence [118].

Recently, Li et al. examined the possibility to detect CNV (copy number variations) in serum and in plasma of CRC patients with stages I-IV. The analysis showed more prominent CNV changes in plasma than in serum. From 79 CRC patients, CNV was detected in 39 of them, most of them were patients in the advanced stage. The most common changes included whole chromosomes gains at chromosomes 2, 7, 13 and 20; partial gains at chromosomes 8, 12, 13, 20 and partial losses at chromosomes 1, 3, 4, 8, 17, 18 and 22 [119].

All the studies observed the higher cfDNA concentration in cancer patients compared to healthy subjects. Moreover, concentration was different between colon cancer and rectal cancer, which could be caused by different treatments strategies. The concentration of cfDNA could also represent a promising tool for early detection of recurrence. The examination of cfDNA also showed that it is possible to detect mutations, CNV and methylation in plasma or serum in CRC patients, and this may lead to the identification of new, noninvasive biomarkers for CRC detection and prognosis.

3.2.1.3. Stomach cancer. Endoscopy is a sensitive screening method for gastric cancer, but higher costs and risk associated with the procedure are considerable. Therefore, the need for a reliable non-invasive test for early diagnosis is obvious.

All studies, focusing on the measurement of cfDNA concentration, reported higher plasma/serum cfDNA levels in cancer patients [7,120–122] (Table 5). Differences in the cfDNA concentration levels were also noticed between the various cancer stages – patients with advanced cancer had higher plasma cfDNA concentration than those with early stages [122]. Another study focused on measurements of pre-operative cfDNA level and cfDNA level 24 h after the surgery. The cfDNA level after surgery significantly decreased in comparison with preoperative levels [7].

Aberrant promoter methylation of candidate genes is frequently detected in gastric cancer and some studies tested the feasibility of detecting aberrant methylation in cfDNA. A study focused on e-*kadherin*, *p15*, *dap-kinase*, *CDKN2A* and *GSTP1* promoter methylation in serum cfDNA detected aberrant methylation in one or more of these genes in 83% cancer patients (stages I-IV) and none in the 30 healthy

Table 4
– Overview of the studies investigating cfDNA in relation with CRC's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[112]	14	–	Switzerland	plasma	mutation	PCR	*KRAS*	diagnostic
[264]	30	28	USA	plasma serum	mutation	PCR	*KRAS*	diagnostic
[265]	44	–	USA	serum	mutation	PCR	*KRAS*	diagnostic
[266]	90	–	Japan	serum	mutation	mismatch ligation assay	*KRAS*	diagnostic
[267]	58	–	France	plasma	mutation methylation	MS-PCR qPCR	*KRAS* *CDKN2A*	prognostic
[268]	94	–	Ireland	serum	mutation		*KRAS*	Diagnostic prognostic
[12]	104	50	Taiwan	serum	mutation concentration	PCR-SSCP	*APC* *KRAS* *TP53*	diagnostic
[269]	49	41	Hong Kong	serum	methylation	MS-PCR	*HTLF* *hMLH1* *APC*	diagnostic
[270]	25	–	Sweden	plasma	mutation	TGEE	*KRAS*	prognostic
[271]	66	–	Italy	plasma	mutation methylation	direct sequencing	*KRAS* *HRAS* *P53* *CDKN2A*	prognostic
[98]	75	75	Italy	serum	concentration	qPCR	*GAPDH*	diagnostic
[99]	70	20	Italy	plasma	concentration	qPCR		prognostic diagnostic
[272]	86		Italy	serum	mutation	qPCR	*KRAS2*	diagnostic
[115]	38	20	Germany	serum	methylation	MS-PCR	*HPP1/TPEF* *HLTF* *hMLH1*	prediction
[97]	67	67	Italy	plasma	concentration	qPCR	*RNAse P*	diagnostic
[273]	94	–	Japan	serum	methylation	MS-PCR	*CDKN2A*	diagnostic
[105]	70	20	Italy	plasma	concentration mutation methylation	qPCR MS-PCR ME-PCR	*KRAS* $p16^{INK4a}$	diagnostic predictive
[274]	133	179	Germany	plasma	methylation	MS-PCR	*TMEFF2* *NGFR* *SEPT9*	diagnostic
[104]	55	20	Germany	serum	concentration	spectrophotometry		diagnostic
[15]	20	–	USA	serum plasma urine	concentration mutation	RE-PCR	*KRAS*	diagnostic
[106]	26	–	Austria	plasma	concentration	qPCR	*18S rRNA gene*	prognostic predictive
[275]	97	172	Germany	plasma	methylation	MS-PCR	*SEPT9*	diagnostic
[107]	67	35	Italy	plasma	concentration DNA integrity	qPCR	β-globin Alu repeats	predictive
[114]	106	–	Germany	plasma	methylation	MS-PCR	*HPP1/TPEF* *HLTF* *NEUROG1*	diagnostic
[276]	71	–	UK	plasma serum	mutation	qPCR	*KRAS*	diagnostic
[116]	311	–	Germany	serum	methylation	MS-PCR	*HPP1* *HLTF*	prognostic
[49]	108	–	Denmark	plasma	concentration mutation	qPCR	*KRAS*	prognostic predictive
[277]	106	–	China	plasma	mutation	PNA-PCR	*KRAS*	diagnostic prognostic
[103]	223	–	Italy	plasma	concentration	qPCR	*RNAse P gene*	prognostic
[278]	101	96	Korea	plasma	methylation	qPCR	*Septin9*	diagnostic
[279]	211	–	Denmark	plasma	mutation	qPCR	*KRAS* *BRAF*	diagnostic predictive prognostic
[108]	34	10	China	plasma	concentration methylation mutation	PCR-RFLP qPCR MS-PCR	*MGMT* *KRAS*	diagnostic predictive
[280]	52	–	Taiwan	plasma	mutation	PNA-PCR	*KRAS*	predictive
[281]	133	–	Taiwan	plasma	concentration	qPCR	*PPIA*	prognostic
[282]	10	–	Austria	plasma	mutation	WGS	*KRAS* *BRAF PIK3CA* *EGFR*	diagnostic predictive
[283]	170	–	Italy	plasma	mutation concentration	qPCR	*KRAS*	diagnostic
[284]	100	100	Denmark	plasma	concentration mutation	PCR	*KRAS*	diagnostic predictive
[118]	150	–	Singapore	serum	methylation	MS-PCR		prognostic

(*continued on next page*)

Table 4 (*continued*)

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
							TAC1 *Septin9* *NELL1*	
[113]	106	29	France	plasma	mutation	qPCR	*KRAS* *BRAF*	diagnostic predictive
[285]	242	–	China	plasma	mutation	sequencing PNA-PCR	*KRAS*	prognostic
[286]	34	–	France	plasma	mutation concentration	digital PCR	*KRAS*	diagnostic prognostic
[100]	229	100	Denmark	plasma	mutation concentration	qPCR	*KRAS*	prognostic diagnostic
[287]	116	–	Singapore	serum	methylation	MS-PCR	*SST*	prognostic
[117]	155	–	Greece	plasma	methylation	MS-PCR	*APC* *RASSF1A*	prognostic
[101]	97	–	France	plasma	mutation concentration	qPCR	*KRAS* *BRAF*	diagnostic prognostic
[288]	230	–	Australia	plasma	mutation		*APC* *KRAS* *TP53*	prognostic
[289]	74	–	Iran	plasma	concentration	qPCR	*GAPDH*	diagnostic
[290]	467	–	Germany	plasma	methylation	MS-PCR	*HPP1*	predictive prognostic
[291]	50	8	Germany	plasma	concentration mutation	qPCR	*KRAS*	diagnostic
[119]	80	35	USA	serum plasma	concentration CNVs	Qubit WGS		prognostic diagnostic
[292]	128	–	USA	plasma	mutation	sequencing	*APC* *TP53* *KRAS*[*]	diagnostic
[293]	273	94	Denmark Norway Sweden	plasma	concentration	fluorescence droplet digital PCR DFA	*β-2-microglobulin*	diagnostic
[294]	28	–	Denmark	plasma	mutation	droplet digital PCR	*KRAS*	prognostic
[295]	98 CRC 101 adenomas 76 nCRC	253	China	plasma	methylation	MS-PCR	*SEPT9*	prognostic
[296]	22	–	Japan	serum	mutation	NGS	*TP53* *KRAS* *APC* *PIK3CA* *BRAF* *FBXW7* *NRAS*	diagnostic prognostic
[297]	20 CRC 20 adenomas	20	Spain	serum	methylation	microarray		diagnostic
[298]	3	–	Germany	plasma	mutation	BEAMing	*BRAF* *PIK3CA*	predictive
[299]	24	25	USA	plasma	CNVs	NGS		diagnostic prognostic
[300]	131	37	UK	plasma stool	concentration mutation	qPCR	*KRAS* *BRAF*	diagnostic
[301]	72	103	Portugal	plasma	methylation	MS-PCR	*APC* *FOXA1* *RARβ2* *RASSF1A* *SCGB3A1* *SEPT9* *SOX17*[**]	prognostic
[302]	50	–	Iran	plasma	methylation	High methylation resolution PCR	*BMP3*	prognostic
[109]	123	–	Denmark	plasma	concentration	fluorescence		diagnostic
[303]	150	–	China	urine	concentration mutation	spectrophotometry droplet digital PCR	*KRAS*	predictive prognostic
[304]	113	25	Japan	serum	methylation	droplet digital PCR	*TWIST1*	diagnostic
[305]	11	–	China	plasma	mutation	NGS	*TP53* *APC* *KRAS*[***]	prognostic
[306]	85	–	Japan	plasma	concentration mutation	digital PCR	*KRAS*	diagnostic predictive
[307]	138	–	Denmark	plasma	concentration mutation	droplet digital PCR	*RAS/RAF*	prognostic
[102]	20	–	Belgium	plasma	concentration	droplet digital PCR	*APC* *KRAS*	predictive

(*continued on next page*)

Table 4 (*continued*)

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[308]	21	–	Japan	plasma	mutation	sequencing	*PIK3CA* *TP53* *APC* *TP53* *PIK3CA* *BRAF*[****]	predictive
[309]	47	–	China	plasma	mutation	NGS	*TP53* *PIK3CA* *EGFR* *APC*[*****]	diagnostic prognostic

MS-PCR – methylation specific PCR, qPCR quantitative PCR, SSP PCR – single specific primer PCR, TGEE – temperature gradient gel electrophoresis, RE-PCR – restriction-enzyme PCR, PNA-PCR – peptide nucleic acid-mediated PCR, WGS – whole genome sequencing, DFA – direct fluorescent assay.

* Complete list of analyzed genes [292]: *ABL1, AKT1, ALK, APC, AR, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTBBB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, MYC, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTPN11, PTEN, PROC, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TERT, TP53, VHL.*

** Complete list of analyzed genes [301]: *APC, FOXA1, MGMT, RARβ2, RASSF1A, SCGB3A1, SEPT9, SHOX2, SOX17.*

*** Complete list of analyzed genes [305]: *ABCB1, AKAP9, AKT1, APC, ARID1A, ATIC, ATM, AXIN2, BARD1, BMPR1A, BRAF, BRCA1, BRCA2, BRIP1, BUB1, CBR3, CDA, CDH1, CHEK2,CREBBP, CTNNB1, CYP19A1, CYP2D6, DPYD, EGFR, EIF3E, EPCAM, ERBB2, ERBB4, ERCC1, FBXW7, GREM1, GSTP1, HRAS, KDR, KMT2C, KMT2D, KRAS, MET, MLH1, MLH3, MRE11A, MSH2, MSH3, MSH5, MSH6, MTHFR, MTRR, MUTYH, NBN, NF1, NRAS, PALB2, PIK3CA, PMS1, PMS2, POLD1, POLE, PTCH1, PTEN, PTPRK, RAD50, RAD51C, RAD51D, RB1, RRM1, RSPO2, RSPO3, SLIT1, SMAD2, SMAD4, SOD2, STK11, STMN1, TOP2A, TP53, TRRAP, TUBB3, TYMP, TYMS, UGT1A1, UMPS, XPC,XRCC1.*

**** Complete list of analyzed genes [308]: *APC, TP53, PIK3CA, BRAF, KRAS, ARID1A, SETD2, ATM, SMARCA4, MET, NRG1, CCND1, FBXW7, PTEN, TSC1, FLT3, MAP3K1, JAK1, VHL.*

***** Complete list of analyzed genes [309]: *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL.*

individuals' samples. The concordance between the presence of DNA methylation in tumor and serum cfDNA exceeded 68% [123]. High levels of methylated cfDNA were frequently detected in patients with more advanced stages; particularly those with III/IV stage had a higher concentration of methylated *ACP*, *TIMP3* and *hMLH1* genes. Moreover, patients with methylated *e-cadherin* showed a longer survival than patients without methylation [124]. A further study compared methylation of *RUNX3* gene in relation with patients' OS. The median methylation index (IM) was 12 times lower in postoperative serum cfDNA than in preoperative in gastric cancer patients (I-IV stages) [125]. In another study, authors examined the promoter methylation status of candidate genes such as *RUNX3*, *CDKN2A, RASSF1A* and *CDH1*. Promoter cfDNA hypermethylation was detected in the serum of all patients with gastric cancer. Authors proved that serum *RUNX3* hypermethylation could be a more reliable marker than other genes in this study [126].

Besides the methylations, gene amplification of specific genes plays an important role in the pathogenesis of gastric cancer. A few studies showed that it is possible to detect these amplifications (e.g. amplification of *MYC*, *HER2* or *GAPDH*) in plasma cfDNA [127,128].

Same as other cancers, the concentration was higher in gastric cancer patients than healthy subjects. One study even measured cfDNA level before surgery and 24 h after surgery and there was a significant difference. Clearly, cfDNA levels could represent a promising prognostic and diagnostic tool for gastric cancer.

3.2.1.4. Pancreatic cancer. Pancreatic cancer exhibits the poorest prognosis with the worst mortality of any solid cancers [129]. Patients with chronic pancreatitis (CP) have an increased risk of pancreatic cancer (PanC), and early detection of PanC is very difficult due to the similarity of symptoms attributable to CP and PanC. A reliable test that could distinguish CP from PanC is therefore urgently needed.

One of the earliest studies investigating the cfDNA in PanC patients dates back to 1998 [130] (Table 6). The authors examined the presence of *KRAS* mutations in plasma cfDNA from PanC patients (stage I-III) and healthy volunteers. CfDNA *KRAS* mutations at codon 12 were detected in 81% PanC patients and in none of the healthy volunteers [131]. Other parallel studies proved similar results [130,132]. The presence of *KRAS* mutations (codon 12) was also compared between PanC patients and patients with CP. In PanC patients *KRAS* mutations in plasma were often detected, but not in CP patients [132]. Patients bearing *KRAS* mutations in plasma cfDNA displayed shorter OS in comparison with those with wild-type *KRAS* gene [133,134]. Carriers of *KRAS* mutations (codon 12) had a significantly shorter OS (60 days) than those without *KRAS* mutations (772 days) [135]. Kinugasa et al. observed 80% concordance rate of *KRAS* mutations (codon 12) detection between tumor tissue and serum (most patients had stage IV) [136]. However, Pishvaian et al. suggested that liquid biopsy is an adequate substitution for tumor tissue biopsy due to the low (39%) concordance of *KRAS* mutations in tissue and in the blood [137]. The cause of low sensitivity could be due to technical limitation (low cfDNA yields).

Differences in presence of *KRAS* mutations between preoperative and postoperative serum samples could represent a promising predictive factor for a patient's survival, treatment response and recurrence. The presence of *KRAS* mutations (at codon12/13) in post-operative serum samples of PanC patients (stage IIA and IIB) was associated with shorter OS and DFS [138].

Besides *KRAS* mutations, plasma cfDNA levels correlated with patients' survival as well – higher plasma cfDNA level was associated with worse OS, the presence of metastases and poor therapy outcome [139–141]. The level of plasma cfDNA was the highest in patients with PanC (I-IV) in comparison to benign pancreatic disease and healthy volunteers [133,142].

Methylation profile of specific genes may distinguish PanC patients from patients with pancreatitis and healthy individuals [143,144]. Liggett et al. recorded promoter methylation of 14 genes in order to distinguish PanC patients from those with pancreatitis (*CND2, DAPK1, ESR1, hMLH1, MGMT, MUC2, MYOD1, CDKN2B, CDKN1C, PGK1, PGR, RARb, RB1,SYK*) [145].

In pancreatic cancer, there are several studies focusing on cfDNA isolated from exosomes. As mentioned above, exosomes are small vesicles which can contain nucleic acids such as DNA. In study of Bernard et al., plasma from patients with localized or metastatic PancA and from

Table 5
– Overview of the studies investigating cfDNA in relation with stomach cancer's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Focus study	Methodology	Target	Clinical relevance
[123]	54	30	Hong Kong	serum	methylation	MS-PCR	*E-kadherin* *p15* *Dap-kinase* *CDKN2A* *GSTP1*	diagnostic prognostic
[124]	60	22	Hong Kong	serum	methylation	MS-PCR	*APC* *E-cadherin* *hMLH1* *TIMP3**	diagnostic
[310]	35 GC 24 benign	10	China	serum	methylation	MS-PCR	*SFRP2*	diagnostic
[122]	53	21	Japan	plasma	concentration	qPCR	*ACTB*	diagnostic prognostic
[126]	4	10	Singapore	serum	methylation	MS-PCR	*RUNX3*	diagnostic
[311]	52	50	Iran	serum	methylation	MS-PCR	*CDKN2A*	diagnostic
[120]	20	22	Russia	plasma	methylation concentration	MS-PCR	*MGMT* *hMLH1* *p15*	diagnostic
[312]	47 GC 30 benign	30	China	serum	methylation	MS-PCR	*RASSF1*	diagnostic
[127]	57	79	Korea	plasma	amplification	qPCR	*MYC*	diagnostic
[125]	65	50	Japan	serum	methylation	MS-PCR	*RUNX3*	diagnostic prognostic
[313]	73	20	Japan	serum	methylation	MS-PCR	*TFPI2*	diagnostic
[314]	65	80	Hong Kong	plasma	methylation	MS-PCR	*SLC19A3*	diagnostic
[315]	32	21	China	serum	methylation	MS-PCR	*BX141696 WT1* *CYP26B1 KCNA4*	diagnostic
[121]	130	59	Korea	serum plasma	concentration	spectrophotomery qPCR	*Alu* fragments	diagnostic
[316]	71	21	Japan	serum	methylation	MS-PCR	*VIM*	diagnostic
[317]	73	–	Greece	serum	methylation	MS-PCR	*SOX17*	prognosis
[318]	202	88	China	serum	methylation	MS-PCR	*XAF1*	diagnostic
[319]	40	22	China	plasma	methylation	MS-PCR	*BCL6B*	diagnostic
[320]	92	–	China	serum	methylation	MS-PCR	*MINT2*	prognostic
[7]	30	34	South Korea	plasma	concentration	qPCR		prognosis prediction
[321]	81cancer patients 8 gastric adenomas 64 gastritis patients	32	South Korea	plasma	gene amplification	qPCR	*HER2* *MYC*	diagnostic
[322]	92	–	China	serum	methylation	MS-PCR	*CDKN2A*	prognostic
[323]	92	–	China	serum	methylation	MS-PCR	*TIMP-3*	Prediction prognosis
[324]	52	40	Japan	plasma	amplification	qPCR	*HER2*	prognostic
[325]	42	–	Japan	plasma	mutation	NGS	*TP53*	prognostic
[326]	277	–	Taiwan	plasma	concentration mutation	qPCR	*ARID1A* *TP53* *PIK3CA* *PTEN***	prognostic
[128]	60	30	Japan	plasma	amplification	droplet digital PCR	*HER2*	prognostic

q-PCR – quantitative PCR, MS-PCR – methylation specific PCR, NGS – next generation sequencing.
* Complete list of analyzed genes [124]: *APC, E-cadherin, GSTP1, hMLH1, MGMT, p15, CDKN2A, SOCS1, TIMP3, TGF-βRII.*
** Complete list of analyzed genes [326]: *ARID1A, TP53, PIK3CA, PTEN, AKT3, BRAF, AKT2, AKT1.*

healthy individuals was collected. Authors determined *KRAS* mutant allele fraction (MAF) from exosomal DNA and cfDNA. An increase in exosomal DNA was associated with disease progression and, on the contrary, cfDNA was not correlated with outcomes. Seventy-one percent of patients, that underwent resection, evinced a decrease in exosomal *KRAS* MAF and 94% of patients without resection displayed increase or no change in *KRAS* MAF in exosomal DNA. Moreover, authors tried to identify *KRAS* mutation (G12 V, G12D. G12R, G12C, G12S, G12A and G13D) in exosomal cfDNA and cfDNA and reached the corcondance of 95.5% for exoDNA and 68.2% for cfDNA [146]. The potential of clinical utility of serum exosomal DNA for the identification specific mutations *(KRAS* - G12D and *TP53* - R273 H) was proved by another study [147].

CfDNA represents a promising marker in the management of PanC patients. There is a clear evidence that genetic alterations presented in the tumors of PanC patients can be also detected in their plasma or serum. In addition, several studies showed that, besides cfDNA, exosomal DNA has prognostic effect and can provide unique predictive information.

3.2.2. Lung cancer

Most lung cancer patients are diagnosed in late stages, when 5-year survival rate is below 5%. A half of patients die within 1 year after diagnosis. Therefore, recent research is focused on discovery of biomarkers enabling early diagnosis of lung cancer [148,149] (Table 7).

There is evidence for diagnostic potential of cfDNA in lung cancer. A higher level of cfDNA in patients with non-small-cell lung cancer (NSCLC) than in individuals with chronic respiratory inflammation and healthy individuals was observed by Szpechcinski et al., 2015. The authors suggest that elevated plasma levels of cfDNA in NSCLC patients (stage I-III) result primarily from tumor development, which implies potential clinical implications for lung cancer screening and early diagnosis [150]. These results were confirmed by others [151–154]. Plasma cfDNA level in lung cancer patients was 318 ng/ml in comparison with 18 ng/ml in healthy subjects; patients with stage I showed significantly higher cfDNA levels when compared to healthy individuals

Table 6
Overview of the studies investigating cfDNA in relation with pancreatic cancer's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[131]	21 3 pancreatitis patients	5	The UK	plasma	mutation	RFLP-PCR	*KRAS*	diagnostic
[130]	21	–	Japan	plasma	mutation	MASA	*KRAS*	predictive
[327]	44 37 chronic pancreatitis	–	Spain	plasma	mutation	RFLP PCR	*KRAS*	diagnostic prognostic
[328]	29 12 chronic pancreatitis	–	Italy	serum	mutation	Mutant -enriched PCR	*KRAS*	diagnostic
[329]	47	31	France	serum	mutation	qPCR	*KRAS2*	diagnostic
[330]	28	–	Japan	plasma	mutation	mismatch ligation assay	*KRAS*	diagnostic
[143]	83	–	USA	plasma	methylation mutation	MS-PCR direct sequencing	*KRAS* *CDKN2A* *pENK*	diagnostic
[132]	56 13 pancreatitis patients	–	Germany	plasma	mutation	qPCR	*KRAS*	diagnostic
[144]	30	30	USA	plasma	methylation	MSER-PCR	*CCND2* *SOCS1* *THBS* *PLAU* *VHL*	diagnostic
[134]	91	–	China	plasma	mutation	direct sequencing	*KRAS*	predictive
[145]	30	30	USA	plasma	methylation	MS-PCR	*BRCA1* *CCND2 HMLH1* *CDKN1C PGR*[*]	diagnostic
[135]	45 20 CP 20 HV	–	Spain	plasma	concentration mutation	digital PCR	*KRAS*	diagnostic
[136]	141	–	Japan	serum	mutation	digital PCR	*KRAS*	predictive
[331]	77	–	USA	plasma	mutation	NGS	*MLL* *MLL2* *MLL3* *ARID1A* *CDKN2A* *KRAS* *SMAD4* *TGFBR2* *TP53*	predictive
[142]	73 23 CP	23	Italy	plasma	concentration DNA integrity	qPCR	*ALU fragments*	diagnostic
[140]	127	–	India	plasma	concentration mutation	qPCR	*KRAS*	predictive
[332]	259	–	Japan	plasma	mutation	droplet PCR	*KRAS*	diagnostic prognostic
[333]	26	–	USA	plasma	mutation	NGS	*KRAS* *TP53* *BRAF* *SMAD4* *GNAS* *FBXW7* *APC*[**]	diagnostic
[1]	50	–	Germany	plasma	mutation	digital PCR	*KRAS*	diagnostic
[139]	105	–	Japan	plasma	mutation	droplet digital PCR	*KRAS*	predictive
[334]	437 141 benign	394	Czech Republic Slovakia	plasma	mutation	qPCR	*KRAS*	diagnostic
[141]	14	29	Norway	plasma	mutation	qPCR	*KRAS*	predictive
[133]	26 14 chronic pancreatitis	12	The UK	plasma	concentration mutation	targeted NGS	*KRAS*	diagnostic
[335]	188	–	China	plasma	mutation	NGS droplet digital PCR	*BRCA2* *EGFR* *KDR* *ERBB2* *K-RAS*	prognostic
[336]	221	182	USA	plasma	mutation	sequencing	*KRAS*	diagnostic
[337]	135	–	France	plasma	concentration	NGS droplet digital PCR	*KRAS*	prognostic
[137]	34	–	USA	plasma	mutation	NGS	*TP53* *SMAD4* *CDKN2A* *KRAS*[***]	diagnostic
[338]	106	–	Korea	plasma	mutation	droplet digital PCR	*KRAS*	prognosis
[138]	45	–	Japan	serum	mutation	qPCR	*KRAS*	predictive
[339]	17	–	Korea	plasma	mutation concentration	targeted deep sequencing	*KRAS*	prognosis
[146]	194	37	USA	plasma	mutation	droplet digital PCR	*KRAS*	predictive prognostic

(*continued on next page*)

Table 6 (*continued*)

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[147]	171	114	Germany	serum	mutation	droplet digital PCR	*KRAS* *TP53*	diagnostic

MASA-mutant allele specific amplification method, RFLP PCR – restriction fragment length polymorphism PCR, qPCR – quantitative PCR, MS-PCR – methylation specific PCR, MSER-PCR – methylation-sensitive enzyme restriction PCR.

* Complete list of analyzed genes [145]: *BRCA1,CCND2, HMLH1, CDKN1C, PGR, SYK, VHL, DAPK1, ESR1, MGMT, MUC2, MYOD1, CDKN2B, CDKN1C, PGK1, RARb, RB1*.

** Complete list of analyzed genes [333]: *APC, AR, ARID1A, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCNE1, CDK4, CDK6, CDKN2A, CDKN2B, EGFR, ERBB2, FGFR1, FGFR2, HRAS, KIT, KRAS, MET, MYC, NF1, NRAS, PDGFRA, PIK3CA, PTEN, RAF1, TP53, AKT1, ALK, ARAF, ATM, CDH1, CTNNB1, ESR1, EZH2, FBXW7, FGFR3, GATA3, GNA11, GNAQ, GNAS, HNF1A, IDH1, IDH2, JAK2, JAK3, MAP2K1, MAP2K2, MLH1, MPL, NFE2L2, NOTCH1, NPM1, NTRK1, PTPN11, RET, RHEB, RHOA, RIT1, ROS1, SMAD4, SMO, SRC, STK11, TERT, VHL*.

*** Complete list of analyzed genes [137]: *AKT1, ALK, APC, AR, ARAF, ARID1A, ATM, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCNE1, CDH1, CDK4, CDK6, CDKN2A, DKN2B, CTNNB1, EGFR, ERBB2, ESR1, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, GATA3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MLH1, MPL, MYC, NF1, NFE2L2, NOTCH1, NPM1, NRAS, NTRK1, PDGFRA, PIK3CA, PTEN, PTPN11, RAF1, RET, RHEB, RHOA, RIT1, ROS1, SMAD4, SMO, SRC, STK11, TERT, TP53, VHL*.

[155,156]. Plasma cfDNA levels after therapy were significantly lower when compared to those at the time of surgery and, interestingly, the levels were comparable with the value observed in healthy subjects [155,157]. Patients with progressive disease (stage III-IV) had significantly higher cfDNA concentration than patients with partial remission or stable disease [152].

The prognostic and/or predictive values of cfDNA in lung cancer have attracted a substantial interest. In patients without recurrence cfDNA levels showed a tendency toward reduction within an interval of 1 to 6-months after surgery. On the other hand, 4 patients showed 2–20 times increased level in the 7–23 months interval post-surgery. Two of them developed liver metastases, one patient had recurrence of carcinoma and the last patient displayed a new primary tumor [155]. This suggests that cfDNA concentration correlates with the clinical status of patients. An elevated cfDNA concentration was associated with higher risk of NSCLC, and a median cfDNA level was significantly lower in 30 disease free individuals when compared to patients with proven cancer relapse [151,158]. Patients with higher cfDNA concentration displayed worse DFS and OS [159–161]. Moreover, patients with metastases (III-IV) had a significantly higher cfDNA level than patients without metastases [157].

The epigenetic inactivation of genes also plays an important role in lung cancer. Fischer et al. investigated the promoter methylation of seven genes (*APC1A*, *DAPK*, *FHIT*, *p14ARF*, *p16INK4a*, *RARβ*, *RASSF1A*) in serum cfDNA. The frequency of methylation varied between 25.9% and 47.3% in stage III-IV patients [162]. The MI for *RASSF1A* and *RARB2* in the cfDNA was elevated 2- to 3-fold in patients with lung cancer (stage I-III) than in healthy donors and decreased in patients after therapy (tumor resection or chemotherapy) [163]. High frequency of *APC* gene promoter methylation in tumor tissue is well known. Usadel et al. documented *APC* promoter methylation in matched serum and plasma samples and tumor tissues from almost a half of patients with lung cancer but in none of 50 healthy individuals [164].

Analysis of mutation in gene epidermal-growth factor receptor gene (*EGFR*) is essential for treatment selection. Especially T790M *EGFR* mutation has been established as a major mechanism of resistence in tyrosin-kinase inhibitor (TKI) of EGFR therapy (such as drug gefitinib). Most of the studies aimed to detect this particular mutation in plasma samples with similar sensitivity as in tumor tissue [165]. In the study of Yu et al., authors examined patients with advanced stage of lung cancer (IIIB/IV). The reached concordance between plasma and tissue was 90% for deletion in exon 19 (ex19del), 95% for mutation L858R and 95% for T790 M mutation [166]. Similar concordance in stage IV patients with lung cancer was described in other study (87.9% for L858R and 86.2% for ex19del) where the mutations presence correlated with progression-free survival (PFS, patients without mutations had median PFS 10.1 months and patients with *EGFR* mutations had 6.3 months) [167]. The pretreatment T790 M mutation status, T790 M mutation status at the disease progression and growth rate of *EGFR* mutations significantly were associated with overal survival (OS) [168]. The presence of *EGFR* mutations (ex19del and L858R) also was associated with OS: patients with *EGFR* mutation in both plasma and tumor tissue displayed significantly shorter OS than patients with mutation only in tumor tissue [169,170]. This observation was further supported by larger study in which more than 300 patients with stage IIIB/IV were examined for the presence of *EGFR* mutation (ex19del, L885R) in plasma and serum. However the concordance of these mutations (ex19del, L885R) rate was lower (28.6% for serum and 60.5% for plasma), patients with at least one of these mutations in blood evinced advanced disease characteristics and poorer prognosis [171]. In relation with *EGFR*, several studies focused on other the sources of cfDNA, such as urine or exosomes from plasma [172–174]. In patients with advanced stages, the concordance between transrenal (from urine) cfDNA and tumor tissue were about 85–93% [173,174].

In lung cancer, several studies showed significantly different cfDNA levels between specific stages of cancer, or even between healthy subjects and patients with stage I. Moreover, plasma cfDNA could represent a very promising predictive biomarker for patient's response to *EGFR* therapy.

3.2.3. Breast cancer

Breast cancer (BC) is the most common cancer in women worldwide. Since metastatic BC is currently incurable, researchers are focused on identifying a biomarker of early cancer detection.

Many authors observed differences in serum and plasma cfDNA levels between BC patients, patients with benign breast disease and healthy woman [6,175–177] (Table 8). BC patients (stages I-IV) showed plasma cfDNA concentration about 65 ng/ml, patients with benign disease 27 ng/ml and healthy controls 13 ng/ml [6]. Moreover, the authors also noticed differences in plasma cfDNA between TNM stages – the positive correlation between the cfDNA level and stages [177]. Higher plasma cfDNA levels in stage I-III patients were associated with worse OS and DFS [176,178], with the presence of distant metastases, larger tumor size, higher TNM stage and involvement of lymph node [177,179,180]. Similarly as for other cancers, the plasma cfDNA level decreased after surgery [69,151,177].

Somatic mutation *PIK3CA* occurs in more than 30% BC patients [181] and can be also detected in plasma or serum cfDNA [178,182]. Board et al. recorded 95% *PIK3CA* mutation (H1047R, H1047 L, E545 K and E542 K) concordance between tumor tissue and plasma and 88% between tumor tissue and serum in stage I-III patients [183]. In another study, concordance in plasma was even 100% (for mutations E545 K, H1047R and H1047L) [184].

More than 25% of BC patients had over-expressed HER2 protein, encoded by human epidermal growth factor receptor 2 gene (*HER2*) [185]. Many studies aimed at investigation whether the amplification

Table 7
Overview of the studies investigating cfDNA in relation with lung cancer's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[340]	43	10	Germany	plasma	MSI LOH	qPCR	*ACTBP-2* *AR* *UT 762*	diagnostic
[155]	84	43	Italy	plasma	concentration MSI	qPCR	microsat. alt.: 3p14.2 3p21 3p23 3p24.2 3p25–26	diagnostic
[341]	14	–	China	plasma	methylation	MS-PCR	*CDKN2A*	prognostic
[164]	89	50	USA	serum plasma	methylation	MS-PCR	*APC*	prognostic
[158]	100	100	Italy	plasma	concentration	qPCR	*hTERT*	diagnostic
[342]	185	46	Switzerland	serum	concentration	qPCR	*GAPDH*	predictive
[343]	25	–	USA	plasma	mutation	RFLP-PCR	*KRAS*	diagnostic prediction
[344]	67	44	China	plasma	concentration	qPCR		diagnostic
[14]	91	100	Japan	serum	methylation	MS-PCR	*MGMT* *p16^{INK4A}* *RASSF1A* *DAPK* *RAR-β*	diagnostic
[162]	92	10	Germany	serum	methylation	MS-PCR	*APC1A* *DAPK* *FHIT* *p14^{ARF}* *p16^{INK4a}* *RARβ* *RASSF1A*	prognostic
[345]	10	10	UK	serum plasma	concentration	qPCR	*AAT* *TP53* *BCL-2*	diagnostic
[151]	104	205	Portugal	plasma	concentration	qPCR	*hTERT*	diagnostic prognostic
[346]	76	66	Italy	plasma	concentration	qPCR	*hTERT* *microsat. markers: 3p14.2, 3p23,3p21,3p24.2*	diagnostic
[347]	151	79	Italy	plasma	concentration	qPCR	*hTERT*	prognostic diagnostic
[156]	102	105	Korea	plasma	concentration	qPCR	*ACTB*	diagnostic
[161]	46	21	Netherlands	plasma	concentration	qPCR	*β-globin*	diagnostic prognostic
[152]	100	100	India	plasma	concentration	qPCR		diagnostic
[348]	446	–	Italy	plasma	concentration	qPCR	*hTERT*	prognostic
[160]	104	205	Portugal	plasma	concentration	qPCR	*hTERT*	diagnostic
[349]	139	50	Korea	serum	mutation	MS-PCR	*TMEFF2*	diagnostic
[153]	88	200	China	plasma	concentration	qPCR	*ACTB*	predictive
[350]	9	–	UK	plasma	mutation	exome sequencing	*EGFR* *TP53* *NFKB1*	diagnostic
[163]	60	32	Russia	plasma	methylation	MS-PCR	*RASSF1A* *RARB2*	diagnostic prognostic
[157]	58	–	Denmark	plasma	concentration mutation	qPCR	*KRAS*	prognostic
[154]	134	32	China	plasma	concentration mutation	qPCR	*EGFR*	diagnostic predictive
[351]	22	22	Italy	plasma	concentration	qPCR	*hTERT*	prognostic
[352]	69	21	Italy	plasma	mutation	qPCR NGS	*EGFR*	diagnostic
[159]	218	–	France	plasma	concentration	fluorimetry		diagnostic prognostic
[150]	50 NSCLC 101 respiratory disease	40	Poland	plasma	concentration	qPCR	*ACTB*	diagnostic
[149]	65 NSCLC 28 benign tumors	16	Poland	plasma	concentration DNA integrity	qPCR	*ACTB*	diagnostic
[353]	307	–	China	plasma	mutation	ddPCR	*EGFR*	prognostic
[354]	79	–	China	plasma	mutation	ddPCR SuperARMS	*EGFR*	predictive
[355]	19	–	Japan	plasma	mutation	qPCR	*EGFR*	predictive
[356]	144	–	Denmark	plasma	mutation	ddPCR	*EGFR*	predictive
[357]	102	–	Israel	plasma	mutation	Ion Torrent PGM Sequencing	*EGFR*	predictive
[358]	85	–	USA	plasma	mutation	ddPCR	*EGFR*	predictive
[359]	56	–	South Korea	serum	mutation	Ultra deep sequencing ddPCR	*EGFR*	predictive

(*continued on next page*)

Table 7 (*continued*)

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[173]	160	–	China	urine plasma	mutation	ddPCR	*EGFR*	predictive
[360]	61	–	France	plasma	mutation	ddPCR Massive parallel sequencing	*EGFR*	predictive
[361]	200	–	China	serum	mutation	ddPCR	*EGFR*	predictive
[166]	22	–	China	plasma	mutation	ddPCR	*EGFR*	predictive
[362]	45	–	China	plasma	mutation	ddPCR	*EGFR*	predictive
[168]	20	–	China	plasma	mutation	ddPCR	*EGFR*	predictive
[363]	168	–	China	plasma	mutation	qPCR	*EGFR*	predictive
[364]	49	–	Japan	plasma	mutation	Chip based dPCR	*EGFR*	predictive
[171]	709	–	China South Korea Thailand	serum plasma	mutation	qPCR	*EGFR*	predictive
[365]	60	–	USA	plasma	mutation	ddPCR	*EGFR*	predictive
[366]	21	–	Japan	plasma	mutation	Ion Torrent PGM	*EGFR*	predictive
[367]	36	–	Spain	plasma	mutation concentration	ddPCR	*EGFR*	predictive
[368]	16	–	Germany	plasma	mutation	ddPCR	*KRAS* *EGFR*	prognostic predictive
[369]	80	–	China	plasma	mutation	qPCR	*EGFR*	prognostic
[167]	81	–	South Korea	plasma	mutation	ddPCR	*EGFR*	predictive prognostic
[370]	198	–	China	plasma	mutation	ARMS	*EGFR*	predictive
[169]	94		China	plasma	mutation	ARMS	*EGFR*	prognostic
[170]	57	–	South Korea	plasma	mutation	PNA-mediated PCR	*EGFR*	predictive
[371]	97	–	Spain	serum	mutation	Multiplex qPCR	*EGFR*	prognostic
[372]	111	–	China	plasma	mutation	ME-PCR	*EGFR*	prognostic
[172]	210	–	USA	exosomes from plasma	mutation	qPCR	*EGFR*	prognostic
[373]	77	–	USA	plasma	mutation	Illumina	*EGFR*	predictive
[374]	19	–	China	serum	mutation	ARMS	*EGFR*	prognostic
[375]	21	–	Spain	plasma	mutation	ddPCR	*EGFR*	prognostic
[174]	160	–	China	plasma urine	mutation	ddPCR	*EGFR*	prognostic

RFLP PCR – restriction fragment length polymorphism PCR, qPCR – quantitative PCR, MS-PCR – methylation specific PCR, ddPCR-droplet digital PCR, ARMS-Amplification-refractory mutation system, PNA-peptide nucleic acid, ME-PCR – mutant-enriched PCR.

of *HER2* can also be detected in cfDNA [186–188]. Page et al.'s results demonstrated that the presence of *HER2* amplifications in stage I-III cancer patients (locus 17q21.1) in cfDNA could be considered as a good biomarker in BC prognosis [186].

Outcomes relating to DNA integrity are controversial. Majority of studies demonstrated the highest DNA integrity in BC patients [189–192]. DNA integrity number (ALU247/ALU115) was higher in stage IV than in earlier stages [189]. On the other hand, Madhavan et al. observed the highest cfDNA integrity number (ALU260/ALU111 and LINE197/LINE1266) in healthy individuals, followed by patients with primary BC and the lowest integrity was in monitored metastatic BC. They suggested that the conflict of their results with other studies reporting an increased cfDNA integrity index in cancer patients could be partly due to the properties of primer pairs and amplicon lengths and due to sample processing and preparation [21]. DNA integrity index positively correlated with tumor size of invasive cancers, with lymph node metastases and TNM stage [190,192].

Hypermethylation of specific genes (*PITX2*, *RASSF1A*, *GSTP1*, *RARβ2*, *BRCA1*, *MGMT* and *SOX17)* can be employed as a biomarker in early diagnosis and prognosis of BC [2,193,194]. BC patients with hypermethylated *GSTP*, *RASSF1A*, *PITX2* and *RARβ2* in cfDNA displayed significantly worse OS [195–197]. *SOX17* hypermethylation in plasma cfDNA was an independent negative prognostic factor for DFS [198].

In breast cancer, many studies were focused on DNA integrity index, however results were controversial. Most of the studies showed that the highest integrity was in BC patients, but other study had a contrary result. It could be caused because of using different target repeated sequencing and processing method. CfDNA has potential as a prognostic, diagnostic and predictive biomarker in breast tumor management.

3.2.4. Ovarian cancer

Ovarian cancer is the most lethal gynecological malignancy and the fifth most common cause of cancer death in women [148]. The high mortality may be caused by the lack of early diagnosis – most patients with ovarian cancer are diagnosed in advanced stages (III-IV) [199]. The most common type of ovarian cancer is epithelial ovarian cancer (EOC).

Several studies were focused on the quantification of cfDNA level and all of them found significantly increased levels in ovarian cancer patients than in healthy individuals or in patients with benign ovarian diseases [200–203] (Table 9). Kamat et al. found that preoperative cfDNA level correlated with patients' survival – cancer patients with the highest cfDNA level had decreased survival [204]. CfDNA level showed the correlation between stages: cfDNA levels were signnificantly increased in stage III and IV compared with those of stages I and II [203]. In addition, cfDNA level significantly decreased after surgery and during succesful chemotherapy [203,205,206].

Other studies attempted to detect the presence of tumor-specific genes in cfDNA in order to evaluate cfDNA potential for early diagnosis and assesment of prognosis. Majority of the studies focused on *TP53* gene and showed that the possibility to detect *TP53* mutations in plasma [207,208]. Mutations in *TP53* are presented in more than 80% of patients with ovarian cancer. In the study of Kim et al., authors examined 61 high-grade patients with *TP53* mutations in tumor tissue (mutations in exons 4, 5, 6, 7, 8, 9, 10) and interestingly, all these patients displayed this mutation in their plasma samples and thus reached the concordance of 100% [29]. In another study, authors

Table 8
Overview of the studies investigating cfDNA in relation with breast cancer's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[376]	61	10	Switzerland	plasma serum	MSI LOH	qPCR	*D7S522 D9S169 D10S197 D17S1325* *ER* *DM1* *TP53*[*]	diagnostic
[13]	71	9	The UK	plasma	LOH MSI	qPCR	*DM-1 D16S400*	diagnostic
[377]	147	–	Spain	plasma	mutation	qPCR	*TP53*	Predictive prognostic
[378]	26 PBC 10 MBC	10	Austria	serum	methylation	MS-PCR	*ESR1* *APC* *RASSF1* *HIC1* *HSD17B4*	prognostic
[175]	96	24	UK	serum	concentration	qPCR	*β-globin*	diagnostic
[6]	61 33 benign	27	China	plasma	concentration	qPCR	*β-globin*	diagnostic
[229]	68	54	Greece	plasma	concentration methylation	qPCR MS-PCR	*RASSF1* *ATM*	diagnostic
[192]	83	51	USA	serum	DNA integrity	qPCR	*Alu* fragments	diagnostic prognostic
[379]	33	50	Switzerland	serum	concentration	qPCR	*GAPDH*	diagnostic
[151]	175	80	Portugal	plasma	concentration	qPCR	*hTERT*	diagnostic prognostic
[380]	36	29	USA	serum	concentration DNA integrity	qPCR	*LINE1*	diagnostic
[381]	29	49	Switzerland	serum plasma	concentration	qPCR	*GAPDH*	diagnostic
[382]	52 26 benign	70	Germany	plasma	concentration	qPCR	*GAPDH* *MTATP8*	diagnostic
[194]	80	20	Belgium	plasma serum	concentration methylation	qPCR MS-PCR	*APC* *RASSF1* *ESR1*	diagnostic predictive
[183]	46 MBC 30 PBC	–	The UK	plasma serum	mutation	qPCR	*PIK3CA*	predictive
[383]	77 PBC 34 MBC	34	Spain	serum	methylation	MS-PCR	*14-3-3σ* *ESR1*	prognostic
[195]	428	–	Austria	plasma	methylation	MS-PCR	*PITX2* *RASSF1A*	prognostic
[314]	98	80	Hong Kong	plasma	concentration methylation	qPCR MS-PCR	*SLC19A3*	diagnostic
[186]	78 PBC 30 MBC	98	The UK	plasma	amplification	qPCR	*HER2*	prognostic
[180]	31 PBC 32 MBC 20 benign	28	Germany	serum	concentration	ELISA		diagnostic
[176]	102 32 benign	53	Germany	serum	concentration	qPCR	*PTEN*	diagnostic
[107]	39	49	Italy	plasma	methylation concentration	qPCR	*RASSF1A* *MAL* *SFRP1*	diagnostic
[196]	336	80	Japan	serum	methylation	MS-PCR	*GSTP1, RASSF1A RARβ2*	prognostic
[384]	200	100	China	serum	concentration	qPCR	*GAPDH*	diagnostic
[179]	42	27	Egypt	plasma	concentration	qPCR	*hTERT*	diagnostic prognostic
[184]	60	–	USA	plasma	mutation	BEAMing	*PIKC3A*	predictive
[197]	100	30	India	serum	methylation	MS-PCR	*ERβ* *RARβ2*	prognostic
[193]	40	30	India	serum	methylation	MS-PCR	*BRCA1* *GSTP1* *MGMT* *MDR1* *Stratifin*	prognostic
[385]	388		Germany	plasma	LOH	qPCR	*D3S1605 D10S1765 D11S4200 D12S1660 D12S1725 D13S218 D16S421 D17S855*	prognostic
[187]	50 15 MBC	50	Denmark	plasma	concentration amplification	qPCR	*HER2*	diagnostic
[2]	79	60	Greece	plasma	methylation	MS-PCR	*Sox17*	prognostic
[38]	52	–	UK	plasma	concentration	digital PCR	*PIK3CA* *TP53*	prognostic
[188]	58	–	UK	plasma	amplification	digital PCR	*HER2*	diagnostic

(*continued on next page*)

Table 8 (*continued*)

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[350]	2	–	UK	plasma	mutation	exosome sequencing	*PIK3CA* *BMI1* *SMC4* *FANCD2* *MED1* *ATM* *PDGFRA* *GAS6* *TP63*	diagnostic
[182]	29	–	USA	plasma	mutation	droplet digital PCR	*PIK3CA*	diagnostic
[21]	82 PBC 201 MBC	100	Germany	plasma	concentration DNA integrity	qPCR	*ALU* *LINE-1*	diagnostic
[191]	112 12 benign	28	Germany	plasma	DNA integrity	qPCR	*ALU*	diagnostic
[198]	155	60	China	plasma	methylation	MS-PCR	*SOX17*	prognostic
[386]	48	–	UK	plasma	mutation	droplet digital PCR	*ESR1*	prognostic
[189]	148	51	India	serum	DNA integrity	qPCR	*PIK3CA*	prognostic
[178]	313	50	Japan	serum	mutation	digital PCR	*PIK3CA*	prognostic
[177]	100	100	Thailand	plasma	concentration	Qubit		diagnostic
[190]	95 95 benign	70	Egypt	plasma	DNA integrity	qPCR	*β-actin*	diagnostic
[387]	79	10	Italy	serum	concentration	qPCR	*HER2* *MYC* *BCAS1* *PI3KCA*	diagnostic
[69]	268	–	Germany	plasma	concentration DNA integrity	qPCR	*ALU* *LINE1*	prognostic
[388]	86	–	Japan	plasma	mutation	droplet digital PCR	*ESR1* *PIK3CA*	prognostic
[389]	25 25 benign	25	China	plasma	methylation	MS-PCR	*NBPF1*	prognostic
[390]	128	–	Japan	plasma	mutations	droplet digital PCR	*PIK3CA* *AKT1* *ESR1*	prognostic

qPCR – quantitative PCR, MS-PCR – methylation specific PCR.

* Complete list of analyzed genes [376]: *D6S311 D7S522 D8S137 D9S169 D10S197 D13S260 D16S402, D16S421 D17S1325 D17S579 THRA,ER, EABMD, DM1, TP53*.

proved, besides possibility to detect *KRAS* and *PIK3CA* mutations, the potential to monitor patient's response to therapy and predict recurrence – higher level of *PIK3CA* mutation (H1047R) preceded recurrence and *KRAS* (G12D) level was decreased after surgery and adjuvant chemotherapy and contrary it increased before death [209].

Several investigators tried to detect epigenetic alterations to evaluate the use of cfDNA in screening and diagnosis [210–212]. We have already mentioned that *RASSF2A*, tumor suppressor gene, is inactivated by hypermethylation in many cancers. In the study of Wu et al., authors examined the detection of promoter hypermethylation *RASSF2A* in plasma. Hypermethylation was detected in 51% EOC tissues (stages I-IV) and in 36% corresponding plasma samples. This aberrant methylation profile was not detected in patients with benign disease and healthy controls [213]. The ability to stratify patients with early stage of EOC and healthy controls was also presented by the methylation profile of the *OPCML* gene. In early stage EOC, the methylation of OPCML was significantly altered than in healthy donors [214]. Promoter methylation of *SLIT2* gene was detected in tumor tissue of 29 patients of stage I-IV but none in the healthy control. Among the patients with *SLIT2* hypermethylation in tumor tissue, 27 patients (concordance 93.1%) also showed this hypermethylation in serum [215]. In the study of Gifford et al., authors studied patients with ovarian cancer with stage I-IV for plasma cfDNA *hMLH1* methylation before chemotherapy and at relapse. Relapse blood samples were collected at the time of suspected progression but before verification of relapse from tumor tissue. At the time of relapse, 25% of patients with relapse had *hMLH1* methylation that was not detected in matched pre-chemotherapy plasma [216].

Regarding the correlation between cfDNA levels and clinicopathological characteristics, it is indicated that cfDNA reflects the burden of ovarian cancer. Many researchers have proved the possibility to distinguish patients with cancer and healthy control based on the cfDNA concentration and its methylation profile.

3.2.5. Prostate cancer

In prostate cancer, only one non-invasive tumor biomarker has been introduced into clinical practice – prostate-specific antigen (PSA). The PSA test can be used for early diagnosis and monitoring of tumor progression, however with insufficient sensitivity and specificity. Therefore, the biomarker which can discriminate patients with cancer and with a benign disease is highly needed.

In the case of plasma cfDNA concentration measurement, differences in plasma levels were observed between prostate cancer patients and patients with benign disease, especially with benign prostatic hyperplasia (BPH) [217,218] (Table 10). The median of cfDNA concentration in patients with BPH was 267 ng/ml and in patients with prostate cancer (stage II-III) 709 ng/ml [219]. Increased cfDNA levels were also monitored in patients with prostatic intraepithelial neoplasia [220]. Higher cfDNA levels were significantly associated with higher stage of tumor, recurrence, patient's survival and presence of metastases [221–223]. Patients with metastases showed the higher cfDNA concentration (663 ng/ul) than those without (188 ng/ul) [224]. The cfDNA concentration was also associated with therapy outcome, the mean plasma cfDNA level in newly diagnosed prostate cancer patients was the highest (240 ng/ml) and decreased after therapy to 60 ng/ml [225].

High concordance (about 90%) of somatic mutations in 72 genes (like *TP53*, *PTEN*, *RB1*, *APC*, *CDKN1B*, *BRCA2*, and *PIK3R1*) between

Table 9
Overview of the studies investigating cfDNA in relation with ovarian cancer's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[210]	50 EOC 10 BOD	20	USA	serum plasma	methylation	MS-PCR	*RASSFA1* *BRCA1*	diagnostic
[216]	138	–	UK	plasma	methylation	MS-PCR	*MLH1*	prognostic
[207]	27	–	Japan	plasma	mutation	F-SCCP	*TP53*	diagnostic
[208]	137	–	USA	plasma serum	mutation	Ligase detection reaction	*TP53*	diagnostic prognostic
[202]	19	12	USA	plasma	concentration	qPCR	*GAPDH* *β-globin* *β-actin*	diagnostic
[206]	22	50	Italy	plasma	concentration	qPCR	*hTERT*	diagnostic
[391]	21 EOC 24 BOD	36	Switzerland	serum plasma	concentration	qPCR	*GADPH* *MTATP 8*	diagnostic
[211]	33	33	USA	plasma	methylation	MethDet56	*BRCA1 HIC1 PAX5 PGR THBS1*	diagnostic
[200]	164 EOC 49 BOD	75	USA	plasma	concentration	qPCR	*GADPH*	diagnostic prognostic
[212]	106	–	USA	serum	methylation	MS-PCR	*RASSF1A*	prognostic
[392]	126	–	Poland	plasma	concentration	Elisa	*TP53*	prognostic
[393]	30 EOC 30 BOD	30	USA	plasma	methylation	MethDet56		diagnostic
[394]	32	28	Germany	serum	concentration	qPCR		predictive prognostic
[395]	36 EOC 16 BOD	–	South Korea	serum	copy number variations	qPCR	*B2M* *RAB25* *CLDN4* *ABCF2*	prognostic
[215]	36	25	China	serum	methylation	MS-PCR	*SLIT2*	diagnostic
[396]	87 EOC 53 BOD	62	China	serum	methylation	Multiplex MS-PCR	*APC* *RASSF1A* *CDH1* *RUNX3* *TFPI2* *SFRP5* *OPCML*	diagnostic
[397]	100	–	India	plasma	concentration	qPCR	*GADPH*	prognostic
[201]	144	–	Denmark	plasma	concentration	qPCR	*Cyclophilin A*	prognostic
[213]	47 EOC 14 BOD	10	China	plasma	methylation	MS-PCR	*RASSF1A*	diagnostic
[398]	22	–	USA	serum	mutation	droplet digital PCR	*TP53* *ANO1* *KRAS**	diagnostic prognostic
[203]	36 EOC 22 BOD	19	China	serum	concentration	bDNA technique	*CA125* *HE-4*	diagnostic
[399]	10	–	USA	plasma	Chromosomal rearrengement	qPCR	*β-actin*	diagnostic
[400]	40	–	UK	plasma	mutation	digital PCR	*TP53*	prognostic
[401]	30	–	Australia	plasma	mutation	Targeted amplicon sequencing	*BRCA1/2*	predictive
[402]	128	–	Greece	plasma	methylation	MS-PCR MS-HRMA	*RASSF1A*	prognostic
[214]	71 EOC 43 BOD	80	China	serum	methylation	MS-PCR	*OPCML*	diagnostic
[403]	19	–	USA	serum	mutation	Massively paralel sequencing	*BRCA1* *BRCA2*	prognostic
[404]	27 EOC 119 BOD	21	UK	serum	methylation	Targeted bisulfite sequencing		diagnostic prognostic
[405]	50	–	Greece	plasma	methylation	MS-PCR	*ESR1*	prognostic
[205]	67	–	Slovakia	plasma	concentration	Qubit		prognostic
[29]	102	–	South Korea	plasma	mutation concentration	droplet digital PCR	*TP53*	prognostic
[209]	33	–	Japan	plasma	mutation	droplet digital PCR	*PIK3CA* *KRAS*	diagnostic predictive
[406]	4	–	South Korea	plasma	mutation	digital PCR	*TP53*	diagnostic

qPCR – quantitative PCR, EOC – epithelial ovarian cancer, BOD – benign ovarian cancer, F-SCCP – fluorescence-based single-strand conformation polymorphism, MS-PCR – methylation specific PCR.

* Complete list of analyzed genes [398]: *FGFR, SPEG, RBKS, TRANK1, TET1, RANGAP, PIK3CA, CELSR1, CDK12, ASPH, ODZ2, PYGM, EPHB6, FANCL, PDGFB, FBXW7, RBM19, MET, TGFBR2, TNC, XRCC3, PTEN, BRAF.*

tumor tissue and plasma was observed by Wyatt et al. All somatic mutations identified in metastatic tissue were also present in matched plasma cfDNA [226].

Besides plasma and serum, seminal cfDNA can be processed as well. As shown by Ponti et al., higher seminal cfDNA levels in cancer patients (about 2200 ng/ul) were recorded, while healthy individuals showed only about 60 ng/ul [227].

Since promoter methylation of glutathione-S-transferase (*GSTP1*)

Table 10
Overview of the studies investigating cfDNA in relation with prostate cancer's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[407]	69 PC 31 BPH	–	Portugal	urine plasma	methylation	MS-PCR	*GSTP1*	prognostic
[220]	37	–	The UK	plasma	concentration	qPCR	β-globin	diagnostic
[225]	31	18	Germany	plasma	concentration methylation	qPCR MS-PCR	*GSTP1*	diagnostic
[219]	142	19	Germany	plasma	concentration	spectrophotometry		diagnostic
[229]	27	13	Greece	plasma	methylation	MS-PCR	*GSTP1* *RASSF1*	diagnostic
[221]	192	–	Germany	serum	concentration	qPCR	*GSTP1* *MDR1* *EBNRB*	prognostic diagnostic
[408]	76	49	Austria	serum	methylation	MS-PCR	*GSTP1* *AR* *14-3-3 σ*	diagnostic
[222]	64	45	Italy	plasma	concentration methylation	qPCR MS-PCR	*GSTP1*	diagnostic prognostic
[230]	10	5	Russia	urine plasma	methylation	MS-PCR	*GSTP1*	diagnostic
[409]	5	22	Russia	plasma	concentration	fluorescence		diagnostic
[410]	168 PC 42 BPH	11	Germany	serum	methylation	MS-PCR	*GSTP1 PTGS2* *Reprimo TIG1*	diagnostic
[411]	83	40	USA	serum	methylation MSI	qPCR MS-PCR	*RASSF1* *RARB2* *GSTP1*	diagnostic
[412]	20	19	Brazil	plasma	concentration	qPCR	ALU	prognostic
[413]	96 PC 112 BPH	–	China	plasma	concentration DNA integrity	qPCR	ALU	diagnostic
[61]	17	25	Austria	plasma	CNV	whole genome sequencing		prognostic predictive
[231]	98	47	China	serum	methylation	MS-PCR	*CDH13*	predictive prognostic
[414]	62	–	Canada	plasma	amplification concentration	aCGH Qubit	AR	predictive
[415]	59	–	Germany	serum	concentration	spectrophotometry		predictive prognostic
[232]	82	–	USA	serum	methylation	pyrosequencing	*GADD45a*	diagnostic
[416]	97	–	Italy	plasma	CNV mutation	targeted NGS		prognostic
[417]	204 PC 10 prostatitis 10 BPH	207	Canada	serum	concentration CIN	NGS		diagnostic
[224]	50 PC 25 BPH	30	Egypt	plasma	concentration DNA integrity	qPCR	ALU	diagnostic
[418]	19	–	USA	urine	CNV	whole genome sequencing		predictive prognostic
[419]	49	–	UK	plasma	mutations concentration	targeted sequencing whole genome sequencing	*ATM* *BRCA2* *PALB2*	predictive prognostic
[226]	45	–	Canada	plasma	mutation CNV	targeted sequencing	*TP53* *PTEN* *RB1* *APC* *CDKN1B* *BRCA2* *PIK3R1*	prognostic predictive
[217]	47	20	Netherlands	plasma	methylation	MS-PCR	*GSTP1* *APC*	prognostic
[218]	76 PC 50 BPH	–	Iran	plasma	concentration	spectrophotometry		diagnostic
[3]	70	–	USA	plasma	amplification	digital PCR	*AR*	prognostic
[223]	571	–	UK	plasma	concentration	spectrophotometry		prognostic
[227]	6	3	Italy	seminal plasma	concentration	electrophoresis		diagnostic

RFLP PCR – restriction fragment length polymorphism PCR, qPCR – quantitative PCR, MS-PCR – methylation specific PCR, aCGH – array comparative genome hybridization, NGS – next generation sequencing.
*Complete list of analyzed genes [226]: *ARID1A, HSD3B1, MDM4, AKT3, MSH2,MSH6, ERCC3, NFE2L2, IDH1, FANCD2, MLH1, CTNNB1, FOXP1, RYBP, PIK3CB, ATR, PIK3CA, FBXW7, PIK3R1, CHD1, APC, FANCE, CDK6, MET, BRAF, CUL1, KMT2C, NKX3-1, CLU, NCOA2, MYC, CDKN2A, FANCG, FANCC, PTEN, FANCF, CCND1, ATM, ZBTB16, CDKN1B, KRAS, KMT2D, CDK4, MDM2, BRCA2, RB1, ERCC5, FOXA1, RAD51B, AKT1, IDH2, ERCC4, ZFHX3, FANCA, TP53, CDK12, BRCA1, SPOP, RNF43, RAD51C, AKT2, ERCC2, ERCC1, ASXL1, GNAS, RUNX1, ERG, TMPRSS2, KDM6A, AR, MED12, SMARCA1*.

was observed in prostate tumor tissues, several studies focused on this signature in cfDNA [225,228]. Hypermethylation of *GSTP1* gene in cfDNA was observed in 75% of newly diagnosed prostate cancer patients and in 37% of patients undergoing therapy, however none of the healthy individuals bore the hypermethylation of *GSTP1* in their cfDNA [229]. Promoter methylation of *GSTP1* gene was also studied in

urine. The C-phosphate-G (CPGs) promoter loci of the *GSTP1* gene in all prostate cancer patients were methylated while no methylation was observed in the group of healthy volunteers and patients with BPH [230]. Plasma hypermethylation of *CDH13* was observed in 50% prostate cancer patients while in none of the healthy subjects. The presence of *CDH13* methylation was associated with increased Gleason score (grading system used to determine the aggressiveness of prostate cancer), advanced stage of the tumor and a higher level of PSA. Moreover, plasma cfDNA methylation of *CDH13* was associated with worse OS in I—IV stage patients [231]. Serum *GADD45A* methylation may also distinguish patients with benign prostate disease and cancer patients [232]. *GADD45A* cfDNA methylation was significantly higher in malignant than in benign patients and authors postulated that combination of PSA and cfDNA level could represent promising tool for non-invasive distinguishing of a benign and malignant status of the disease [232].

CfDNA plasma level showed the potential as a diagnostic and prognostic biomarker in patients with prostate cancer with most promising the detection of *GSTP1* methylation that described large differences between patients and healthy controls.

3.2.6. Head and neck cancer

The detection and quantification of cfDNA in body fluids of patients with head and neck cancer are also feasible. The cfDNA level was higher in patients with head and neck squamous cell carcinoma (HNSCC) and also showed significantly greater DNA integrity index than healthy subjects [233] (Table 11). Mazurek et al. compared cfDNA levels between HNSCC patients and specific clinical characteristics. The cfDNA levels were higher in patients with lymph node metastases status than in patients with N0. The higher cfDNA levels were also observed in patients with IV stage than in I-III stages. Moreover, patients with oropharyngeal squamous cell carcinoma (OPSCC) displayed higher cfDNA levels than patients with HNSCC [234].

Regarding the presence of mutations in cfDNA, feasibility to detect *TP53* mutations in plasma samples of HNSCC patients was recently proved. Van Ginkel et al. first determined presence of mutation *TP53* in tumor tissue from six patients with a high stage (II-IV). After that, authors designed specific droplet digital PCR (ddPCR) assay and examined the presence of this mutation in pretreatment plasma of HNSCC patients. In all patients, *TP53* mutation was detected in tumor tissue by NGS and in plasma by ddPCR. Authors showed that detection of *TP53* in plasma using ddPCR is technically feasible [235].

Significantly higher plasma cfDNA levels were associated with smoking habits and consumption of alcohol [236]. Recently, Zwirner et al. recorded that cfDNA levels during cancer treatment may be influenced by infection or drugs (antibiotics) intake [237].

Molecular evidence suggests a role for human papillomavirus (HPV) in the pathogenesis of OPSCC [238], [239]. There was significant effort to identify HPV sequence in cfDNA. Wang et al. attempted to identify HPV in cfDNA isolated from plasma and saliva. From 21 patients with HPV positive tumors, 18 (86%) patients showed HPV sequence in plasma cfDNA. The concordance between saliva and tumor was lower, about 40% [240].

In patients with thyroid cancer, higher plasma cfDNA level was also observed. CfDNA level significantly correlated with stages: in the early stage the median level was 20.67 ng/ml while for late stages, the median was 37.45 ng/ml) [241]. However, the possibility to detect mutation is still questionable. Kwak et al. detected *BRAF* mutation

Table 11
Overview of the studies investigating cfDNA in relation with head and neck cancer's clinical outcome.

References	Patients	Controls	Origin of the study	Source of cfDNA	Study focus	Methodology	Target	Clinical relevance
[233]	58	47	USA	plasma	DNA integrity	qPCR	β-actin	diagnostic
[234]	200	–	Caucasians	plasma	concentration	qPCR	*hTERT*	diagnostic
[235]	6	–	Netherlands	plasma	concentration point mutation	droplet digital PCR	*TP53*	diagnostic
[236]	50	50	India	plasma	concentration	qPCR	*GAPDH*	diagnostic
[240]	93	–	USA	plasma saliva	mutation	qPCR	*HPV16* *HPV18*	diagnostic
[420]	116	–	Germany	plasma	CNA	NGS		prognostic
[237]	20	–	Germany	plasma	concentration	Qubit		prognostic
[421]	103 PTC 16 NNT	49	Italy	plasma	mutation	qPCR	*BRAF*	prognostic
[244]	28	–	USA	serum	mutation	qPCR	*BRAF*	prognostic
[243]	193	–	USA	serum	mutation	Real-time allele specific PCR	*BRAF*	diagnostic
[422]	96	–	USA	serum	methylation	MS-PCR	*β-actin* *CALCA* *CDH1* *TIMP3* *DAPK* *RARβ2*	diagnostic prognostic
[242]	94	–	South Korea	serum	mutation	qPCR	*BRAF*	diagnostic
[241]	181	19	Italy	plasma	concentration mutation	qPCR MS-PCR	*SLC5A* *SLC26A4* *BRAF* *ALU244* *ALU83*	diagnostic
[423]	97	49	Italy	plasma	concentration DNA integrity	qPCR	*APP*	diagnostic prognostic
[245]	75	–	USA	plasma	mutation	droplet digital PCR	*RET*	diagnostic
[424]	13 malignant 43 benign	–	USA	plasma	mutation	Illumina Miseq	*KRAS* *BRAF* *CTNNB1* *FOXL2* *GNAS* *NRAS* *PIK3CA* *TP53*	diagnostic

qPCR – quantitative PCR, NGS – next generation sequencing, PTC – papillary thyroid carcinoma, NNT - non-nodular thyroid, MS-PCR – methylation-specific PCR.

(T1799A) in serum cfDNA in patients with thyroid cancer while no mutation was found in patients with benign disease. In the contrary, none of the patients had detectable *BRAF* mutation V600E in their serum samples although all of these patients bearded this mutation in their tumor tissue [242]. Authors hypothesized that different results were obtained due to the use of another analytical method and moreover pointed to possibility of false positive result in previous studies [243,244]. The aggressive form of thyroid cancer, medullary thyroid carcinoma (MTC), is caused by germinal mutation of the *RET* gene. In the study of Cote et al., authors analyzed the detection of *RET* M918 T mutation in plasma samples. *RET* (M918 T) mutation was detected in 32% plasma samples with a positive *RET* mutation tissue biopsy. Authors observed that patients with a IV stage were more likely to have a positive *RET* cfDNA assay and postulated that the presence of *RET* mutation in cfDNA might indicate poor survival [245].

Beside the cfDNA concentration and the possibility to detect the mutation in cfDNA, the correlation between external factors (like smoking and alcohol consumption) and the cfDNA level was observed in head and neck cancer.

4. Conclusion

The aim of this review was to sum up previous studies focusing on cfDNA and cancer and to illustrate the potential of cfDNA as a cancer biomarker. In the line with the findings from other studies, cfDNA concentration is higher in cancer patients than in patients with benign diseases or in healthy subjects. Recent studies proved that the same mutations located in tumor tissues can be detected in plasma/serum samples. Moreover, most of the articles showed direct connection with patients' prognosis – higher cfDNA level in plasma/serum is associated with worse patients' outcome.

Tissue biopsy possesses several limitations such as invasiveness, high financial cost and can cause pain and risk for patients. Liquid biopsy, especially analysis of cfDNA, has rapidly emerged as a new approach with promising clinical application due to its favorable characteristics like a lack of risk, minimal invasiveness, low-cost. The overviewed results support the possibility to apply cfDNA as a cancer biomarker for early diagnosis, evaluation of treatment response and overall? prognosis of cancer patients. Prior to its clinical applications, several important issues must be taken into consideration. One of the important limitations is lower specificity and sensitivity in comparison with tissue biopsy thus it is critical to standardized pre-analytical methodologies (including body fluid collection, extraction of cfDNA and storage). Today, the potential of cfDNA is validated by several clinical trials [425–427].

Declarations of interest

None.

Acknowledgements

This study was supported by the Grant Agency of the Charles University (GAUK 302119), the Grant Agency of the Ministry of Health of the Czech Republic (AZV 17- 30920A), Charles University Research Centre program UNCE/MED/006 " University Center of Clinical and Experimental Liver Surgery" and by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic. This article is based upon work from COST Action CA17118, supported by COST (European Cooperation in Science and Technology, www.cost.eu).

References

[1] N. Brychta, T. Krahn, O. Von Ahsen, Detection of KRAS mutations in circulating tumor DNA by digital PCR in early stages of pancreatic cancer, Clin. Chem. 62 (11) (2016) 1482–1491, https://doi.org/10.1373/clinchem.2016.257469 PMID: 27591291.

[2] M. Chimonidou, A. Strati, N. Malamos, V. Georgoulias, E.S. Lianidou, SOX17 promoter methylation in circulating tumor cells and matched cell-free DNA isolated from plasma of patients with breast cancer, Clin. Chem. 59 (1) (2013) 270–279, https://doi.org/10.1373/clinchem.2012.191551 PMID: 23136251.

[3] M. Kohli, J. Li, M. Du, D.W. Hillman, S.M. Dehm, W. Tan, et al., Prognostic association of plasma cell-free DNA-based androgen receptor amplification and circulating tumor cells in pre-chemotherapy metastatic castration-resistant prostate cancer patients, Prostate Cancer Prostatic Dis. (2018), https://doi.org/10.1038/s41391-018-0043-z 1–8. PMID: 29858592.

[4] J. Atamaniuk, C. Kopecky, S. Skoupy, M.D. Saemann, T. Weichhart, Apoptotic cell-free DNA promotes inflammation in haemodialysis patients, Nephrol. Dial. Transplant. 27 (3) (2012) 902–905, https://doi.org/10.1093/ndt/gfr695 PMID:22167588.

[5] S. Tug, S. Helmig, J. Menke, D. Zahn, T. Kubiak, A. Schwarting, P. Simon, Correlation between cell free DNA levels and medical evaluation of disease progression in systemic lupus erythematosus patients, Cell. Immunol. 292 (1–2) (2014) 32–39, https://doi.org/10.1016/j.cellimm.2014.08.002 PMID: 25243646.

[6] Z.H. Huang, L.H. Li, D. Hua, Quantitative analysis of plasma circulating DNA at diagnosis and during follow-up of breast cancer patients, Cancer Lett. 243 (1) (2006) 64–70, https://doi.org/10.1016/j.canlet.2005.11.027 PMID: 16412565.

[7] K. Kim, D.G. Shin, M.K. Park, S.H. Baik, T.H. Kim, S. Kim, S. Lee, Circulating cell-free DNA as a promising biomarker in patients with gastric cancer: diagnostic validity and significant reduction of cfDNA after surgical resection, Ann. Surg. Treat. Res. 86 (3) (2014) 136, https://doi.org/10.4174/astr.2014.86.3.136 PMID: 24761422.

[8] B. Shapiro, M. Chakrabarty, E.M. Cohn, S.A. Leon, Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease, Cancer 51 (11) (1983) 2116–2120, https://doi.org/10.1002/1097-0142(19830601)51:11<2116::AID-CNCR2820511127>3.0.CO;2-S PMID: 6188527.

[9] X. Wang, X.Q. Shi, P.W. Zeng, F.M. Mo, Z.H. Chen, Circulating cell free DNA as the diagnostic marker for colorectal cancer: a systematic review and meta-analysis, Oncotarget (2018), https://doi.org/10.18632/oncotarget.25314 PMID: 29849957.

[10] P. Mandel, P. Metais, Les acides nucleiques du plasma sanguin chez l'homme, Comptes Rendus Des Séances de La Société de Biologie et de Ses Filiales 142 (3–4) (1948) 241–243 PMID: 18875018.

[11] S.A. Leon, B. Shapiro, D.M. Sklaroff, M.J. Yaros, Free DNA in the serum of cancer patients and the effect of therapy, Cancer Res. 37 (3) (1977) 646–650 PMID: 837366.

[12] J.Y. Wang, J.S. Hsieh, M.Y. Chang, T.J. Huang, F.M. Chen, T.L. Cheng, et al., Molecular detection of APC, K-ras, and p53 mutations in the serum of colorectal Cancer patients as circulating biomarkers, World J. Surg. 28 (7) (2004) 721–726, https://doi.org/10.1007/s00268-004-7366-8 PMID: 15185002.

[13] J.A. Shaw, B.M. Smith, T. Walsh, S. Johnson, L. Primrose, M.J. Slade, et al., Microsatellite alterations plasma DNA of primary breast cancer patients, Clin Cancer Res: An Official Journal of the American Association for Cancer Research 6 (March) (2000) 1119–1124 PMID: 10741742.

[14] K. Fujiwara, N. Fujimoto, M. Tabata, K. Nishii, K. Matsuo, K. Hotta, et al., Identification of epigenetic aberrant promoter methylation in serum DNA is useful for early detection of lung cancer, Clin. Cancer Res. 11 (3) (2005) 1219–1225, https://doi.org/10.1378/chest.111.6.1710 PMID: 15709192.

[15] Y.H. Su, M. Wang, D.E. Brenner, P.A. Norton, T.M. Block, Detection of mutated K-ras DNA in urine, plasma, and serum of patients with colorectal carcinoma or adenomatous polyps, Ann. N. Y. Acad. Sci. 1137 (1) (2008) 197–206, https://doi.org/10.1196/annals.1448.027 PMID: 18837947.

[16] W. Yao, C. Mei, X. Nan, L. Hui, Evaluation and comparison of in vitro degradation kinetics of DNA in serum, urine and saliva: a qualitative study, Gene 590 (1) (2016) 142–148, https://doi.org/10.1016/j.gene.2016.06.033 PMID: 27317895.

[17] D.A. Haber, V.E. Velculescu, Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA, Cancer Disc. American Association for Cancer Research. (2014), https://doi.org/10.1158/2159-8290.CD-13-1014 PMID: 24801577.

[18] S. Jahr, H. Hentze, S. Englisch, D. Hardt, F.O. Fackelmayer, R. Hesch, R. Knippers, DNA fragments in the blood plasma of cancer patients : quantitations and evidence for their origin from apoptotic and necrotic cells, Cancer Res. 61 (4) (2001) 1659–1665, https://doi.org/10.1016/0022-1759(75)90106-4 PMID: 11245480.

[19] V. Vymetalkova, K. Cervena, L. Bartu, P. Vodicka, Circulating cell-free DNA and colorectal Cancer: a systematic review, Int. J. Mol. Sci. 19 (11) (2018) 3356, https://doi.org/10.3390/ijms19113356 PMID: 30373199.

[20] H.C. Fan, Y.J. Blumenfeld, U. Chitkara, L. Hudgins, S.R. Quake, Analysis of the size distributions of fetal and maternal cell-free DNA by paired-end sequencing, Clin. Chem. 56 (8) (2010) 1279–1286, https://doi.org/10.1373/clinchem.2010.144188 PMID: 20558635.

[21] D. Madhavan, M. Wallwiener, K. Bents, M. Zucknick, J. Nees, S. Schott, et al., Plasma DNA integrity as a biomarker for primary and metastatic breast cancer and potential marker for early diagnosis, Breast Cancer Res. Treat. 146 (1) (2014) 163–174, https://doi.org/10.1007/s10549-014-2946-2 PMID: 24838941.

[22] F. Mouliere, N. Rosenfeld, Circulating tumor-derived DNA is shorter than somatic DNA in plasma, Proc Natl Acad Sci U S A 112 (11) (2015) 3178–3179, https://doi.org/10.1073/pnas.1501321112 PMID: 25733911.

[23] A. Esposito, C. Criscitiello, D. Trapani, G. Curigliano, The emerging role of 'Liquid biopsies,' circulating tumor cells, and circulating cell-free tumor DNA in lung Cancer diagnosis and identification of resistance mutations, Curr. Oncol. Rep. 19 (1) (2017) 1, https://doi.org/10.1007/s11912-017-0564-y PMID: 28110461.

[24] J. Phallen, M. Sausen, V. Adleff, A. Leal, C. Hruban, et al., Direct detection of early-stage cancers using circulating tumor DNA, Sci. Transl. Med. 9 (403) (2017), https://doi.org/10.1126/scitranslmed.aan2415 PMID: 28814544.
[25] T.H. Lee, L. Montalvo, V. Chrebtow, M.P. Busch, Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma, Transfusion 41 (2) (2001) 276–282, https://doi.org/10.1046/j.1537-2995.2001.41020276.x PMID: 11239235.
[26] Y.M.D. Lo, M.S.C. Tein, T.K. Lau, C.J. Haines, T.N. Leung, P.M.K. Poon, et al., Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis, Am J Hum Gen 62 (4) (1998) 768–775, https://doi.org/10.1086/301800 PMID: 9529358.
[27] M. Jung, S. Klotzek, M. Lewandowski, M. Fleischhacker, K. Jung, Changes in concentration of DNA in serum and plasma during storage of blood samples, Clin. Chem. 49 (6 Pt 1) (2003) 1028–1029, https://doi.org/10.1373/49.6.1028 PMID: 12766024.
[28] A.J. Bronkhorst, J. Aucamp, P.J. Pretorius, Cell-free DNA: preanalytical variables, Clin. Chim. Acta 450 (2015) 243–253, https://doi.org/10.1016/j.cca.2015.08.028 PMID: 26341895.
[29] Y.M. Kim, Y.J. Lee, S.W. Lee, H.Y. Lee, J.E. Lee, E.K. Choi, Prospective study of the efficacy and utility of TP53 mutations in circulating tumor DNA as a non-invasive biomarker of treatment response monitoring in patients with high-grade serous ovarian carcinoma, J. Gynecol. Oncol. 30 (3) (2018), https://doi.org/10.3802/jgo.2019.30.e32 PMID: 30887755.
[30] Won D. Ko, T.D. Jeong, W. Lee, S. Chun, W.K. Min, Comparison of red blood cell hemolysis using plasma and serum separation tubes for outpatient specimens, Ann. Lab. Med. 35 (2) (2015) 194, https://doi.org/10.3343/alm.2015.35.2.194 PMID: 25729720.
[31] S. Volik, M. Alcaide, R.D. Morin, C. Collins, Cell-free DNA (cfDNA): clinical significance and utility in Cancer Shaped by emerging technologies, Mol. Cancer Res. 14 (10) (2016) 898–908, https://doi.org/10.1158/1541-7786.MCR-16-0044 PMID: 27422709.
[32] N. Umetani, A. Hiramatsu, D.S.B. Hoon, Higher amount of free circulating DNA in serum than in plasma is not mainly caused by contaminated extraneous DNA during separation, Ann. N. Y. Acad. Sci. 1075 (1) (2006) 299–307, https://doi.org/10.1196/annals.1368.040 PMID: 17108224.
[33] A. Vallée, M. Marcq, A. Bizieux, C.E. Kouri, H. Lacroix, J. Bennouna, J.Y. Douillard, M.G. Denis, Plasma is a better source of tumor-derived circulating cell-free DNA than serum for the detection of EGFR alterations in lung tumor patients, Lung Cancer 82 (2) (2013) 373–374, https://doi.org/10.1016/j.lungcan.2013.08.014 PMID: 24007628.
[34] Y. Shu, X. Wu, X. Tong, X. Wang, Z. Chang, Y. Mao, et al., Circulating tumor DNA mutation profiling by targeted next generation sequencing provides guidance for personalized treatments in multiple Cancer types, Sci. Rep. 7 (1) (2017) 583, https://doi.org/10.1038/s41598-017-00520-1 PMID: 28373672.
[35] S. Roychowdhury, M.K. Iyer, D.R. Robinson, R.J. Lonigro, Y.M. Wu, et al., Personalized oncology through integrative high-throughput sequencing: a pilot study, Sci. Transl. Med. 3 (111) (2011), https://doi.org/10.1126/scitranslmed.3003161 PMID: 22133722.
[36] E. Yong, Cancer biomarkers: written in blood, Nature 511 (7511) (2014) 524–526, https://doi.org/10.1126/scitranslmed.3003161 PMID: 22133722.
[37] L.A. Diaz, A. Bardelli, Liquid biopsies: genotyping circulating tumor DNA, J. Clin. Oncol. 32 (6) (2014) 579–586, https://doi.org/10.1126/scitranslmed.3003161 PMID: 22133722.
[38] S.J. Dawson, D.W.Y. Tsui, M. Murtaza, H. Biggs, O.M. Rueda, S.F. Chin, et al., Analysis of circulating tumor DNA to monitor metastatic breast Cancer, N Eng J Med 368 (13) (2013) 1199–1209, https://doi.org/10.1056/NEJMoa1213261 PMID: 23484797.
[39] S. Khakoo, A. Georgiou, M. Gerlinger, D. Cunningham, N. Starling, Circulating tumour DNA, a promising biomarker for the management of colorectal cancer, Crit. Rev. Oncol. Hematol. 122 (2018) 72–82, https://doi.org/10.1016/j.critrevonc.2017.12.002 PMID: 29458792.
[40] M.F. Sanmamed, S. Ferández-Landázuri, C. Rodríguez, R. Zárate, M.D. Lozano, et al., Quantitative cell-free circulating BRAFV600E mutation analysis by use of droplet digital PCR in the follow-up of patients with melanoma being treated with BRAF inhibitors, Clin. Chem. 61 (1) (2015) 297–304, https://doi.org/10.1373/clinchem.2014.230235 PMID: 25411185.
[41] G. Zhu, X. Ye, Z. Dong, Y.C. Lu, Y. Sun, Y. Liu, R. McCormack, Y. Gu, X. Liu, Highly sensitive droplet digital PCR method for detection of EGFR-Activating mutations in plasma cell–Free DNA from patients with advanced non–Small cell lung Cancer, J. Mol. Diagn. 17 (3) (2015) 265–272, https://doi.org/10.1016/j.jmoldx.2015.01.004 PMID: 25769900.
[42] E. Day, P.H. Dear, F. McCaughan, Digital PCR strategies in the development and analysis of molecular biomarkers for personalized medicine, Methods 59 (1) (2013) 101–107, https://doi.org/10.1016/j.ymeth.2012.08.001 PMID: 22926236.
[43] J.F. Huggett, A. Whale, Digital PCR as a novel technology and its potential implications for molecular diagnostics, Clin. Chem. 59 (12) (2013) 1691–1693, https://doi.org/10.1373/clinchem.2013.214742 PMID: 24100808.
[44] X. Yi, J. Ma, Y. Guan, R. Chen, L. Yang, X. Xia, "The feasibility of using mutation detection in ctDNA to assess tumor dynamics.,", Int. J. Cancer 140 (June 12) (2017) 2642–2647, https://doi.org/10.1002/ijc.30620 PMID: 28124376.
[45] V. Taly, D. Pekin, L. Benhaim, S.K. Kotsopoulos, D. Le Corre, X. Li, et al., Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from the plasma of colorectal Cancer patients, Clin. Chem. 59 (12) (2013) 1722–1731, https://doi.org/10.1373/clinchem.2013.206359 PMID: 23938455.
[46] S.V. Bratman, A.M. Newman, A.A. Alizadeh, M. Diehn, Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq, Expert Rev. Mol. Diagn. 15 (6) (2015) 715–719, https://doi.org/10.1586/14737159.2015.1019476 PMID: 25773944.
[47] X. Yi, J. Ma, Y. Guan, R. Chen, L. Yang, X. Xia, The feasibility of using mutation detection in ctDNA to assess tumor dynamics, Int. J. Cancer 140 (12) (2017) 2642–2647, https://doi.org/10.1002/ijc.30620 PMID: 28124376.
[48] F. Diehl, K. Schmidt, M.A. Choti, K. Romans, S. Goodman, M. Li, et al., Circulating mutant DNA to assess tumor dynamics, Nat. Med. 14 (9) (2008) 985–990, https://doi.org/10.1038/nm.1789 PMID: 18670422.
[49] K.L.G. Spindler, N. Pallisgaard, I. Vogelius, A. Jakobsen, Quantitative cell-free DNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal Cancer during treatment with Cetuximab and irinotecan, Clin. Cancer Res. 18 (4) (2012) 1177–1185, https://doi.org/10.1158/1078-0432.CCR-11-0564 PMID: 22228631.
[50] S. Schlücker, Surface-enhanced raman spectroscopy: concepts and chemical applications, Angew. Chemie Int. Ed. 53 (19) (2014) 4756–4795, https://doi.org/10.1002/anie.201205748 PMID: 24711218.
[51] M.J. Mosko, A.A. Nakorchevsky, E. Flores, H. Metzler, M. Ehrich, et al., Ultrasensitive detection of multiplexed somatic mutations using MALDI-TOF mass spectrometry, J. Mol. Diagn. 18 (1) (2016) 23–31, https://doi.org/10.1016/j.jmoldx.2015.08.001 PMID: 26596526.
[52] C. Bettegowda, M. sausen, R.J. Leary, I. Kinde, Y. Wang, N. Agrawal, et al., Detection of circulating tumor DNA in early- and late-stage human malignancies, Sci. Transl. Med. 6 (224) (2014), https://doi.org/10.1126/scitranslmed.3007094 PMID: 24553385.
[53] A. Narayan, N.J. Carriero, S.N. Gettinger, J. Kluytenaar, K.R. Kozak, et al., Ultrasensitive measurement of hotspot mutations in tumor DNA in blood using error-suppressed multiplexed deep sequencing, Cancer Res. 72 (14) (2012) 3492–3498 Jul. 2012. PMID: 22581825 10.1158%2F0008-5472.CAN-11-4037.
[54] R. Lebofsky, C. Decraene, V. Bernard, M. Kamal, A. Blin, Q. Leroy, et al., Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types, Mol. Oncol. 9 (4) (2015) 783–790, https://doi.org/10.1016/j.molonc.2014.12.003 PMID: 25579085.
[55] J.S. Frenel, S. Carreira, J. Goodall, D. Roda, R. Perez-Lopez, N. Tunariu, et al., Serial next-generation sequencing of circulating cell-free DNA evaluating tumor clone response to molecularly targeted drug administration, Clin. Cancer Res. 21 (20) (2015) 4586–4596, https://doi.org/10.1158/1078-0432.CCR-15-0584 PMID: 26085511.
[56] J. Tabernero, H.J. Lenz, S. Siena, A. Sobreto, A. Falcone, M. Ychou, et al., Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal cancer: a retrospective, exploratory analysis of the CORRECT trial, Lancet Oncol. 16 (8) (2015) 937–948, https://doi.org/10.1016/S1470-2045(15)00138-2 PMID: 26184520.
[57] T. Forshew, M. Murtaza, C. Parkinson, D. Gale, D.W.Y. Tsui, F. Kaper, et al., Noninvasive identification and monitoring of Cancer mutations by targeted deep sequencing of plasma DNA, Sci. Transl. Med. 4 (136) (2012), https://doi.org/10.1126/scitranslmed.3003726 136ra68-136ra68. PMID: 22649089.
[58] A.M. Newman, S.V. bratman, J. to, J.F. Wynne, N.C. Eclov, L.A. Modlin, et al., An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage, Nat. Med. 20 (5) (2014) 548–554, https://doi.org/10.1038/nm.3519 PMID: 24705333.
[59] W. Lv, X. Wei, R. guo, Q. Liu, Y. Zheng, J. Chang, T. Bai, et al., Noninvasive prenatal testing for Wilson disease by use of circulating single-molecule amplification and resequencing technology (cSMART), Clin. Chem. 61 (1) (2015) 172–181, https://doi.org/10.1373/clinchem.2014.229328 PMID: 25376582.
[60] R.J. Leary, M. Sausen, I. Kinde, N. Papadopoulos, J.D. Carpten, D. Craig, et al., Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing, Sci. Transl. Med. 4 (162) (2012), https://doi.org/10.1126/scitranslmed.3004742 PMID: 23197571.
[61] E. Heitzer, P. Ulz, J. Belic, S. Gutschi, F. Quehenberger, K. Fischereder, et al., Tumor-associated copy number changes in the circulation of patients with prostate cancer identified through whole-genome sequencing, Genome Med. 5 (4) (2013), https://doi.org/10.1186/gm434 PMID: 23561577.
[62] E. Heitzer, M. Auer, E.M. Hoffmann, M. Pichler, C. Gasch, P. Ulz, et al., Establishment of tumor-specific copy number alterations from plasma DNA of patients with cancer, Int. J. Cancer 133 (2) (2013) 346–356, https://doi.org/10.1002/ijc.28030 PMID: 23319339.
[63] V.L. Costa, R. Henrique, S.A. Danielsan, S. Duarte-Pereira, M. Eknaes, et al., Three epigenetic biomarkers, GDF15, TMEFF2, and VIM, accurately predict bladder cancer from DNA-based analyses of urine samples, Clin. Cancer Res. 16 (23) (2010) 5842–5851, https://doi.org/10.1158/1078-0432.CCR-10-1312 PMID: 20975101.
[64] S. Eissa, M. Swellam, I.M. El-Khouly, S.K. Kassim, H. Shehata, et al., Aberrant methylation of RARbeta2 and APC genes in voided urine as molecular markers for early detection of bilharzial and nonbilharzial bladder cancer, Cancer Epidemiol. Biomarkers Prev. 20 (8) (2011) 1657–1664, https://doi.org/10.1158/1055-9965.EPI-11-0237 Aug. 2011. PMID: 21680534.
[65] T. Reinert, C. Modin, F.M. Castano, P. Lamy, T.K. Wojdacz, et al., Comprehensive genome methylation analysis in bladder cancer: identification and validation of novel methylated genes and application of these as urinary tumor markers, Clin. Cancer Res. 17 (17) (2011) 5582–5592, https://doi.org/10.1158/1078-0432.CCR-10-2659 Sep. 2011. PMID: 21788354.
[66] C.A. Eads, K.D. Danenberg, K. Kawakami, L.B. Saltz, C. Blake, et al., MethyLight: a high-throughput assay to measure DNA methylation, Nucleic Acids Res. 28 (8) (2000), https://doi.org/10.1093/nar/28.8.e32 PMID: 10734209.

[67] N. Umetani, J. Kim, S. Hiramatsu, H.A. Reber, O.J. Hines, A.J. Bilchik, D.S.B. Hoon, Increased integrity of free circulating DNA in sera of patients with colorectal or periampullary cancer: direct quantitative PCR for ALU repeats, Clin. Chem. 52 (6) (2006) 1062–1069, https://doi.org/10.1373/clinchem.2006.068577 PMID: 16723681.
[68] H.R. Hwu, J.W. Roberts, E.H. Davidson, R.J. Britten, Insertion and/or deletion of many repeated DNA sequences in human and higher ape evolution, Proc Natl Acad Sci U S A 83 (11) (1986) 3875–3879, https://doi.org/10.1073/pnas.83.11.3875 PMID: 3012563.
[69] J. Cheng, T. Holland-Letz, M. Wallwiener, H. Surowy, K. Cuk, S. Schott, et al., Circulating free DNA integrity and concentration as independent prognostic markers in metastatic breast cancer, Breast Cancer Res. Treat. 169 (1) (2018) 69–82, https://doi.org/10.1007/s10549-018-4666-5 PMID: 29340881.
[70] V. Vasioukhin, P. Anker, P. Maurice, J. Lyautey, C. Lederrey, M. Stroun, Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia, Br J Hematol 86 (4) (1994) 774–779, https://doi.org/10.1111/j.1365-2141.1994.tb04828.x PMID: 7918071.
[71] G. Hosny, N. Farahat, P. Hainaut, TP53 mutations in circulating free DNA from Egyptian patients with non-Hodgkin's lymphoma, Cancer Lett. 275 (2) (2009) 234–239, https://doi.org/10.1016/j.canlet.2008.10.029 PMID: 19046801.
[72] J. Quan, Y.J. Gao, Z.L. Yang, H. Chen, J.R. Xian, S.S. Zhang, et al., Quantitative detection of circulating nucleophosmin mutations DNA in the plasma of patients with acute myeloid leukemia, Int. J. Med. Sci. 12 (1) (2015) 17–22, https://doi.org/10.7150/ijms.10144 PMID: 25552914.
[73] S. Zorofchian, G. Lu, J.J. Zhu, D.Y. Duose, J. Windham, Y. Esquenazi, L.Y. Ballester, Detection of the MYD88 p.L265P mutation in the CSF of a patient with secondary central nervous system lymphoma, Front. Oncol. 8 (2018) 382, https://doi.org/10.3389/fonc.2018.00382 PMID: 30294590.
[74] L.S. Hiemcke-Jiwa, R.J. Leguit, J.E.C. Bromberg, S. Nierkens, et al., MYD88 p.(L265P) detection on cell-free DNA in liquid biopsies of patients with primary central nervous system lymphoma, Br. J. Haematol. (2018), https://doi.org/10.1111/bjh.15674 PMID: 30408153.
[75] M. Fontanilles, F. Marguet, É Bohers, P.J. Viailly, S. Dubois, P. Bertrand, et al., Non-invasive detection of somatic mutations using next-generation sequencing in primary central nervous system lymphoma, Oncotarget 8 (29) (2017) 48157–48168, https://doi.org/10.18632/oncotarget.18325 PMID:28636991.
[76] S. Hohaus, M. Giachelia, G. Massini, G. Mansueto, B. Vannata, V. Bozzoli, et al., Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas, Ann. Oncol. 20 (8) (2009) 1408–1413, https://doi.org/10.1093/annonc/mdp006 PMID: 19465421.
[77] L. Mussolin, R. Burnelli, M. Pillon, E. Carraro, P. Farruggia, A. Todesco, et al., Plasma cell-free DNA in paediatric lymphomas, J. Cancer 4 (4) (2013) 323–329, https://doi.org/10.7150/jca.6226 PMID: 23678368.
[78] A.K. Schwarz, M. Stanulla, G. Cario, T. Flohr, R. Sutton, A. Möricke, et al., Quantification of free total plasma DNA and minimal residual disease detection in the plasma of children with acute lymphoblastic leukemia, Ann. Hematol. 88 (9) (2009) 897–905, https://doi.org/10.1007/s00277-009-0698-6 PMID: 19165483.
[79] S. Mueller, S. Holdenrieder, P. Stieber, T. Haferlach, A. Schalhorn, J. Braess, et al., Early prediction of therapy response in patients with acute myeloid leukemia by nucleosomal DNA fragments, BMC Cancer 6 (2006), https://doi.org/10.1186/1471-2407-6-143 PMID: 16734907.
[80] A. Rogers, Y. Joe, T. Manshouri, A. Dey, I. Jilani, F. Giles, et al., Relative increase in leukemia-specific DNA in peripheral blood plasma from patients with acute myeloid leukemia and myelodysplasia, Neoplasia 103 (7) (2004), https://doi.org/10.1182/blood-2003-06-1840 PMID: 14576069.
[81] R.K. Sterling, L. Jeffers, F. Gordon, A.P. Venook, K.R. Reddy, S. Satomura, et al., Utility of Lens culinaris agglutinin-reactive fraction of α-Fetoprotein and des-gamma-Carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma, Clin. Gastroenterol. Hepatol. 7 (1) (2009) 104–113, https://doi.org/10.1016/j.cgh.2008.08.041 PMID: 18849011.
[82] G. Hosny, N. Farahat, H. Tayel, P. Hainaut, Ser-249 TP53 and CTNNB1 mutations in circulating free DNA of Egyptian patients with hepatocellular carcinoma versus chronic liver diseases, Cancer Lett. 264 (2) (2008) 201–208, https://doi.org/10.1016/j.canlet.2008.01.031 PMID: 18313840.
[83] Z. Huang, D. Hua, Y. Hu, Z. Cheng, X. Zhou, Q. Xie, et al., Quantitation of plasma circulating DNA using quantitative PCR for the detection of hepatocellular carcinoma, Pathol. Oncol. Res. 18 (2) (2012) 271–276, https://doi.org/10.1007/s12253-011-9438-z PMID: 21779787.
[84] N. Iizuka, I. Sakaida, T. Moribe, N. Fujita, T. Miura, M. Stark, et al., Elevated levels of circulating cell-free DNA in the blood of patients with hepatitis C virus-associated hepatocellular carcinoma, Anticancer Res. 26 (6) (2006) 4713–4719 PMID: 17214331.
[85] I.H.N. Wong, Y.M.D. Lo, W. Yeo, W.Y. Lau, P.J. Johnson, Frequent p15 promoter methylation in tumor and peripheral blood from hepatocellular carcinoma patients, Clin. Cancer Res. 6 (9) (2000) 3516–3521 PMID: 10999738.
[86] L. Yan, Y. Chen, J. Zhou, H. Zhao, H. Zhang, G. Wang, Diagnostic value of circulating cell-free DNA levels for hepatocellular carcinoma, Int. J. Infect. Dis. 67 (2017) 92–97, https://doi.org/10.1016/j.ijid.2017.12.002 PMID: 29229500.
[87] N. Ren, Q.H. Ye, L.X. Qin, B.H. Zhang, Y.K. Liu, Z.Y. Tang, Circulating DNA level is negatively associated with the long-term survival of hepatocellular carcinoma patients, World J. Gastroenterol. 12 (24) (2006) 3911–3914, https://doi.org/10.3748/wjg.v12.i24.3911 PMID: 16804981.
[88] W. Yeo, N. Wong, W.L. Wong, P.B.S. Lai, S. Zhong, P.J. Johnson, High frequency of promoter hypermethylation of RASSF1A in tumor and plasma of patients with hepatocellular carcinoma, Liver Int. 25 (2) (2005) 266–272, https://doi.org/10.1111/j.1478-3231.2005.01084.x PMID: 15780049.
[89] K.C.A. Chan, P.B.S. Lai, T.S.K. Mok, H.L.Y. Chan, C. Ding, S.W. Yeung, Y.M.D. Lo, Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma, Clin. Chem. 54 (9) (2008) 1528–1536, https://doi.org/10.1373/clinchem.2008.104653 PMID: 18653827.
[90] N.A. Mohamed, E.M. Swify, N.F. Amin, M.M. Soliman, L.M. Tag-Eldin, N.M. Elsherbiny, Is serum level of methylated RASSF1A valuable in diagnosing hepatocellular carcinoma in patients with chronic viral hepatitis C? Arab J. Gastroenterol. 13 (3) (2012) 111–115, https://doi.org/10.1016/j.ajg.2012.06.009 PMID: 23122451.
[91] I.H.N. Wong, Y.M.D. Lo, J. Zhang, C.T. Liew, M.H.L. Ng, N. Wong, et al., Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients, Cancer Res. 59 (1) (1999) 71–73 PMID: 9892188.
[92] I.H.N. Wong, J. Zhang, P.B.S. Lai, W.Y. Lau, Y.M.D. Lo, Quantitative analysis of tumor-derived methylated p16INK4a sequences in plasma, serum, and blood cells of hepatocellular carcinoma patients, Clin. Cancer Res. 9 (3) (2003) 1047–1052 PMID: 12631605.
[93] P. Tangkijvanich, N. Hourpai, P. Rattanatanyong, N. Wisedopas, V. Mahachai, A. Mutirangura, Serum LINE-1 hypomethylation as a potential prognostic marker for hepatocellular carcinoma, Clin. Chim. Acta 379 (1–2) (2007) 127–133, https://doi.org/10.1016/j.cca.2006.12.029 PMID: 17303099.
[94] A. Huang, X. Zhang, S.L. Zhou, Y. Cao, X.W. Huang, J. Fan, et al., Plasma circulating cell-free DNA integrity as a promising biomarker for diagnosis and surveillance in patients with hepatocellular carcinoma, J. Cancer 7 (13) (2016) 1798–1803, https://doi.org/10.7150/jca.15618 PMID: 27698918.
[95] B. Moghimi-Dehkordi, A. Safaee, An overview of colorectal cancer survival rates and prognosis in Asia, World J. Gastrointest. Oncol. 4 (4) (2012) 71, https://doi.org/10.4251/wjgo.v4.i4.71 PMID: 22532879.
[96] R.M. Goldberg, M.L. Rothenberg, E. Van Cutsem, A.B. Benson, C.D. Blanke, R.B. Diasio, et al., The continuum of care: a paradigm for the management of metastatic colorectal cancer, Oncologist 12 (1) (2007) 38–50, https://doi.org/10.1634/theoncologist.12-1-38 PMID: 17227899.
[97] L. Boni, E. Cassinotti, M. Canziani, G. Dionigi, F. Rovera, R. Dionigi, Free circulating DNA as possible tumour marker in colorectal cancer, Surg. Oncol. 16 (Suppl 1) (2007) S29–31, https://doi.org/10.1016/j.suronc.2007.10.004 PMID: 18024018.
[98] E. Flamini, L. Mercatali, O. Nanni, D. Calistri, R. Nunziatini, W. Zoli, et al., Free DNA and carcinoembryonic antigen serum levels: an important combination for diagnosis of colorectal cancer, Clin. Cancer Res. 12 (23) (2006) 6985–6988, https://doi.org/10.1158/1078-0432.CCR-06-1931 PMID: 17145818.
[99] M. Frattini, G. Gallino, S. Signoroni, D. Balestra, L. Battaglia, G. Sozzi, et al., Quantitative analysis of plasma DNA in colorectal cancer patients: a novel prognostic tool, Ann. N. Y. Acad. Sci. 1075 (2006) 185–190, https://doi.org/10.1196/annals.1368.025 PMID: 17108210.
[100] K.L.G. Spindler, N. Pallisgaard, R.F. Andersen, I. Brandslund, A. Jakobsen, Circulating free DNA as biomarker and source for mutation detection in metastatic colorectal cancer, PLoS One 10 (4) (2015) e0108247, , https://doi.org/10.1371/journal.pone.0108247 PMID: 25875772.
[101] S. El Messaoudi, F. Mouliere, S. Du Manoir, C. Bascoul-Mollevi, B. Gillet, M. Nouaille, et al., Circulating DNA as a strong multimarker prognostic tool for metastatic colorectal Cancer patient management care, Clin. Cancer Res. 22 (12) (2016) 3067–3077, https://doi.org/10.1158/1078-0432.CCR-15-0297 PMID: 26847055.
[102] C. Vandeputte, P. Kehagias, H.E. Housni, L. Ameye, J.F. Laes, C. Desmedt, et al., Circulating tumor DNA in early response assessment and monitoring of advanced colorectal cancer treated with a multi-kinase inhibitor, Oncotarget 9 (25) (2018) 17756–17769, https://doi.org/10.18632/oncotarget.24879 PMID: 29707145.
[103] E. Cassinotti, L. Boni, S. Segato, S. Rausei, A. Marzorati, F. Rovera, et al., Free circulating DNA as a biomarker of colorectal cancer, Int. J. Surg. 11 (S1) (2013) S54–S57, https://doi.org/10.1016/S1743-9191(13)60017-5 PMID: 24380554.
[104] H. Schwarzenbach, J. Stoehlmacher, K. Pantel, E. Goekkurt, Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer, Ann. N. Y. Acad. Sci. 1137 (2008) 190–196, https://doi.org/10.1196/annals.1448.025 PMID: 18837946.
[105] M. Frattini, G. Gallino, S. Signoroni, D. Balestra, L. Lusa, L. Battaglia, et al., Quantitative and qualitative characterization of plasma DNA identifies primary and recurrent colorectal cancer, Cancer Lett. 263 (2) (2008) 170–181, https://doi.org/10.1016/j.canlet.2008.03.021 PMID: 18395974.
[106] M. Zitt, H.M. Müller, M. Rochel, V. Schwendinger, M. Zitt, G. Goebel, et al., Circulating cell-free DNA in plasma of locally advanced rectal cancer patients undergoing preoperative chemoradiation: a potential diagnostic tool for therapy monitoring, Dis. Markers 25 (3) (2008) 159–165, https://doi.org/10.1155/2008/598071 PMID: 19096128.
[107] M. Agostini, S. Pucciarelli, M.V. Enzo, P. Del Bianco, M. Briarava, C. Bedin, et al., Circulating cell-free DNA: a promising marker of pathologic tumor response in rectal cancer patients receiving preoperative chemoradiotherapy, Ann. Surg. Oncol. 18 (9) (2011) 2461–2468, https://doi.org/10.1245/s10434-011-1638-y PMID: 21416156.
[108] W. Sun, Y. Sun, M. Zhu, Z. Wang, H. Zhang, Y. Xin, et al., The role of plasma cell-free DNA detection in predicting preoperative chemoradiotherapy response in rectal cancer patients, Oncol. Rep. 31 (3) (2014) 1466–1472, https://doi.org/10.3892/or.2013.294993 PMID: 24378613.
[109] J.V. Schou, F.O. Larsen, B.S. Sørensen, R. Abrantes, A.K. Boysen, J.S. Johansen, et al., Circulating cell-free DNA as predictor of treatment failure after neoadjuvant chemo-radiotherapy before surgery in patients with locally advanced rectal cancer, Ann. Oncol. 29 (3) (2018) 610–615, https://doi.org/10.1093/annonc/mdx778 PMID: 29253083.

[110] C.J. Allegra, J. Jessup, M. Somerfield, S. Hamilton, E.H. Hammond, D.F. Hayes, P.K. McAllister, R.F. Morton, R.L. schilsky, American society of clinical oncology provisional clinical opinion: Testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy, J. Clin. Oncol. 27 (12) (2009) 2091–2096, https://doi.org/10.1200/JCO.2009.21.9170 PMID: 19188670.
[111] A. Lievre, KRAS mutation status is predictive of response to cetuximab therapy in colorectal Cancer, Cancer Res. 66 (8) (2006) 3992–3995, https://doi.org/10.1158/0008-5472.CAN-06-0191 PMID: 16618717.
[112] P. Anker, F. Lefort, V. Vasioukhin, J. Lyautey, C. Lederrey, X. Chen, et al., K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer, Gastroenterology 112 (4) (1997) 1114–1120, https://doi.org/10.1016/S0016-5085(97)70121-5 PMID: 9097993.
[113] A.R. Thierry, F. Mouliere, S. El Messaoudi, C. Mollevi, E. Lopez-Crapez, F. Rolet, et al., Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA, Nat. Med. 20 (4) (2014) 430–435, https://doi.org/10.1038/nm.3511 PMID: 24658074.
[114] A. Herbst, K. Rahmig, P. Stieber, A. Philipp, A. Jung, A. Ofner, et al., Methylation of NEUROG1 in serum is a sensitive marker for the detection of early colorectal cancer, Am. J. Gastroenterol. 106 (6) (2011) 1110–1118, https://doi.org/10.1038/ajg.2011.6 PMID: 21326223.
[115] M. Wallner, A. Herbst, A. Behrens, A. Crispin, P. Stieber, B. Göke, et al., Methylation of serum DNA is an independent prognostic marker in colorectal cancer, Clin. Cancer Res. 12 (24) (2006) 7347–7352, https://doi.org/10.1158/1078-0432.CCR-06-1264 PMID: 17189406.
[116] A.B. Philipp, P. Stieber, D. Nagel, J. Neumann, F. Spelsberg, A. Jung, et al., Prognostic role of methylated free circulating DNA in colorectal cancer, Int. J. Cancer 131 (10) (2012) 2308–2319, https://doi.org/10.1002/ijc.27505 PMID: 22362391.
[117] D. Matthaios, I. Balgkouranidou, A. Karayiannakis, H. Bolanaki, N. Xenidis, K. Amarantidis, et al., Methylation status of the APC and RASSF1A promoter in cell-free circulating DNA and its prognostic role in patients with colorectal cancer, Oncol. Lett. 12 (1) (2016) 748–756, https://doi.org/10.3892/ol.2016.4649 PMID: 27347211.
[118] C. Tham, M. Chew, R. Soong, J. Lim, M. Ang, C. Tang, et al., Postoperative serum methylation levels of *TAC1* and *SEPT9* are independent predictors of recurrence and survival of patients with colorectal cancer, Cancer 120 (20) (2014) 3131–3141, https://doi.org/10.1002/cncr.28802 PMID: 24925595.
[119] J. Li, R.L. Dittmar, S. Xia, H. Zhang, M. Du, C.C. Huang, et al., Cell-free DNA copy number variations in plasma from colorectal cancer patients, Mol. Oncol. 11 (8) (2017) 1099–1111, https://doi.org/10.1002/1878-0261.12077 PMID: 28504856.
[120] E.V. Kolesnikova, S.N. Tamkovich, O.E. Bryzgunova, P.I. Shelestyuk, V.I. Permyakova, et al., Circulating DNA in the blood of gastric cancer patients, Ann. N. Y. Acad. Sci. 1137 (1) (2008) 226–231, https://doi.org/10.1196/annals.1448.009 PMID: 18837952.
[121] J.L. Park, H.J. Kim, B.Y. Choi, H.C. Lee, H.R. Jang, K.S. Song, et al., Quantitative analysis of cell-free DNA in the plasma of gastric cancer patients, Oncol. Lett. 3 (4) (2012) 921–926, https://doi.org/10.3892/ol.2012.592 Apr. 2012. PMID: 22741019.
[122] S. Sai, D. Ichikawa, H. Tomita, D. Ikoma, N. Tani, H. Ikoma, et al., Quantification of plasma cell-free DNA in patients with gastric cancer, Anticancer Res. 27 (4) (2007) 2747–2751 PMID: 17695442.
[123] T.L. Lee, W.K. Leung, M.W. Chan, E.K. Ng, J.H. Tong, et al., Detection of gene promoter hypermethylation in the tumor and serum of patients with gastric carcinoma, Clin. Cancer Res. 8 (6) (2002) 1761–1766 PMID: 12060614.
[124] W.K. Leung, K.F. To, E.S.H. Chu, M.W.Y. Chan, A.H.C. Bai, E.K.W. Ng, et al., Potential diagnostic and prognostic values of detecting promoter hypermethylation in the serum of patients with gastric cancer, Br. J. Cancer 92 (12) (2005) 2190–2194, https://doi.org/10.1038/sj.bjc.6602636 PMID: 15942635.
[125] C. Sakakura, T. Hamada, K. Miyagawa, M. Nishio, A. Miyashita, H. Nagata, et al., Quantitative analysis of tumor-derived methylated RUNX3 sequences in the serum of gastric cancer patients, Anticancer Res. 29 (7) (2009) 2619–2625 PMID: 19596937.
[126] S.H. Tan, H. Ida, Q.C. Lau, B.C. Goh, W.S. Chieng, M. Loh, Y. Ito, Detection of promoter hypermethylation in serum samples of cancer patients by methylation-specific polymerase chain reaction for tumour suppressor genes including RUNX3, Oncol. Rep. 18 (5) (2007) 1225–1230, https://doi.org/10.3892/or.18.5.1225 PMID: 17914577.
[127] K.U. Park, H.E. Lee, D.J. Park, E.J. Jung, J. Song, H.H. Kim, et al., MYC quantitation in cell-free plasma DNA by real-time PCR for gastric cancer diagnosis, Clin. Chem. Lab. Med. 47 (5) (2009), https://doi.org/10.1515/CCLM.2009.126 PMID: 19302034.
[128] K. Shoda, D. Ichikawa, Y. Fujita, K. Masuda, H. Hiramoto, J. Hamada, et al.,) Monitoring the HER2 copy number status in circulating tumor DNA by droplet digital PCR in patients with gastric cancer, Gastric Cancer 20 (1) (2017) 126–135, https://doi.org/10.1007/s10120-016-0599-z PMID: 26874951.
[129] O. González-Santiago, M.L. Yeverino-Gutiérrez, M. Del Rosario González-González, R. Corral-Symes, P.C. Morales-San-Claudio, Mortality assessment of patients with pancreatic cancer in Mexico, 2000-2014, Ecancermedicalscience 11 (2017) 788, https://doi.org/10.3332/ecancer.2017.788 PMID: 29290757.
[130] T. Yamada, S. Nakamori, H. Ohzato, S. Oshuma, A. Taro, N. Higaki, et al., Detection with of K-ras gene mutations in plasma with pancreatic adenocarcinoma: correlation with clinicopathological features, Clin. Cancer Res. 4 (6) (1998) 1527–1532 PMID: 9626473.
[131] H.E. Mulcahy, J. Lyautey, C. Lederrey, X. qi Chen, P. Anker, E.M. Alstead, et al., A prospective study of K-ras mutations in the plasma of pancreatic cancer patients, Clin. Cancer Res. 4 (2) (1998) 271–275 PMID: 9516910.
[132] J. Däbritz, R. Preston, J. Hänfler, H. Oettle, Follow-up study of K-ras mutations in the plasma of patients with pancreatic cancer: correlation with clinical features and carbohydrate antigen 19-9, Pancreas 38 (5) (2009) 534–541, https://doi.org/10.1097/MPA.0b013e31819f6376 PMID: 19295453.
[133] P. Adamo, C.M. Cowley, C.P. Neal, V. Mistry, K. Page, A.R. Dennison, et al., Profiling tumour heterogeneity through circulating tumour DNA in patients with pancreatic cancer, Oncotarget 8 (50) (2017) 87221–87233, https://doi.org/10.18632/oncotarget.20250 PMID: 29152076.
[134] H. Chen, H. Tu, Z.Q. Meng, Z. Chen, P. Wang, L.M. Liu, K-ras mutational status predicts poor prognosis in unresectable pancreatic cancer, Eur. J. Surg. Oncol. 36 (7) (2010) 657–662, https://doi.org/10.1016/j.ejso.2010.05.014 PMID: 20542658.
[135] J. Earl, S. Garcia-Nieto, J.C. Martinez-Avila, J. Montans, A. Sanjuanbenito, M. Rodríguez-Garrote, et al., Circulating tumor cells (Ctc) and kras mutant circulating free Dna (cfdna) detection in peripheral blood as biomarkers in patients diagnosed with exocrine pancreatic cancer, BMC Cancer 15 (2015) 797, https://doi.org/10.1186/s12885-015-1779-7 PMID: 26498594.
[136] H. Kinugasa, K. Nouso, K. Miyahara, Y. Morimoto, C. Dohi, K. Tsutsumi, et al., Detection of K-ras gene mutation by liquid biopsy in patients with pancreatic cancer, Cancer 121 (13) (2015) 2271–2280, https://doi.org/10.1002/cncr.29364 PMID: 25823825.
[137] M.J. Pishvaian, R.J. Bender, L.M. Matrisian, L. Rahib, A. Hendifar, W.A. Hoos, et al., A pilot study evaluating concordance between blood-based and patient-matched tumor molecular testing within pancreatic cancer patients participating in the Know Your Tumor (KYT) initiative, Oncotarget 8 (48) (2017), https://doi.org/10.18632/oncotarget.13225 PMID: 29137355.
[138] Y. Nakano, M. Kitago, S. Matsuda, Y. Nakamura, Y. Fujita, S. Imai, et al., KRAS mutations in cell-free DNA from preoperative and postoperative sera as a pancreatic cancer marker: a retrospective study, Br. J. Cancer 118 (5) (2018) 662–669, https://doi.org/10.1038/bjc.2017.479 PMID: 29360815.
[139] N. Hadano, Y. Murakami, K. Uemura, Y. Hashimoto, N. Kondo, N. Nakagawa, et al.,) Prognostic value of circulating tumour DNA in patients undergoing curative resection for pancreatic cancer, Br. J. Cancer 115 (1) (2016) 59–65, https://doi.org/10.1038/bjc.2016.175 PMID: 27280632.
[140] N. Singh, S. Gupta, R.M. Pandey, S.S. Chauhan, A. Saraya, High levels of cell-free circulating nucleic acids in pancreatic Cancer are associated with vascular encasement, metastasis and poor survival, Cancer Invest. 33 (3) (2015) 78–85, https://doi.org/10.3109/07357907.2014.1001894 PMID: 25647443.
[141] K. Tjensvoll, M. Lapin, T. Buhl, S. Oltedal, K. Steen-Ottosen Berry, B. Gilje, et al., Clinical relevance of circulating KRAS mutated DNA in plasma from patients with advanced pancreatic cancer, Mol. Oncol. 10 (4) (2016) 635–643, https://doi.org/10.1016/j.molonc.2015.11.012 PMID: 26725968.
[142] K. Sikora, C. Bedin, C. Vicentini, G. Malpeli, E. D'Angelo, N. Sperandio, et al., Evaluation of cell-free DNA as a biomarker for pancreatic malignancies, Int. J. Biol. Markers 30 (1) (2015) 136–141, https://doi.org/10.5301/jbm.5000088 PMID: 24832178.
[143] L. Jiao, J. Zhu, M.M. Hassan, D.B. Evans, J.L. Abbruzzese, D. Li, K-ras mutation and p16 and preproenkephalin promoter hypermethylation in plasma DNA of pancreatic cancer patients: in relation to cigarette smoking, Pancreas 34 (1) (2007) 55–62, https://doi.org/10.1097/01.mpa.0000246665.68869.d4 PMID: 17198183.
[144] A.A. Melnikov, D. Scholtens, M.S. Talamonti, D.J. Bentrem, V.V. Levenson, Methylation profile of circulating plasma DNA in patients with pancreatic cancer, J. Surg. Oncol. 99 (2) (2009) 119–122, https://doi.org/10.1002/jso.21208 PMID: 19065632.
[145] T. Liggett, A. Melnikov, Q.L. Yi, C. Replogle, R. Brand, K. Kaul, et al., Differential methylation of cell-free circulating DNA among patients with pancreatic cancer versus chronic pancreatitis, Cancer 116 (7) (2010) 1674–1680, https://doi.org/10.1002/cncr.24893 PMID: 20143430.
[146] V. Bernard, D.U. Kim, F.A. San Lucas, J. Castillo, K. Allenson, F.C. Mulu, et al., Circulating nucleic acids are associated with outcomes of patients with pancreatic Cancer, Gastroenterology 156 (1) (2019) 108–118, https://doi.org/10.1053/j.gastro.2018.09.022 PMID: 30240661.
[147] S. Yang, S.P. Che, P. Kurywchak, J.L. Tavormina, L.B. Gansmo, P. Correa de Sampaio, et al., Detection of mutant KRAS and TP53 DNA in circulating exosomes from healthy individuals and patients with pancreatic cancer, Cancer Biol. Ther. 18 (3) (2017) 158–165, https://doi.org/10.1080/15384047.2017.1281499 PMID: 28121262.
[148] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2017, CA Cancer J. Clin. 67 (1) (2017) 7–30, https://doi.org/10.3322/caac.21387 PMID: 28055103.
[149] A. Szpechcinski, P. Rudzinski, W. Kupis, R. Langfort, T. Orlowski, J. Chorostowska-Wynimko, Plasma cell-free DNA levels and integrity in patients with chest radiological findings: NSCLC versus benign lung nodules, Cancer Lett. 374 (2) (2016) 202–207, https://doi.org/10.1016/j.canlet.2016.02.002 PMID: 26854716.
[150] A. Szpechcinski, J. Chorostowska-Wynimko, R. Struniawski, W. Kupis, P. Rudzinski, R. Langfort, et al., Cell-free DNA levels in plasma of patients with non-small-cell lung cancer and inflammatory lung disease, Br. J. Cancer 113 (3) (2015) 476–483, https://doi.org/10.1038/bjc.2015.225 PMID: 26125447.
[151] R. Catarino, M.M. Ferreira, H. Rodrigues, A. Coelho, A. Nogal, A. Sousa, R. Medeiros, Quantification of free circulating tumor DNA as a diagnostic marker for breast Cancer, DNA Cell Biol. 27 (8) (2008) 415–421, https://doi.org/10.1089/dna.2008.0744 PMID: 18694299.
[152] S. Kumar, R. Guleria, V. Singh, A.C. Bharti, A. Mohan, B.C. Das, Efficacy of circulating plasma DNA as a diagnostic tool for advanced non-small cell lung cancer

and its predictive utility for survival and response to chemotherapy, Lung Cancer 70 (2) (2010) 211–217, https://doi.org/10.1016/j.lungcan.2010.01.021 PMID: 20181407.
[153] S. Pan, W. Xia, Q. Ding, Y. Shu, T. Xu, Y. Geng, et al., Can plasma DNA monitoring be employed in personalized chemotherapy for patients with advanced lung cancer? Biomed. Pharmacother. 66 (2) (2012) 131–137, https://doi.org/10.1016/j.biopha.2011.11.022 PMID: 22401927.
[154] S. Wang, X. Han, X. Hu, X. Wang, L. Zhao, L. Tang, et al., Clinical significance of pretreatment plasma biomarkers in advanced non-small cell lung cancer patients, Clin. Chim. Acta 430 (2014) 63–70, https://doi.org/10.1016/j.cca.2013.12.026 PMID: 24378285.
[155] G. Sozzi, D. Conte, L. Mariani, S. Lo Vullo, L. Roz, C. Lombardo, et al., Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients, Cancer Res. 61 (12) (2001) 4675–4678 PMID: 11406535.
[156] K.A. Yoon, S. Park, S.H. Lee, J.H. Kim, J.S. Lee, Comparison of circulating plasma DNA levels between lung Cancer patients and healthy controls, J. Mol. Diagn. 11 (3) (2009) 182–185, https://doi.org/10.2353/jmoldx.2009.080098 PMID: 19324991.
[157] A. Dowler Nygaard, K.L.G. Spindler, N. Pallisgaard, R.F. Andersen, A. Jakobsen, Levels of cell-free DNA and plasma KRAS during treatment of advanced NSCLC, Oncol. Rep. 31 (2) (2014) 969–974, https://doi.org/10.3892/or.2013.2906 PMID: 24316734.
[158] G. Sozzi, D. Conte, M.E. Leon, R. Cirincione, L. Roz, C. Ratcliffe, et al., Quantification of free circulating DNA as a diagnostic marker in lung cancer, J. Clin. Oncol. 21 (21) (2003) 3902–3908, https://doi.org/10.1200/JCO.2003.02.006 PMID: 14507943.
[159] C. Tissot, A.C. Toffart, S. Villar, P.J. Souquet, P. Merle, D. Moro-Sibilot, et al., Circulating free DNA concentration is an independent prognostic biomarker in lung cancer, Eur. Respir. J. 46 (6) (2015) 1773–1780, https://doi.org/10.1183/13993003.00676-2015 PMID: 26493785.
[160] R. Catarino, A. Coelho, A. Araújo, M. Gomes, A. Nogueira, C. Lopes, R. Medeiros, Circulating DNA: diagnostic tool and predictive marker for overall survival of NSCLC patients, PLoS One 7 (6) (2012), https://doi.org/10.1371/journal.pone.0038559 e38559. PMID: 22701665.
[161] M.A. van der Drift, B.E.A. Hol, C.H.W. Klaassen, C.F.M. Prinsen, Y.A.W.G. van Aarssen, R. Donders, et al., Circulating DNA is a non-invasive prognostic factor for survival in non-small cell lung cancer, Lung Cancer 68 (2) (2010) 283–287, https://doi.org/10.1016/J.LUNGCAN.2009.06.021 PMID: 19632736.
[162] J.R. Fischer, U. Ohnmacht, N. Rieger, M. Zemaitis, C. Stoffregen, C. Manegold, H. Lahm, Prognostic significance of RASSF1A promoter methylation on survival of non-small cell lung cancer patients treated with gemcitabine, Lung Cancer 56 (1) (2007) 115–123, https://doi.org/10.1016/j.lungcan.2006.11.016 PMID: 17196704.
[163] A.A. Ponomaryova, E.Y. Rykova, N.V. Cherdyntseva, T.E. Skvortsova, A.Y. Dobrodeev, et al.,) Potentialities of aberrantly methylated circulating DNA for diagnostics and post-treatment follow-up of lung cancer patients, Lung Cancer 81 (3) (2013) 397–403, https://doi.org/10.1016/j.lungcan.2013.05.016 PMID: 23806794.
[164] H. Usadel, J. Brabender, K.D. Danenberg, C. Jerónimo, S. Harden, J. Engles, et al., Quantitative adenomatous polyposis coli promoter methylation analysis in tumor tissue, serum, and plasma DNA of patients with lung cancer, Cancer Res. 62 (2) (2002) 371–375 PMID: 11809682.
[165] M. Murtaza, S.J. Dawson, D.W. Tsui, D. Gale, T. Forshew, A.M. Piskorz, et al., Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA, Nature 497 (7447) (2013) 108–112, https://doi.org/10.1038/nature12065 PMID: 23563269.
[166] Q. Yu, F. Huang, M. Zhang, H. Ji, S. Wu, Y. Zhao, C. Zhang, et al., Multiplex picoliter-droplet digital PCR for quantitative assessment of EGFR mutations in circulating cell-free DNA derived from advanced non-small cell lung cancer patients, Mol. Med. Rep. 16 (2) (2017) 1157–1166, https://doi.org/10.3892/mmr.2017.6712 PMID: 29067441.
[167] J.Y. Lee, X. Qing, W. Xiumin, B. Yali, S. Chi, S.H. Bak, H.Y. Lee, et al., Longitudinal monitoring of EGFR mutations in plasma predicts outcomes of NSCLC patients treated with EGFR TKIs: korean Lung Cancer consortium (KLCC-12-02), Oncotarget 7 (6) (2016) 6984–6993, https://doi.org/10.18632/oncotarget.6874 PMID: 26755650.
[168] J. Ni, L. Weng, Y. Liu, Z. Sun, C. Bai, Y. Wang, Dynamic monitoring of EGFR mutations in circulating cell-free DNA for EGFR-mutant metastatic patients with lung cancer: early detection of drug resistance and prognostic significance, Oncol. Lett. 13 (6) (2017) 4549–4557, https://doi.org/10.3892/ol.2017.6022 PMID: 28599456.
[169] H. Duan, Junliang Lu, L. Tao, J. Gao, J. Zhang, Y. Xu, et al., Comparison of EGFR mutation status between plasma and tumor tissue in non-small cell lung cancer using the Scorpion ARMS method and the possible prognostic significance of plasma EGFR mutation status, Int. J. Clin. Exp. Pathol. 8 (10) (2015) 13136–13145 PMID: 26722512.
[170] Y. Lee, S. Park, W.S. Kin, J.C. Lee, S.J. Jang, et al., Correlation between progression-free survival, tumor burden, and circulating tumor DNA in the initial diagnosis of advanced-stage *EGFR* -mutated non-small cell lung cancer, Thorac. Cancer 9 (9) (2018) 1104–1110, https://doi.org/10.1111/1759-7714.12793 PMID: 29989342.
[171] Y.L. Wu, L.V. Sequist, C.P. Hu, J. Feng, Y. Lu Sm Huang, et al., EGFR mutation detection in circulating cell-free DNA of lung adenocarcinoma patients: analysis of LUX-Lung 3 and 6, Br. J. Cancer 116 (2) (2017) 175–185, https://doi.org/10.1038/bjc.2016.420 PMID: 28006816.
[172] E. Castellanos-Rizaldos, D.G. Grimm, V. Tadigotla, J. Hurley, J. Healy, et al., Exosome-based detection of *EGFR* T790M in plasma from non–Small cell lung Cancer patients, Clin. Cancer Res. 24 (12) (2018) 2944–2950, https://doi.org/10.1158/1078-0432.CCR-17-3369 PMID: 29535126.
[173] F. Li, J. Huang, D. Ji, Q. Meng, C. Wang, S. Chen, et al., Utility of urinary circulating tumor DNA for EGFR mutation detection in different stages of non-small cell lung cancer patients, Clin. Transl. Oncol. 19 (10) (2017) 1283–1291, https://doi.org/10.1007/s12094-017-1669-3 PMID: 28497422.
[174] H. Zhang, B. He, J. Cui, M. Zhao, Z. Zhang, Comparison of circulating DNA from plasma and urine for EGFR mutations in NSCLC patients, Cancer Biomark. 23 (3) (2018) 427–436, https://doi.org/10.3233/CBM-181511 PMID: 30223392.
[175] S. Gal, C. Fidler, Y. Lo, M. Taylor, C. Han, J. Moore, et al., Quantitation of circulating DNA in the serum of breast cancer patients by real-time PCR, Br. J. Cancer 90 (2004) 1211–1215, https://doi.org/10.1038/sj.bjc.6601609 PMID: 15026803.
[176] H. Schwarzenbach, V. Müller, K. Milde-Langosch, B. Steinbach, K. Pantel, Evaluation of cell-free tumour DNA and RNA in patients with breast cancer and benign breast disease, Mol. Biosyst. 7 (10) (2011) 2848–2854, https://doi.org/10.1039/c1mb05197k PMID: 21785770.
[177] O. Tangvarasittichai, W. Jaiwang, S. Tangvarasittichai, The plasma DNA concentration as a potential breast Cancer Screening marker, Indian J. Clin. Biochem. 30 (1) (2015) 55–58, https://doi.org/10.1007/s12291-013-0407-z PMID: 25646041.
[178] C. Oshiro, N. Kagara, Y. Naoi, M. Shimoda, A. Shimomura, N. Maruyama, et al., PIK3CA mutations in serum DNA are predictive of recurrence in primary breast cancer patients, Breast Cancer Res. Treat. 150 (2) (2015) 299–307, https://doi.org/10.1007/s10549-015-3322-6 PMID: 25736040.
[179] D. Hashad, A. Sorour, A. Ghazal, I. Talaat, Free circulating tumor DNA as a diagnostic marker for breast Cancer, J. Clin. Lab. Anal. 26 (6) (2012) 467–472, https://doi.org/10.1002/jcla.21548 PMID: 23143630.
[180] C. Roth, K. Pantel, V. Müller, B. Rack, S. Kasimir-Bauer, W. Janni, H. Schwarzenbach, Apoptosis-related deregulation of proteolytic activities and high serum levels of circulating nucleosomes and DNA in blood correlate with breast cancer progression, BMC Cancer 11 (2011) 4, https://doi.org/10.1186/1471-2407-11-4 PMID: 21211028.
[181] T. Mukohara, PI3K mutations in breast cancer: prognostic and therapeutic implications, Breast Cancer 7 (2015) 111–123, https://doi.org/10.2147/BCTT.S60696 PMID: 26028978.
[182] J.A. Beaver, D. Jelovac, S. Balukrishna, R.L. Cochran, S. Croessmann, D.J. Zabransky, et al.,) Detection of cancer DNA in plasma of patients with early-stage breast cancer, Clin. Cancer Res. 20 (10) (2014) 2643–2650, https://doi.org/10.1158/1078-0432.CCR-13-2933 PMID: 24504125.
[183] R.E. Board, A.M. Wardley, J.M. Dixon, A.C. Armstrong, S. Howell, L. Renshaw, et al., Detection of PIK3CA mutations in circulating free DNA in patients with breast cancer, Breast Cancer Res. Treat. 120 (2) (2010) 461–467, https://doi.org/10.1007/s10549-010-0747-9 PMID: 20107891.
[184] M.J. Higgins, D. Jelovac, E. Barnathan, B. Blair, S. Slater, P. Powers, et al., Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood, Clin. Cancer Res. 18 (12) (2012) 3462–3469, https://doi.org/10.1158/1078-0432.CCR-11-2696 PMID:22421194.
[185] D.J. Slamon, G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich, W.L. McGuire, Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, Science 235 (4785) (1987) 177–182, https://doi.org/10.1126/science.3798106 PMID: 3798106.
[186] K. Page, N. Hava, B. Ward, J. Brown, D. Guttery, C. Ruangpratheep, et al., Detection of HER2 amplification in circulating free DNA in patients with breast cancer, Br. J. Cancer 104 (2011) 1342–1348, https://doi.org/10.1038/bjc.2011.89 PMID: 21427727.
[187] T. Bechmann, R.F. Andersen, N. Pallisgaard, J.S. Madsen, E. Maae, E.H. Jakobsen, et al., Plasma HER2 amplification in cell-free DNA during neoadjuvant chemotherapy in breast cancer, J. Cancer Res. Clin. Oncol. 139 (6) (2013) 995–1003, https://doi.org/10.1007/s00432-013-1413-5 PMID: 23479212.
[188] H. Gevensleben, I. Garcia-Murillas, M.K. Graeser, G. Schiavon, P. Osin, M. Parton, et al., Noninvasive detection of HER2 amplification with plasma DNA digital PCR, Clin. Cancer Res. 19 (12) (2013) 3276–3284, https://doi.org/10.1158/1078-0432.CCR-12-3768 PMID: 23637122.
[189] S. Iqbal, S. Vishnubhatla, V. Raina, S. Sharma, A. Gogia, S.S.V. Deo, et al., Circulating cell-free DNA and its integrity as a prognostic marker for breast cancer, SpringerPlus 4 (1) (2015) 265, https://doi.org/10.1186/s40064-015-1071-y PMID: 26090312.
[190] A.M. Kamel, S. Teama, A. Fawzy, M. El Deftar, Plasma DNA integrity index as a potential molecular diagnostic marker for breast cancer, J. Immunother. Emphasis Tumor Immunol. 37 (6) (2016) 7565–7572, https://doi.org/10.1007/s13277-015-4624-3 PMID: 26684805.
[191] O.J. Stotzer, J. Lehner, D. Fersching-Gierlich, D. Nagel, S. Holdenrieder, Diagnostic relevance of plasma DNA and DNA integrity for breast cancer, J. Immunother. Emphasis Tumor Immunol. 35 (2) (2014) 1183–1191, https://doi.org/10.1007/s13277-013-1158-4 PMID: 24018822.
[192] N. Umetani, A.E. Giuliano, S.H. Hiramatsu, F. Amersi, T. Nakagawa, S. Martino, D.S.B. Hoon, Prediction of breast tumor progression by integrity of free circulating DNA in serum, J. Clin. Oncol. (26) (2006) 4270–4276, https://doi.org/10.1200/JCO.2006.05.9493 PMID: 16963729.
[193] G. Sharma, S. Mirza, R. Parshad, S.D. Gupta, R. Ralhan, DNA methylation of circulating DNA: a marker for monitoring efficacy of neoadjuvant chemotherapy in breast cancer patients, J. Immunother. Emphasis Tumor Immunol. 33 (6) (2012) 1837–1843, https://doi.org/10.1007/s13277-012-0443-y PMID: 22744714.
[194] I. Van der Auwera, H.J. Elst, S.J. Van Laere, H. Maes, P. Huget, P. van Dam, et al., The presence of circulating total DNA and methylated genes is associated with

circulating tumour cells in blood from breast cancer patients, Br. J. Cancer 100 (8) (2009) 1277–1286, https://doi.org/10.1038/sj.bjc.6605013 PMID: 19367284.
[195] G. Göbel, D. Auer, I. Gaugg, A. Schneitter, R. Lesche, E. Müller-Holzner, et al., Prognostic significance of methylated RASSF1A and PITX2 genes in blood- and bone marrow plasma of breast cancer patients, Breast Cancer Res. Treat. 130 (1) (2011) 109–117, https://doi.org/10.1007/s10549-010-1335-8 PMID: 21221769.
[196] N. Fujita, T. Nakayama, N. Yamamoto, S.J. Kim, K. Shimazu, A. Shimomura, et al., Methylated DNA and total DNA in serum detected by one-step methylation-specific pcr is predictive of poor prognosis for breast cancer patients, Oncol 83 (5) (2012) 273–282, https://doi.org/10.1159/000342083 PMID: 22964822.
[197] S. Mirza, G. Sharma, R. Parshad, A. Srivastava, S.D. Gupta, R. Ralhan, Clinical significance of promoter hypermethylation of ERβ and RARβ2 in tumor and serum DNA in Indian breast cancer patients, Ann. Surg. Oncol. 19 (9) (2012) 3107–3115, https://doi.org/10.1245/s10434-012-2323-5 PMID: 22451234.
[198] D. Fu, C. Ren, H. Tan, J. Wei, Y. Zhu, C. He, et al., Sox17 promoter methylation in plasma DNA is associated with poor survival and can be used as a prognostic factor in breast cancer, Medicine 94 (11) (2015) e637, https://doi.org/10.1097/md.0000000000000637 PMID: 25789956.
[199] D. Jelovac, D.K. Armstrong, Recent progress in the diagnosis and treatment of ovarian cancer, CA Cancer J. Clin. 61 (3) (2011) 183–203, https://doi.org/10.3322/caac.20113 PMID: 21521830.
[200] A.A. Kamat, M. Baldwin, D. Urbauer, D. Dang, L.Y. Han, A. Godwin, et al., Plasma cell-free DNA in ovarian cancer: an independent prognostic biomarker, Cancer 116 (8) (2010) 1918–1925, https://doi.org/10.1002/cncr.24997 PMID: 20166213.
[201] K.D. Steffensen, C.V. Madsen, R.F. Andersen, M. Waldstrøm, P. Adimi, A. Jakobsen, Prognostic importance of cell-free DNA in chemotherapy resistant ovarian cancer treated with bevacizumab, Eur. J. Cancer 50 (15) (2014) 2611–2618, https://doi.org/10.1016/j.ejca.2014.06.022 PMID: 25087181.
[202] A.A. Kamat, A.K. Sood, D. Dang, D.M. Gershenson, J.L. Simpson, F.Z. Bischoff, Quantification of total plasma cell-free DNA in ovarian cancer using real-time PCR, Ann. N. Y. Acad. Sci. 1075 (1) (2006) 230–234, https://doi.org/10.1196/annals.1368.031 PMID: 17108216.
[203] X. Shao, Y. He, M. Ji, X. Chen, J. Qi, et al., Quantitative analysis of cell-free DNA in ovarian cancer, Oncol. Lett. 10 (6) (2015) 3478–3482, https://doi.org/10.3892/ol.2015.3771 PMID: 26788153.
[204] A.A. Kamat, M. Baldwin, D. Urbauer, D. Dang, L.Y. Han, A. Godwin, et al., Plasma cell-free DNA in ovarian Cancer: an independent prognostic biomarker, Cancer 116 (8) (2010) 1918–1925, https://doi.org/10.1002/cncr.24997 PMID: 20166213.
[205] K. Kalavska, T. Minarik, B. Vlkova, D. Manasova, M. Kubickova, et al., Prognostic value of various subtypes of extracellular DNA in ovarian cancer patients, J. Ovarian Res. 11 (1) (2018), https://doi.org/10.1186/s13048-018-0459-z PMID: 30243303.
[206] E. Capizzi, E. Gabusi, A.D. Grigioni, P. De laco, M. Rosati, et al., Quantification of free plasma DNA before and after chemotherapy in patients with advanced epithelial ovarian cancer, Diagn. Mol. Pathol. 17 (1) (2008) 34–38, https://doi.org/10.1097/PDM.0b013e3181359e1f PMID: 18303408.
[207] J. Otsuka, T. Okuda, A. Sekizawa, S. Amemiya, H. saito, et al., Detection of p53 mutations in the plasma DNA of patients with ovarian cancer, Int. J. Gynecol. Cancer 14 (3) (2004) 459–464, https://doi.org/10.1111/j.1048-891x.2004.014305.x PMID: 15228418.
[208] E.M. Swisher, M. Wollan, S.M. Mahtani, J.B. Willner, R. Garcia, et al., Tumor-specific p53 sequences in blood and peritoneal fluid of women with epithelial ovarian cancer, Am. J. Obstet. Gynecol. 193 (3) (2005) 662–667, https://doi.org/10.1016/j.ajog.2005.01.054 PMID: 16150257.
[209] A. Morikawa, T. Hayashi, N. Shimizu, M. Kobayashi, K. Taniue, A. Takahashi, et al., PIK3CA and KRAS mutations in cell free circulating DNA are useful markers for monitoring ovarian clear cell carcinoma, Oncotarget 9 (20) (2018) 15266–15274, https://doi.org/10.18632/oncotarget.24555 PMID: 29632642.
[210] I. Ibanez De Caceres, C. Battagli, M. Esteller, J.G. Herman, E. Dulaimi, et al., Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma, and peritoneal fluid from ovarian cancer patients, Cancer Res. 64 (18) (2004) 6476–6481, https://doi.org/10.1158/0008-5472.CAN-04-1529 PMID: 15374957.
[211] A. Melnikov, D. Scholtens, A. Godwin, V. Levenson, Differential methylation profile of ovarian Cancer in tissues and plasma, J. Mol. Diagn. 11 (1) (2009) 60–65, https://doi.org/10.2353/jmoldx.2009.080072 PMID: 19074590.
[212] A.E. BonDurant, Z. Huang, R.S. Whitaker, L.R. Simel, A. Berchuck, S.K. Murphy, Quantitative detection of RASSF1A DNA promoter methylation in tumors and serum of patients with serous epithelial ovarian cancer, Gynecol. Oncol. 123 (3) (2011) 581–587, https://doi.org/10.1016/j.ygyno.2011.08.029 PMID: 21955482.
[213] Y. Wu, X. Zhang, L. Lin, X.P. Ma, Y.C. Ma, P.S. Liu, Aberrant methylation of RASSF2A in tumors and plasma of patients with epithelial ovarian cancer, Asian Pac. J. Cancer Prev. 15 (3) (2014) 1171–1176 PMID: 24606436.
[214] B. Wang, L. Yu, X. Luo, L. Huang, Q.S. Li, X.S. Shao, et al., Detection of OPCML methylation, a possible epigenetic marker, from free serum circulating DNA to improve the diagnosis of early-stage ovarian epithelial cancer, Oncol. Lett. 14 (1) (2017) 217–223, https://doi.org/10.3892/ol.2017.6111 PMID: 28693156.
[215] R. Dong, J. Yu, H. Pu, Z. Zhang, X. Xu, "Frequent SLIT2 promoter methylation in the serum of patients with ovarian cancer, J. Int. Med. Res. 40 (2) (2012) 681–686, https://doi.org/10.1177/147323001204000231 PMID: 22613430.
[216] G. Gifford, J. Paul, P.A. Vasey, S.B. Kaye, R. Brown, The acquisition of hMLH1 methylation in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients, Clin. Cancer Res. 10 (13) (2004) 4420–4426, https://doi.org/10.1158/1078-0432.CCR-03-0732 PMID: 15240532.
[217] R.J. Hendriks, S. Dijkstra, F.P. Smit, J. Vandersmissen, H. Van De Voorde, et al., Epigenetic markers in circulating cell-free DNA as prognostic markers for survival of castration-resistant prostate cancer patients, Prostate (2018).
[218] S.M. Seyedolmohadessin, M.T. Akbari, Z. Nourmohammadi, A. Basiri, G. Pourmand, Assessing the diagnostic value of plasma-free DNA in prostate Cancer Screening, Iran. Biomed. J. (2018), https://doi.org/10.29252/ibj.22.5.331 PMID: 29475366.
[219] F.K.H. Chun, I. Müller, I. Lange, M.G. Friedrich, A. Erbersdobler, P.I. Karakiewicz, et al., Circulating tumour-associated plasma DNA represents an independent and informative predictor of prostate cancer, BJU Int. 98 (3) (2006) 544–548, https://doi.org/10.1111/j.1464-410X.2006.06352.x PMID: 16925751.
[220] D. Allen, A. Butt, D. Cahill, M. Wheeler, R. Popert, R. Swaminathan, Role of cell-free plasma DNA as a diagnostic marker for prostate cancer, Ann. N. Y. Acad. Sci. 1022 (2004) 76–80, https://doi.org/10.1196/annals.1318.013 PMID: 15251943.
[221] P.J. Bastian, G.S. Palapattu, S. Yegnasubramanian, X. Lin, C.G. Rogers, L.A. Mangold, et al., Prognostic value of preoperative serum cell-free circulating DNA in men with prostate cancer undergoing radical prostatectomy, Clin. Cancer Res. 13 (18 Pt 1) (2007) 5361–5367, https://doi.org/10.1158/1078-0432.CCR-06-2781 PMID: 17875764.
[222] A. Altimari, A.D. Grigioni, E. Benedettini, E. Gabusi, R. Schiavina, A. Martinelli, et al., Diagnostic role of circulating free plasma DNA detection in patients with localized prostate cancer, Am. J. Clin. Pathol. 129 (5) (2008) 756–762, https://doi.org/10.1309/DBPX1MFNDDJBW1FL PMID: 18426736.
[223] N. Mehra, D. Dolling, S. Sumanasuriya, R. Christova, L. Pope, S. Carreira, et al., Plasma cell-free DNA concentration and outcomes from taxane therapy in metastatic castration-resistant prostate Cancer from two phase III trials (FIRSTANA and PROSELICA), Eur. Urol. 74 (2018) 283–291, https://doi.org/10.1016/j.eururo.2018.02.013 PMID: 29500065.
[224] A. Fawzy, K.M. Sweify, H.M. El-Fayoumy, N. Nofal, Quantitative analysis of plasma cell-free DNA and its DNA integrity in patients with metastatic prostate cancer using ALU sequence, J Egypt Natl Cancer Inst 28 (4) (2016) 235–242, https://doi.org/10.1016/J.JNCI.2016.08.003 PMID: 27634416.
[225] E. Papadopoulou, E. Davilas, V. Sotiriou, A. Koliopanos, F. Aggelakis, K. Dardoufas, et al., Cell-free DNA and RNA in plasma as a new molecular marker for prostate cancer, Oncol. Res. 14 (9) (2004) 439–445, https://doi.org/10.3727/0965040041791473 PMID: 15490975.
[226] A.W. Wyatt, M. Annala, R. Aggarwal, K. Beja, F. Feng, J. Youngren, et al., Concordance of circulating tumor DNA and matched metastatic tissue biopsy in prostate Cancer, J. Natl. Cancer Inst. 109 (12) (2017), https://doi.org/10.1093/jnci/djx118 PMID: 29206995.
[227] G. Ponti, M. Maccaferri, M. Mandrioli, M. Manfredini, S. Micali, M. Cotugno, et al., Seminal cell-free DNA assessment as a novel prostate Cancer biomarker, Pathol. Oncol. Res. (2018), https://doi.org/10.1007/s12253-018-0416-6 PMID: 29730802.
[228] F. Martignano, G. Gurioli, S. Salvi, D. Calistri, M. Costantini, R. Gunelli, et al., GSTP1 methylation and protein expression in prostate Cancer: diagnostic implications, Dis. Markers 2016 (2016) 4358292, , https://doi.org/10.1155/2016/4358292 PMID: 27594734.
[229] E. Papadopoulou, E. Davilas, V. Sotiriou, E. Georgakopoulos, et al., Cell-free DNA and RNA in plasma as a new molecular marker for prostate and breast cancer, Ann. N. Y. Acad. Sci. 1075 (1) (2006) 235–243, https://doi.org/10.1196/annals.1368.032 PMID: 17108217.
[230] O.E. Bryzgunova, E.S. Morozkin, S.V. Yarmoschuk, V.V. Vlassov, P.P. Laktionov, Methylation-specific sequencing of GSTP1 gene promoter in circulating/extracellular DNA from blood and urine of healthy donors and prostate cancer patients, Ann. N. Y. Acad. Sci. 1137 (2008) 222–225, https://doi.org/10.1196/annals.1448.039 PMID: 18837951.
[231] L. Wang, Y.L. Lin, B. Li, Y.Z. Wang, W.P. Li, J.G. Ma, Aberrant promoter methylation of the cadherin 13 gene in serum and its relationship with clinicopathological features of prostate cancer, J. Int. Med. Res. 42 (5) (2014) 1085–1092, https://doi.org/10.1177/0300060514540631 PMID: 25015764.
[232] I.M. Reis, K. Ramachandran, C. Speer, E. Gordian, R. Singal, Serum GADD45a methylation is a useful biomarker to distinguish benign vs malignant prostate disease, Br. J. Cancer 113 (3) (2015) 460–468, https://doi.org/10.1038/bjc.2015.240 PMID: 26171936.
[233] W.W. Jiang, M. Zahurak, D. Goldenberg, Y. Milman, H.L. Park, W.H. Westra, et al., Increased plasma DNA integrity index in head and neck cancer patients, Int. J. Cancer 119 (11) (2006) 2673–2676, https://doi.org/10.1002/ijc.22250 PMID: 16991120.
[234] A.M. Mazurek, T. Rutkowski, A. Fiszer-Kierzkowska, E. Małusecka, K. Składowski, Assessment of the total cfDNA and HPV16/18 detection in plasma samples of head and neck squamous cell carcinoma patients, Oral Oncol. 54 (2016) 36–41, https://doi.org/10.1016/j.oraloncology.2015.12.002 PMID: 26786940.
[235] J.H. van Ginkel, M.M.H. Huibers, R.J.J. van Es, R. de Bree, S.M. Willems, Droplet digital PCR for detection and quantification of circulating tumor DNA in plasma of head and neck cancer patients, BMC Cancer 17 (1) (2017) 428, https://doi.org/10.1186/s12885-017-3424-0 PMID: 28629339.
[236] M. Kumar, S. Srivastava, S.A. Singh, A.K. Das, G.C. Das, B. Dhar, et al., Cell-free mitochondrial DNA copy number variation in head and neck squamous cell carcinoma: a study of non-invasive biomarker from Northeast India, J. Immunother. Emphasis Tumor Immunol. 39 (10) (2017), https://doi.org/10.1177/1010428317736643 101042831773664 PMID: 29072129.
[237] K. Zwirner, F.J. Hilke, G. Demidov, S. Ossowski, C. Gani, O. Rieß, et al., Circulating cell-free DNA: a potential biomarker to differentiate inflammation and infection during radiochemotherapy, Radiother. Oncol. (2018), https://doi.org/10.1016/j.radonc.2018.07.016 PMID: 30097252.
[238] A.K. Chaturvedi, E.A. Engels, R.M. Pfeiffer, B.Y. Hernandez, W. Xiao, E. Kim, et al., Human papillomavirus and rising oropharyngeal cancer incidence in the United

States, J. Clin. Oncol. 29 (32) (2011) 4294–4301, https://doi.org/10.1200/JCO.2011.36.4596 PMID: 21969503.
[239] G. D'Souza, A.R. Kreimer, R. Viscidi, M. Pawlita, C. Fakhry, W.M. Koch, et al., Case–Control study of human papillomavirus and oropharyngeal Cancer, N Eng J Med 356 (19) (2007) 1944–1956, https://doi.org/10.1056/NEJMoa065497 PMID: 17494927.
[240] Y. Wang, S. Springer, C.L. Mulvey, N. Silliman, J. Schaefer, M. Sausen, et al., Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck squamous cell carcinomas, Sci. Transl. Med. 7 (293) (2015), https://doi.org/10.1126/scitranslmed.aaa8507 293ra104. PMID: 26109104.
[241] M. Zane, M. Agostini, M.V. Enzo, E. Casal Ide, P. Del Bianco, F. Torresan, et al., Circulating cell-free DNA, SLC5A8 and SLC26A4 hypermethylation, BRAFV600E: a non-invasive tool panel for early detection of thyroid cancer, Biomed. Pharmacother. 67 (8) (2013) 723–730, https://doi.org/10.1016/j.biopha.2013.06.007 PMID: 23931930.
[242] J.Y. Kwak, J.J. Jeong, S.W. Kang, S. Park, J.R. Choi, et al., Study of peripheral BRAFV600E mutation as a possible novel marker for papillary thyroid carcinomas, Head Neck 35 (11) (2013) 1630–1633, https://doi.org/10.1002/hed.23195 PMID: 23161556.
[243] K.W. Cradic, D. Milosevic, A.M. Rosenberg, L.A. Erickson, et al., Mutant BRAFT1799A can be detected in the blood of papillary thyroid carcinoma patients and correlates with disease status, J. Clin. Endocrinol. Metab. 94 (12) (2013) 5001–5009, https://doi.org/10.1210/jc.2009-1349 PMID:19850689.
[244] T.C.Y. Chuang, A.Y.C. Chuang, L. Poeta, W.M. Koch, J.A. Califano, R.P. Tufano, Detectable BRAF mutation in serum DNA samples from patients with papillary thyroid carcinomas, Head Neck 32 (2) (2009), https://doi.org/10.1002/hed.21178 PMID: 19686635.
[245] G.J. Cote, C. Evers, M.I. Hu, E.G. Grubbs, M.D. Williams, T. Hai, et al., Prognostic significance of circulating RET M918T mutated tumor DNA in patients with advanced medullary thyroid carcinoma, J. Clin. Endocrinol. Metab. 102 (9) (2017) 3591–3599, https://doi.org/10.1210/jc.2017-01039 PMID: 28911154.
[246] S.J. Dawson, D.W.Y. Tsui, M. Murtaza, H. Biggs, O.M. Rueda, S.F. Chin, et al., Analysis of circulating tumor DNA to monitor metastatic breast Cancer, N Eng J Med 368 (13) (2013) 1199–1209, https://doi.org/10.1056/NEJMoa1213261 PMID: 23484797.
[247] Y.J. Gao, Y.J. He, Z.L. Yang, H.Y. Shao, Y. Zuo, Y. Bai, et al., Increased integrity of circulating cell-free DNA in plasma of patients with acute leukemia, Clin. Chem. Lab. Med. 48 (11) (2010) 1651–1656, https://doi.org/10.1515/CCLM.2010.311 PMID: 20831457.
[248] A. Greystoke, J. O'connor, K. Linton, M.B. Taylor, J. Cummings, T. Ward, et al., Assessment of circulating biomarkers for potential pharmacodynamic utility in patients with lymphoma, Br. J. Cancer 104 (2011) 719–725, https://doi.org/10.1038/sj.bjc.6606082 PMID: 21245866.
[249] C. Iriyama, A. Tomita, H. Hoshino, M. Adachi-Shirahata, Y. Furukawa-Hibi, K. Yamada, et al., Using peripheral blood circulating DNAs to detect CpG global methylation status and genetic mutations in patients with myelodysplastic syndrome, Bioch Biophys Res Commun 419 (4) (2012) 662–669, https://doi.org/10.1016/j.bbrc.2012.02.071 PMID: 22382018.
[250] Y. Jiang, S.Y. Pan, W.Y. Xia, D. Chen, H. Wang, L.X. Zhang, et al., Dynamic monitoring of plasma circulating DNA in patients with acute myeloid leukemia and its clinical significance, J Exp Hematol 20 (1) (2012) 53–56 PMID: 22391164.
[251] V. Camus, N. Sarafan-Vasseur, E. Bohers, S. Dubois, S. Mareschal, P. Bertrand, et al., Digital PCR for quantification of recurrent and potentially actionable somatic mutations in circulating free DNA from patients with diffuse large B-cell lymphoma, Leuk. Lymphoma 57 (9) (2016) 2171–2179, https://doi.org/10.3109/10428194.2016.1139703 PMID: 26883583.
[252] G.D. Kirk, O.A. Lesi, M. Mendy, K. Szymańska, H. Whittle, J.J. Goedert, et al., 249ser TP53 mutation in plasma DNA, hepatitis B viral infection, and risk of hepatocellular carcinoma, Oncogene 24 (38) (2005) 5858–5867, https://doi.org/10.1038/sj.onc.1208732 PMID: 16007211.
[253] J. Wang, Y. Qin, B. Li, Z. Sun, B. Yang, Detection of aberrant promoter methylation of GSTP1 in the tumor and serum of Chinese human primary hepatocellular carcinoma patients, Clin. Biochem. 39 (4) (2006) 344–348, https://doi.org/10.1016/j.clinbiochem.2006.01.008 PMID: 16527261.
[254] Y. Tokuhisa, N. Iizuka, I. Sakaida, T. Moribe, N. Fujita, T. Miura, et al., Circulating cell-free DNA as a predictive marker for distant metastasis of hepatitis C virus-related hepatocellular carcinoma, Br. J. Cancer 97 (2007) 1399–1403, https://doi.org/10.1038/sj.bjc.6604034 PMID: 17940509.
[255] Y.J. Zhang, H.C. Wu, J. Shen, H. Ahsan, Y.T. Wei, H.I. Yang, et al., Predicting hepatocellular carcinoma by detection of aberrant promoter methylation in serum DNA, Clin. Cancer Res. 13 (8) (2007) 2378–2384, https://doi.org/10.1158/1078-0432.CCR-06-1900 PMID.
[256] S.F. El-Shazly, M.A. Eid, H.A. El-Sourogy, G.F. Attia, S.A. Ezzat, Evaluation of serum dna integrity as a screening and prognostic tool in patients with hepatitis C virus-related hepatocellular carcinoma, Int. J. Biol. Markers 25 (2) (2010) 79–86, https://doi.org/10.1177/172460081002500204 PMID: 20544686.
[257] K. Chen, H. Zhang, L.N. Zhang, S.Q. Ju, J. Qi, D.F. Huang, et al., Value of circulating cell-free DNA in diagnosis of hepatocelluar carcinoma, World J. Gastroenterol. 19 (20) (2013) 3143–3149, https://doi.org/10.3748/wjg.v19.i20.3143 PMID: 23716996.
[258] M. Piciocchi, R. Cardin, A. Vitale, V. Vanin, A. Giacomin, C. Pozzan, et al., Circulating free DNA in the progression of liver damage to hepatocellular carcinoma, Hepatol. Int. 7 (4) (2013) 1050–1057, https://doi.org/10.1007/s12072-013-9481-9 PMID: 26202034.
[259] F.K. Sun, Y.C. Fan, J. Zhao, F. Zhang, S. Gao, Z.H. Zhao, et al., Detection of TFPI2 methylation in the serum of hepatocellular carcinoma patients, Dig. Dis. Sci. 58 (4) (2013) 1010–1015, https://doi.org/10.1007/s10620-012-2462-3 PMID: 23108564.
[260] G. Huang, J.D. Krocker, J.L. Kirk, S.N. Merwat, H. Ju, R.D. Soloway, et al., Evaluation of INK4A promoter methylation using pyrosequencing and circulating cell-free DNA from patients with hepatocellular carcinoma, Clin. Chem. Lab. Med. 52 (6) (2014) 899–909, https://doi.org/10.1515/cclm-2013-0885 PMID: 24406287.
[261] X.F. Ji, Y.C. Fan, S. Gao, Y. Yang, J.J. Zhang, K. Wang, MT1M and MT1G promoter methylation as biomarkers for hepatocellular carcinoma, World J Gastroenterology 20 (16) (2014) 4723–4729, https://doi.org/10.3748/wjg.v20.i16.4723 PMID: 24782625.
[262] P. Jiang, C.W.M. Chan, K.C.A. Chan, S.H. Cheng, J. Wong, V.W.S. Wong, et al., Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients, Proc Natl Acad Sci U S A 112 (11) (2015) E1317–E1325, https://doi.org/10.1073/pnas.1500076112 PMID: 25646427.
[263] H. Xu, X. Zhu, Z. Xu, Y. Hu, S. Bo, T. Xing, K. Zhu, Non-invasive analysis of genomic copy number variation in patients with hepatocellular carcinoma by next generation DNA sequencing, J. Cancer 6 (3) (2015) 247–253, https://doi.org/10.7150/jca.10747 PMID: 25663942.
[264] M.S. Kopreski, F.A. Benko, C. Kwee, K.E. Leitzel, E. Eskander, A. Lipton, C.D. Gocke, Detection of mutant K-ras DNA in plasma or serum of patients with colorectal cancer, Br. J. Cancer 76 (10) (1997) 1293–1299 PMID: 9374374.
[265] K. Hibi, C.R. Robinson, S. Booker, L. Wu, S.R. Hamilton, D. Sidransky, J. Jen, Molecular detection of genetic alterations in the serum of colorectal Cancer patients, Cancer Res. 58 (1998) 1405–1407 PMID: 9537240.
[266] S. Ito, K. Hibi, H. Nakayama, Y. Kodera, K. Ito, S. Akiyama, A. Nakao, Detection of tumor DNA in serum of colorectal Cancer patients, Jpn. J. Cancer Res. 93 (2002) 1266–1269 PMID: 12460469.
[267] T. Lecomte, A. Berger, F. Zinzindohoué, S. Micard, B. Landi, H. Blons, et al., Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis, Int. J. Cancer 100 (5) (2002) 542–548, https://doi.org/10.1002/ijc.10526 PMID: 12124803.
[268] B.M. Ryan, F. Lefort, R. McManus, J. Daly, P.W.N. Keeling, D.G. Weir, D. Kelleher, A prospective study of circulating mutant KRAS2 in the serum of patients with colorectal neoplasia: strong prognostic indicator in postoperative follow up, Gut 52 (1) (2003) 101–108, https://doi.org/10.1136/gut.52.1.101 PMID: 12477769.
[269] W.K. Leung, K.F. To, E.P.S. Man, M.W.Y. Chan, A.H.C. Bai, A.J. Hui, et al., Quantitative detection of promoter hypermethylation in multiple genes in the serum of patients with colorectal Cancer, Am. J. Gastroenterol. 100 (10) (2005) 2274–2279, https://doi.org/10.1111/j.1572-0241.2005.50412.x PMID: 16181380.
[270] U. Lindforss, H. Zetterquist, N. Papadogiannakis, H. Olivecrona, Persistence of K-ras mutations in plasma after colorectal tumor resection, Anticancer Res. 25 (1 B) (2005) 657–661, https://doi.org/10.1038/nm.1789.Circulating PMID: 15816642.
[271] V. Bazan, L. Bruno, C. Augello, V. Agnese, V. Calò, S. Corsale, et al., Molecular detection of TP53, Ki-Ras and p16INK4Apromoter methylation in plasma of patients with colorectal cancer and its association with prognosis. Results of a 3-year GOIM (Gruppo Oncologico dell'Italia Meridionale) prospective study, Ann. Oncol. 17 (Suppl. 7) (2006) 84–90, https://doi.org/10.1093/annonc/mdl958 PMID: 16760301.
[272] C. Trevisiol, F. Di Fabio, R. Nascimbeni, L. Peloso, C. Salbe, E. Ferruzzi, et al., Prognostic value of circulating KRAS2 gene mutations in colorectal cancer with distant metastases, Int. J. Biol. Markers 21 (4) (2006) 223–228 PMID: 17177160.
[273] G. Nakayama, K. Hibi, H. Nakayama, Y. Kodera, K. Ito, S. Akiyama, A. Nakao, A highly sensitive method for the detection of p16 methylation in the serum of colorectal cancer patients, Anticancer Res. 27 (3 B) (2007) 1459–1463 PMID: 17595762.
[274] C. Lofton-Day, F. Model, T. DeVos, R. Tetzner, J. Distler, M. Schuster, et al., DNA methylation biomarkers for blood-based colorectal cancer screening, Clin. Chem. 54 (2) (2008) 414–423, https://doi.org/10.1373/clinchem.2007.095992 PMID: 18089654.
[275] T. DeVos, R. Tetzner, F. Model, G. Weiss, M. Schuster, J. Distler, et al., Circulating methylated SEPT9 DNA in plasma is a biomarker for colorectal cancer, Clin. Chem. 55 (7) (2009) 1337–1346, https://doi.org/10.1373/clinchem.2008.115808 PMID: 19406918.
[276] S.R. Morgan, J. Whiteley, E. Donald, J. Smith, M.T. Eisenberg, E. Kallam, L. Kam-Morgan, Comparison of KRAS mutation assessment in tumor DNA and circulating free DNA in plasma and serum samples, Clin Med Insights. Pathology 5 (2012) 15–22, https://doi.org/10.4137/CPath.S8798 PMID: 22661904.
[277] Y. Bai, X. Liu, Y. Wang, F. Ge, C. Zhao, Y. Fu, et al., Correlation analysis between abundance of K-ras mutation in plasma free DNA and its correlation with clinical outcome and prognosis in patients with metastatic colorectal cancer, Chin J Oncol 35 (9) (2013) 666–671, https://doi.org/10.3760/cma.j.issn.0253-3766.2013.09.006 PMID: 24332053.
[278] H.S. Lee, S.M. Hwang, T.S. Kim, D.W. Kim, J. Park, S.B. Kang, et al., Circulating methylated septin 9 nucleic acid in the plasma of patients with gastrointestinal Cancer in the stomach and Colon, Transl. Oncol. 6 (2013) 290–296, https://doi.org/10.1593/tlo.13118 PMID: 23730408.
[279] K.L.G. Spindler, A.L. Appelt, N. Pallisgaard, R.F. Andersen, A. Jakobsen, KRAS-mutated plasma DNA as predictor of outcome from irinotecan monotherapy in metastatic colorectal cancer, Br. J. Cancer 109 (12) (2013) 3067–3072, https://doi.org/10.1038/bjc.2013.633 PMID: 24332053.
[280] Y.B. Kuo, J.S. Chen, C.W. Fan, Y.S. Li, E.C. Chan, Comparison of KRAS mutation analysis of primary tumors and matched circulating cell-free DNA in plasmas of patients with colorectal cancer, Clin. Chim. Acta 433 (2014) 284–289, https://doi.org/10.1016/J.CCA.2014.03.024 PMID: 24685572.

[281] J.K. Lin, P.C. Lin, C.H. Lin, J.K. Jiang, S.H. Yang, W.Y. Liang, et al., Clinical relevance of alterations in quantity and quality of plasma DNA in colorectal Cancer patients: based on the mutation spectra detected in primary tumors, Ann. Surg. Oncol. 21 (S4) (2014) 680–686, https://doi.org/10.1245/s10434-014-3804-5 PMID: 24841357.
[282] S. Mohan, E. Heitzer, P. Ulz, I. Lafer, S. Lax, M. Auer, et al., Changes in colorectal carcinoma genomes under Anti-EGFR therapy identified by whole-genome plasma DNA sequencing, PLoS Genet. 10 (3) (2014), https://doi.org/10.1371/journal.pgen.1004271 e1004271. PMID: 24841357.
[283] F. Perrone, et al., Circulating free DNA in a screening program for early colorectal cancer detection,", Tumori 100 (2) (2014) 115–121.
[284] K.L.G. Spindler, A.L. Appelt, N. Pallisgaard, R.F. Andersen, I. Brandslund, A. Jakobsen, Cell-free DNA in healthy individuals, noncancerous disease and strong prognostic value in colorectal cancer, Int. J. Cancer 135 (12) (2014) 2984–2991, https://doi.org/10.1002/ijc.28946 PMID: 24798213.
[285] Xu JM, Liu XJ, Ge FJ, Lin L, Wang Y, Sharma MR et al. (2014). KRAS mutations in tumor tissue and plasma by different assays predict survival of patients with metastatic colorectal cancer. PMID: 25491325 10.1186/s13046-014-0104-7.
[286] D. Sefrioui, N. Sarafan-Vasseur, L. Beaussire, M. Baretti, A. Gangloff, F. Blanchard, et al., Clinical value of chip-based digital-PCR platform for the detection of circulating DNA in metastatic colorectal cancer, Dig. Liver Dis. 47 (10) (2015) 884–890, https://doi.org/10.1016/j.dld.2015.05.023 PMID: 26160500.
[287] Y. Liu, M.H. Chew, C.K. Tham, C.L. Tang, S.Y. Ong, Y. Zhao, Methylation of serum SST gene is an independent prognostic marker in colorectal cancer, Am. J. Cancer Res. 6 (9) (2016) 2098–2108, https://doi.org/10.1515/cclm-2014-4013 PMID: 27725914.
[288] J. Tie, Y. Wang, C. Tomasetti, L. Li, S. Springer, I. Kinde, et al., Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer, Sci. Transl. Med. 8 (346) (2016), https://doi.org/10.1126/scitranslmed.aaf6219 346ra92. PMID: 27384348.
[289] S. Agah, A. Akbari, A. Talebi, M. Masoudi, A. Sarveazad, A. Mirzaei, F. Nazmi, Quantification of plasma cell-free circulating DNA at different stages of colorectal Cancer, Cancer Invest. 35 (10) (2017) 625–632, https://doi.org/10.1080/07357907.2017.1408814 PMID: 29243990.
[290] A. Herbst, N. Vdovin, S. Gacesa, A. Philipp, D. Nagel, L.M. Holdt, et al., Methylated free-circulating HPP1 DNA is an early response marker in patients with metastatic colorectal cancer, Int. J. Cancer 140 (9) (2017) 2134–2144, https://doi.org/10.1002/ijc.30625 PMID: 28124380.
[291] V. Kloten, N. Rüchel, N.O. Brüchle, J. Gasthaus, F. Steib, J. Mijnes, et al., Liquid biopsy in colon cancer : comparison of different circulating DNA extraction systems following absolute quantification of KRAS mutations using Intplex allele-specific PCR, Oncotarget 8 (49) (2017) 86253–86263, https://doi.org/10.18632/oncotarget.21134 PMID: 29156792.
[292] A.A.L. Pereira, M.P. Morelli, M. Overman, B. Kee, D. Fogelman, E. Vilar, et al., Clinical utility of circulating cell-free DNA in advanced colorectal cancer, PLoS One 12 (8) (2017), https://doi.org/10.1371/journal.pone.0183949 PMID: 28850629.
[293] A.K. Boysen, B.S. Sørensen, A.C. Lefevre, R. Abrantes, J.S. Johansen, B.V. Jensen, et al., Methodological development and biological observations of cell free DNA with a simple direct fluorescent assay in colorectal cancer, Clin. Chim. Acta 487 (2018) 107–111, https://doi.org/10.1016/j.cca.2018.09.029 PMID: 30240586.
[294] C. Demuth, K.L.G. Spindler, J.S. Johansen, N. Pallisgaard, D. Nielsen, E. Hogdall, et al., Measuring KRAS mutations in circulating tumor DNA by droplet digital PCR and next-generation sequencing, Transl. Oncol. 11 (5) (2018) 1220–1224, https://doi.org/10.1016/j.tranon.2018.07.013 PMID: 30086420.
[295] B. Fu, P. Yan, S. Zhang, Y. Lu, L. Pan, W. Tang, et al., Cell-free circulating methylated SEPT9 for noninvasive diagnosis and monitoring of colorectal Cancer, Dis. Markers (2018), https://doi.org/10.1155/2018/6437104 6437104. PMID:29849824.
[296] H. Furuki, T. Yamada, G. Takahashi, T. Iwai, M. Koizumi, S. Shinji, et al., Evaluation of liquid biopsies for detection of emerging mutated genes in metastatic colorectal cancer, EJSO 44 (7) (2018) 975–982, https://doi.org/10.1016/j.ejso.2018.01.224 PMID: 29849824.
[297] M. Gallardo-Gómez, S. Moran, M. Páez de la Cadena, V.S. Martínez-Zorzano, F.J. Rodríguez-Berrocal, M. Rodríguez-Girondo, et al., A new approach to epigenome-wide discovery of non-invasive methylation biomarkers for colorectal cancer screening in circulating cell-free DNA using pooled samples, Clin. Epigenetics 10 (1) (2018), https://doi.org/10.1186/s13148-018-0487-y PMID: 29686738.
[298] S. Klein-Scory, M. Maslova, M. Pohl, C. Eilert-Micus, R. Schroers, W. Schmiegel, A. Baraniskin, Significance of liquid biopsy for monitoring and therapy decision of colorectal Cancer, Transl. Oncol. 11 (2018) 213–220, https://doi.org/10.1016/j.tranon.2017.12.010 PMID: 29367069.
[299] B. Molparia, G. Oliveira, J.L. Wagner, E.G. Spencer, et al., A feasibility study of colorectal cancer diagnosis via circulating tumor DNA derived CNV detection, PLoS One 13 (5) (2018), https://doi.org/10.1371/journal.pone.0196826 PMID: 29791457.
[300] N.N.M. Myint, A.M. Verma, D. Fernandez-Garcia, P. Sarmah, P.S. Tarpey, S.S. Al-Aqbi, et al., Circulating tumor DNA in patients with colorectal adenomas: assessment of detectability and genetic heterogeneity, Cell Death Dis. 9 (9) (2018) 894, https://doi.org/10.1038/s41419-018-0934-x PMID: 30166531.
[301] S. Nunes, C. Moreira-Barbosa, S. Salta, S. Palma de Sousa, I. Pousa, J. Oliveira, et al., Cell-free DNA methylation of selected genes allows for early detection of the major cancers in women, Cancers 10 (10) (2018) 357, https://doi.org/10.3390/cancers10100357 PMID: 30261643.
[302] P. Rokni, A.M. Shariatpanahi, E. Sakhinia, M.A. Kerachian, BMP3 promoter hypermethylation in plasma-derived cell-free DNA in colorectal cancer patients, Genes Genomics 40 (4) (2018) 423–428, https://doi.org/10.1007/s13258-017-0644-2 PMID: 29892846.
[303] T. Song, F. Mao, L. Shi, X. Xu, Z. Wu, J. Zhou, M. Xiao, Urinary measurement of circulating tumor DNA for treatment monitoring and prognosis of metastatic colorectal cancer patients, Clin Chem Lab Med (CCLM) (2018), https://doi.org/10.1515/cclm-2017-0675 PMID: 30016269.
[304] Y. Suehiro, S. Hashimoto, S. Higaki, I. Fujii, C. Suzuki, T. Hoshida, et al., Blood free-circulating DNA testing by highly sensitive methylation assay to diagnose colorectal neoplasias, Oncotarget 9 (24) (2018) 16974–16987, https://doi.org/10.18632/oncotarget.24768 PMID: 29682198.
[305] X. Sun, T. Huang, F. Cheng, K. Huang, M. Liu, W. He, et al., Monitoring colorectal cancer following surgery using plasma circulating tumor DNA, Oncol. Lett. 15 (4) (2018) 4365–4375, https://doi.org/10.3892/ol.2018.7837 PMID: 29541205.
[306] Y. Takayama, K. Suzuki, Y. Muto, K. Ichida, T. Fukui, N. Kakizawa, et al., Monitoring circulating tumor DNA revealed dynamic changes in KRAS status in patients with metastatic colorectal cancer, Oncotarget 9 (36) (2018) 24398–24413, https://doi.org/10.18632/oncotarget.25309 PMID: 29849949.
[307] C.B. Thomsen, T.F. Hansen, R.F. Andersen, J. Lindebjerg, L.H. Jensen, A. Jakobsen, Monitoring the effect of first line treatment in RAS/RAF mutated metastatic colorectal cancer by serial analysis of tumor specific DNA in plasma, J. Exp. Clin. Cancer Res. 37 (1) (2018) 55, https://doi.org/10.1186/s13046-018-0723-5 PMID: 29530101.
[308] M. Yamauchi, Y. Urabe, A. Ono, D. Miki, H. Ochi, K. Chayama, Serial profiling of circulating tumor DNA for optimization of anti-VEGF chemotherapy in metastatic colorectal cancer patients, Int. J. Cancer 142 (7) (2018) 1418–1426, https://doi.org/10.1002/ijc.31154 PMID: 29134647.
[309] Y.C. Yang, D. Wang, L. Jin, H.W. Yao, J.H. Zhang, J. Wang, et al., Circulating tumor DNA detectable in early- and late-stage colorectal cancer patients, Biosci. Rep. (2018), https://doi.org/10.1042/BSR20180322 BSR20180322. PMID: 29914973.
[310] Y.Y. Cheng, J. Yu, Y.P. Wong, E.P.S. Man, K.F. To, V.X. Jin, et al., Frequent epigenetic inactivation of secreted frizzled-related protein 2 (SFRP2) by promoter methylation in human gastric cancer, Br. J. Cancer 97 (7) (2007) 895–901, https://doi.org/10.1038/sj.bjc.6603968 PMID: 17848950.
[311] M.R. Abbaszadegan, O. Moaven, H.R. Sima, K. Ghafarzadegan, A. A'rabi, M.N. Forghani, et al., p16 promoter hypermethylation: a useful serum marker for early detection of gastric cancer, World J. Gastroenterol. 14 (13) (2008) 2055–2060, https://doi.org/10.3748/wjg.14.2055 PMID: 18395906.
[312] Y.C. Wang, Liu C. Yu ZH, L.Z. Xu, W. Yu, J. Lu, et al., Detection of RASSF1A promoter hypermethylation in serum from gastric and colorectal adenocarcinoma patients, World J. Gastroenterol. 14 (19) (2008) 3074–3080, https://doi.org/10.3748/wjg.14.3074 PMID: 18494062.
[313] K. Hibi, T. Goto, A. Shirahata, M. Saito, G. Kigawa, H. Nemoto, Y. Sanada, Detection of TFPI2 methylation in the serum of gastric cancer patients, Anticancer Res. 31 (11) (2011) 3835–3838 PMID: 22110206.
[314] E.K.O. Ng, C.P.H. Leung, V.Y. Shin, C.L.P. Wong, E.S.K. Ma, H.C. Jin, et al., Quantitative analysis and diagnostic significance of methylated SLC19A3 DNA in the plasma of breast and gastric cancer patients, PLoS One 6 (7) (2011) e22233, https://doi.org/10.1371/journal.pone.0022233 PMID: 21789241.
[315] Y. Zheng, L. Chen, J. Li, B. Yu, L. Su, X. Chen, et al., Hypermethylated DNA as potential biomarkers for gastric cancer diagnosis, Clin Bioch 44 (17–18) (2011) 1405–1411, https://doi.org/10.1016/j.clinbiochem.2011.09.006 PMID: 21945024.
[316] A. Shirahata, K. Sakuraba, Y. Kitamura, K. Yokomizo, T. Gotou, M. Saitou, et al., Detection of vimentin methylation in the serum of patients with gastric cancer, Anticancer Res. 32 (3) (2012) 791–794 PMID: 22399595.
[317] I. Balgkouranidou, A. Karayiannakis, D. Matthaios, H. Bolanaki, G. Tripsianis, A.A. Tentes, et al., Assessment of SOX17 DNA methylation in cell free DNA from patients with operable gastric cancer. Association with prognostic variables and survival, Clin. Chem. Lab. Med. 51 (7) (2013) 1505–1510, https://doi.org/10.1515/cclm-2012-0320 PMID: 23403728.
[318] Z.Q. Ling, P. Lv, X.X. Lu, Han J. Yu JL, L.S. Ying, et al., Circulating methylated XAF1 DNA indicates poor prognosis for gastric Cancer, PLoS One 8 (6) (2013), https://doi.org/10.1371/journal.pone.0067195 PMID: 23826230.
[319] Q. Yang, J. Gao, L. Xu, Z. Zeng, J.J.Y. Sung, J. Yu, Promoter hypermethylation of BCL6B gene is a potential plasma DNA biomarker for gastric cancer, Biomarkers 18 (8) (2013) 721–725, https://doi.org/10.3109/1354750X.2013.853839 PMID: 24191714.
[320] J. Han, P. Lv, Wu Y.C. Yu JL, X. Zhu, L.L. Hong, et al., Circulating methylated MINT2 promoter DNA is a potential poor prognostic factor in gastric cancer, Dig. Dis. Sci. 59 (6) (2014) 1160–1168, https://doi.org/10.1007/s10620-013-3007-0 PMID: 24385013.
[321] K.U. Park, H.E. Lee, S.K. Nam, K.H. Nam, D.J. Park, H.H. Kim, et al., The quantification of HER2 and MYC gene fragments in cell-free plasma as putative biomarkers for gastric cancer diagnosis, Clin. Chem. Lab. Med. 52 (7) (2014) 1033–1040, https://doi.org/10.1515/cclm-2013-0988 PMID: 24670359.
[322] Y.C. Wu, P. Lv, J. Han, Zhu X. Yu JL, L.L. Hong, et al., Enhanced serum methylated p16 DNAs is associated with the progression of gastric cancer, Int. J. Clin. Exp. Pathol. 7 (4) (2014) 1553–1562 PMID: 24817951.
[323] Lv P. Yu JL, J. Han, X. Zhu, L.L. Hong, W.Y. Zhu, et al., Methylated TIMP-3 DNA in body fluids is an independent prognostic factor for gastric cancer, Arch. Pathol. Lab. Med. 138 (11) (2014) 1466–1473, https://doi.org/10.5858/arpa.2013-0285-OA PMID: 25357107.
[324] K. Shoda, K. Masuda, D. Ichikawa, T. Arita, Y. Miyakami, M. Watanabe, et al., HER2 amplification detected in the circulating DNA of patients with gastric

cancer: a retrospective pilot study, Gastric Cancer 18 (4) (2015) 698–710, https://doi.org/10.1007/s10120-014-0432-5 PMID: 253222965.
[325] T. Hamakawa, Y. Kukita, Y. Kurokawa, Y. Miyazaki, T. Takahashi, M. Yamasaki, et al., Monitoring gastric cancer progression with circulating tumour DNA, Br. J. Cancer 112 (2) (2015) 352–356, https://doi.org/10.1038/bjc.2014.609 PMID: 25490524.
[326] W.L. Fang, Y.T. Lan, K.H. Huang, C.A. Liu, Y.P. Hung, C.H. Lin, et al., Clinical significance of circulating plasma DNA in gastric cancer, Int. J. Cancer 138 (12) (2016) 2974–2983, https://doi.org/10.1002/ijc.30018 PMID: 26815009.
[327] A. Castells, P. Puig, J. Móra, J. Boadas, L. Boix, E. Urgell, et al., K- ras mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic utility and prognostic significance, J. Clin. Oncol. 17 (2) (1999) 578, https://doi.org/10.1200/JCO.1999.17.2.578 PMID: 10080602.
[328] C.F. Zambon, F. Navaglia, D. Basso, N. Gallo, E. Greco, M.G. Piva, et al., ME-PCR for the identification of mutated K-ras in serum and bile of pancreatic cancer patients: an unsatisfactory technique for clinical applications, Clin. Chim. Acta 302 (1–2) (2000) 35–48, https://doi.org/10.1016/S0009-8981(00)00351-X PMID: 11074062.
[329] F. Maire, S. Micard, P. Hammel, H. Voitot, P. Lé Vy, P.H. Cugnenc, et al., Differential diagnosis between chronic pancreatitis and pancreatic cancer: value of the detection of KRAS2 mutations in circulating DNA, Br. J. Cancer 87 (2002) 551–554, https://doi.org/10.1038/sj.bjc.6600475 PMID: 12189555.
[330] T. Uemura, K. Hibi, T. Kaneko, S. Takeda, S. Inoue, O. Okochi, et al., Detection of K-ras mutations in the plasma DNA of pancreatic cancer patients, J. Gastroenterol. 39 (1) (2004) 56–60, https://doi.org/10.1007/s00535-003-1245-1 PMID: 14767735.
[331] M. Sausen, J. Phallen, V. Adleff, S. Jones, R.J. Leary, M.T. Barrett, et al., Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients, Nat. Commun. 6 (2015), https://doi.org/10.1038/ncomms8686 PMID: 26154128.
[332] E. Takai, Y. Totoki, H. Nakamura, C. Morizane, S. Nara, N. Hama, et al., Clinical utility of circulating tumor DNA for molecular assessment in pancreatic cancer, Nature Publishing Group (2015), https://doi.org/10.1038/srep18425 PMID: 27753011.
[333] O.A. Zill, C. Greene, D. Sebisanovic, L.M. Siew, J. Leng, M. Vu, et al., Cell-free DNA next-generation sequencing in pancreatobiliary carcinomas, Cancer Discov. 5 (10) (2015) 1040–1048, https://doi.org/10.1158/2159-8290.CD-15-0274 PMID: 26109333.
[334] F. Le Calvez-Kelm, M. Foll, M.B. Wozniak, T.M. Delhomme, G. Durand, P. Chopard, et al., KRAS mutations in blood circulating cell-free DNA: a pancreatic cancer case-control, Oncotarget 7 (48) (2016) 78827–78840, https://doi.org/10.18632/oncotarget.12386 PMID: 27705932.
[335] H. Cheng, C. Liu, J. Jiang, G. Luo, Y. Lu, K. Jin, et al., Analysis of ctDNA to predict prognosis and monitor treatment responses in metastatic pancreatic cancer patients, Int. J. Cancer 140 (10) (2017) 2344–2350, https://doi.org/10.1002/ijc.30650 PMID: 28205231.
[336] J.D. Cohen, A.A. Javed, C. Thoburn, F. Wong, J. Tie, P. Gibbs, et al., Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers, Proc Natl Acad Sci U S A (2017), https://doi.org/10.1073/pnas.1704961114] 201704961. PMID: 28874546.
[337] D. Pietrasz, N. Pécuchet, F. Garlan, A. Didelot, O. Dubreuil, S. Doat, et al., Plasma circulating tumor DNA in pancreatic cancer patients is a prognostic marker, Clin. Cancer Res. 23 (1) (2017) 116–123, https://doi.org/10.1158/1078-0432.CCR-16-0806 PMID: 27993964.
[338] M.K. Kim, S.M. Woo, B. Park, K.A. Yoon, Y.H. Kim, J. Joo, et al., Prognostic implications of multiplex detection of KRAS mutations in cell-free DNA from patients with pancreatic ductal adenocarcinoma, Clin. Chem. 64 (4) (2018) 726–734, https://doi.org/10.1373/clinchem.2017.283721 PMID: 29352043.
[339] G. Park, J.K. Park, D.S. Son, S.H. Shin, Y.J. Kim, H.J. Jeon, et al., Utility of targeted deep sequencing for detecting circulating tumor DNA in pancreatic cancer patient, Sci. Rep. 8 (1) (2018) 11631, https://doi.org/10.1038/s41598-018-30100-w PMID: 30072705.
[340] N. Bruhn, T. Beinert, C. Oehm, B. Jandrig, I. Petersen, X.Q. Chen, et al.,) Detection of microsatellite alterations in the DNA isolated from tumor cells and from plasma DNA of patients with lung cancer, Ann. N. Y. Acad. Sci. 906 (2000) 72–82, https://doi.org/10.1111/j.1749-6632.2000.tb06594.x PMID: 10818600.
[341] C.S.H. Ng, J. Zhang, S. Wan, T.W. Lee, A.A. Arifi, T. Mok, et al., Tumor p16M is a possible marker of advanced stage in non-small cell lung cancer, J. Surg. Oncol. 79 (2) (2002) 101–106 PMID: 11815997.
[342] O. Gautschi, C. Bigosch, B. Huegli, M. Jermann, A. Marx, E. Chassé, et al., Circulating deoxyribonucleic acid as prognostic marker in non-small-cell lung cancer patients undergoing chemotherapy, J. Clin. Oncol. 22 (20) (2004) 4157–4164, https://doi.org/10.1200/JCO.2004.11.123 PMID: 15483026.
[343] T. Kimura, W.S. Holland, T. Kawaguchi, S.K. Williamson, K. Chansky, J.J. Crowley, et al., Mutant DNA in plasma of lung cancer patients: potential for monitoring response to therapy, Ann. N. Y. Acad. Sci. 1022 (2004) 55–60, https://doi.org/10.1196/annals.1318.010 PMID: 15251940.
[344] G. Xie, A. Hou, L. Li, Y. Gao, S. Cheng, Quantification of plasma DNA as a screening tool for lung cancer, Chin. Med. J. (Engl) 117 (October (10)) (2004) 1485–1488.
[345] R.E. Board, V.S. Williams, L. Knight, J. Shaw, A. Greystoke, M. Ranson, et al., Isolation and extraction of circulating tumor DNA from patients with small cell lung cancer, Ann. N. Y. Acad. Sci. 1137 (2008) 98–107, https://doi.org/10.1196/annals.1448.020 PMID: 18837931.
[346] V. Ludovini, L. Pistola, V. Gregorc, I. Floriani, E. Rulli, S. Piattoni, et al., Plasma DNA, microsatellite alterations, and p53 tumor mutations are associated with disease-free survival in radically resected non-small cell lung Cancer patients: a study of the perugia multidisciplinary team for thoracic oncology, J. Thorac. Oncol. 3 (2008), https://doi.org/10.1097/JTO.0b013e318168c7d0 PMID: 18379354.
[347] M. Paci, S. Maramotti, E. Bellesia, D. Formisano, L. Albertazzi, T. Ricchetti, et al., Circulating plasma DNA as diagnostic biomarker in non-small cell lung cancer, Lung Cancer 64 (1) (2009) 92–97, https://doi.org/10.1016/j.lungcan.2008.07.012 PMID: 18804892.
[348] R. Sirera, R.M. Bremnes, A. Cabrera, E. Jantus-Lewintre, E. Sanmartín, A. Blasco, et al., Circulating DNA is a useful prognostic factor in patients with advanced non-small cell lung cancer, J. Thorac. Oncol. 6 (2) (2011) 286–290, https://doi.org/10.1097/JTO.0b013e31820189a5 PMID: 21252717.
[349] S.M. Lee, J.Y. Park, D.S. Kim, Methylation of TMEFF2 gene in tissue and serum DNA from patients with non-small cell lung cancer, Mol. Cells 34 (2) (2012) 171–176, https://doi.org/10.1007/s10059-012-0083-5 PMID: 22814847.
[350] M. Murtaza, S.J. Dawson, D.W.Y. Tsui, D. Gale, T. Forshew, A.M. Piskorz, et al., Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA, Nature 497 (7447) (2013) 108–112, https://doi.org/10.1038/nature12065 PMID: 23563269.
[351] M.T. Bortolin, R. Tedeschi, E. Bidoli, C. Furlan, G. Basaglia, E. Minatel, et al., Cell-free DNA as a prognostic marker in stage I non-small-cell lung cancer patients undergoing stereotactic body radiotherapy, Biomarkers 20 (6–7) (2015) 422–428, https://doi.org/10.3109/1354750X.2015.1094139 PMID: 26526078.
[352] A. Marchetti, J.F. Palma, L. Felicioni, T.M. De Pas, R. Chiari, M. Del Grammastro, et al.,) Early prediction of response to tyrosine kinase inhibitors by quantification of EGFR mutations in plasma of NSCLC patients, J. Thorac. Oncol. 10 (10) (2015) 1437–1443, https://doi.org/10.1097/JTO.0000000000000643 PMID: 26295376.
[353] S. Zhang, L. Zhu, B. Xia, E. Chen, Q. Zhao, X. Zhang, et al., Epidermal growth factor receptor (EGFR) T790M mutation identified in plasma indicates failure sites and predicts clinical prognosis in non-small cell lung cancer progression during first-generation tyrosine kinase inhibitor therapy: a prospective observational study, Cancer Commun. 38 (1) (2018) 28, https://doi.org/10.1186/s40880-018-0303-2 PMID: 29789021.
[354] W.N. Feng, W.Q. Gu, N. Zhao, Y.M. Pan, W. Luo, H. Zhang, et al., Comparison of the SuperARMS and droplet digital PCR for detecting EGFR mutation in ctDNA from NSCLC patients, Transl. Oncol. 11 (2) (2018) 542–545, https://doi.org/10.1016/j.tranon.2018.02.007 PMID: 29525631.
[355] S. Nishikawa, H. Kimura, H. Koba, T. Yoneda, S. Watanabe, T. Sakai, et al., Selective gene amplification to detect the T790M mutation in plasma from patients with advanced non-small cell lung cancer (NSCLC) who have developed epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) resistance, J. Thorac. Dis. 10 (3) (2018) 1431–1439, https://doi.org/10.21037/jtd.2018.01.144 PMID: 29707292.
[356] C. Demuth, A.T. Madsen, B. Weber, L. Wu, P. Meldgaard, B.S. Sorensen, The T790M resistance mutation in EGFR is only found in cfDNA from erlotinib-treated NSCLC patients that harbored an activating EGFR mutation before treatment, BMC Cancer 18 (1) (2018) 191 PMID: 29448920 10.1186%2Fs12885-018-4108-0.
[357] I. Fahoum, R. Forer, D. Volodarsky, I. Vulih, T. Bick, S. Sarji, Z. Bamberger, et al., Characterization of factors affecting the detection limit of EGFR p.T790M in circulating tumor DNA, Technol. Cancer Res. Treat. 17 (2018), https://doi.org/10.1177/1533033818793653 PMID: 30099961.
[358] Y. Hu, R.S. Alden, J.I. Odegaard, S.R. Fairclough, R. Chen, J. Heng, N. Feeney, et al., Discrimination of germline EGFR T790M mutations in plasma cell-free DNA allows study of prevalence across 31,414 Cancer patients, Clin. Cancer Res. 23 (23) (2017) 7351–7359, https://doi.org/10.1158/1078-0432.CCR-17-1745 PMID: 28947568.
[359] J.S. Sung, H.Y. Chong, N.J. Kwon, H.M. Kim, J.W. Lee, B. Kim, et al., Detection of somatic variants and EGFR mutations in cell-free DNA from non-small cell lung cancer patients by ultra-deep sequencing using the ion ampliseq cancer hotspot panel and droplet digital polymerase chain reaction, Oncotarget 8 (63) (2017) 106901–106912, https://doi.org/10.18632/oncotarget.22456 PMID: 29290998.
[360] C. Jovelet, J. Madic, J. Remon, A. Honoré, R. Girard, E. Rouleau, 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 12 (8) (2017), https://doi.org/10.1371/journal.pone.0183319 PMID: 28829811.
[361] Z. Wei, W. Wang, Z. Shu, X. Zhou, Y. Zhang, Correlation between circulating tumor DNA levels and response to tyrosine kinase inhibitors (TKI) treatment in non-small cell lung Cancer, Med. Sci. Monit. 23 (2017) 3627–3634, https://doi.org/10.12659/MSM.902265 PMID: 28742791.
[362] L. Xiong, S. Cui, J. Ding, Y. Sun, L. Zhang, Y. Zhao, A. Gu, et al., Dynamics of EGFR mutations in plasma recapitulates the clinical response to EGFR-TKIs in NSCLC patients, Oncotarget 8 (38) (2017) 63846–63856, https://doi.org/10.18632/oncotarget.19139 PMID: 28969034.
[363] W. Hu, Y. Yang, L. Zhang, J. Yin, J. Huang, et al., Post surgery circulating free tumor DNA is a predictive biomarker for relapse of lung cancer, Cancer Med. 6 (5) (2017) 962–974, https://doi.org/10.1002/cam4.980 PMID: 28382702.
[364] N. Kasahara, H. Kenmotsu, M. Serizawa, R. Umhera, A. Ono, Y. Hisamatsu, K. Wakuda, et al.,) Plasma epidermal growth factor receptor mutation testing with a chip-based digital PCR system in patients with advanced non-small cell lung cancer, Lung Cancer 106 (2017) 138–144, https://doi.org/10.1016/j.lungcan.2017.02.001 PMID: 28285688.
[365] M. Yanagita, A.J. Redig, Paweletz Cp, S.E. Dahlberg, A. O'Connell, et al., A prospective evaluation of circulating tumor cells and cell-free DNA in EGFR-Mutant non-small cell lung Cancer patients treated with erlotinib on a phase II trial, Clin. Cancer Res. 22 (24) (2016) 6010–6020, https://doi.org/10.1158/1078-0432.CCR-16-0909 PMID: 27281561.

[366] F. Imamura, J. uchida, Y. Kukita, T. Kumagai, K. Nishino, et al., Early responses of EGFR circulating tumor DNA to EGFR tyrosine kinase inhibitors in lung cancer treatment, Oncotarget 7 (44) (2016) 71782–71789, https://doi.org/10.18632/oncotarget.12373 PMID: 27708242.
[367] E. Alegre, J.P. Fusco, P. Restituto, D. Salas-Benito, M.E. Rodríguez-Ruiz, M.P. Andueza, et al., Total and mutated EGFR quantification in cell-free DNA from non-small cell lung cancer patients detects tumor heterogeneity and presents prognostic value, J. Immunother. Emphasis Tumor Immunol. 37 (10) (2016) 13687–13694, https://doi.org/10.1007/s13277-016-5282-9 PMID: 27473086.
[368] A.L. Riediger, S. Dietz, U. Schirmer, M. Meister, I. Heinzmann-Groth, et al., Mutation analysis of circulating plasma DNA to determine response to EGFR tyrosine kinase inhibitor therapy of lung adenocarcinoma patients, Nat. Publ. Gr. (2016), https://doi.org/10.1038/srep33505 PMID: 27640882.
[369] Q. Zhou, J.J. Yang, Z.H. Chen, X.C. Zhang, H.H. Yan, C.R. Xu, et al., Serial cfDNA assessment of response and resistance to EGFR-TKI for patients with EGFR-L858R mutant lung cancer from a prospective clinical trial, J. Hematol. Oncol. 9 (1) (2016) 86, https://doi.org/10.1186/s13045-016-0316-8 PMID: 27619632.
[370] K. Guo, Z. zhang, L. Han, J. Han, J. Wang, et al., Detection of epidermal growth factor receptor mutation in plasma as a biomarker in Chinese patients with early-stage non-small cell lung cancer, Onco. Ther. 8 (2015) 3289, https://doi.org/10.2147/OTT.S94297 PMID: 26609241.
[371] N. Karachaliou, C. Mayo-de Las Casas, C. Queralt, I. de Aguirre, B. Melloni, et al., Association of EGFR L858R mutation in circulating free DNA with survival in the EURTAC trial, JAMA Oncol. 1 (2) (2015) 149–157, https://doi.org/10.1001/jamaoncol.2014.257 PMID: 26181014.
[372] X. Zhao, R.B. Han, J. Zhao, J. Wang, F. Yang, W. Zhing, et al., Comparison of epidermal growth factor receptor mutation statuses in tissue and plasma in stage I-IV non-small cell lung cancer patients, Respiration 85 (2) (2013) 119–125, https://doi.org/10.1159/000338790 PMID: 22797485.
[373] E. Helman, M. Nguyen, C.A. Karlovich, D. Despain, A.K. Choquette, A.I. Spira, et al.,) cell-free DNA next-generation sequencing prediction of response and resistance to third-generation EGFR inhibitor, Clin. Lung Cancer 19 (6) (2018) 518–530, https://doi.org/10.1016/j.cllc.2018.07.008 PMID: 30279111.
[374] Z. Xiang, R. Wan, B. Zou, X. Qi, Q. Huang, S. Kumar, et al., Highly sensitive and specific real-time PCR by employing serial invasive reaction as a sequence identifier for quantifying EGFR mutation abundance in cfDNA, Anal. Bioanal. Chem. 410 (26) (2018) 6751–6759, https://doi.org/10.1007/s00216-018-1316-z PMID: 30128808.
[375] M. Macías, E. Alegre, G. Alkorta-Aranburu, A. Patino-García, B. Mateos, M.P. Andueza, et al., The dynamic use of EGFR mutation analysis in cell-free DNA as a follow-up biomarker during different treatment lines in non-small-Cell lung Cancer patients, Dis. Markers (2019) 1–7, https://doi.org/10.1155/2019/7954921 PMID: 30809319.
[376] X. Chen, H. Bonnefoi, S. Diebold-Berger, J. Lyautey, C. Lederrey, E. Faltin-Traub, et al.,) Detecting tumor-related alterations in plasma or serum DNA of patients diagnosed with breast cancer, Clin. Cancer Res. 5 (9) (1999) 2297–2303 PMID: 10499596.
[377] J.M. Silva, J. Silva, A. Sanchez, J.M. Silva, J. Silva, A. Sanchez, et al., Tumor DNA in plasma at diagnosis of breast Cancer patients is a valuable predictor of disease-free survival, Clin. Cancer Res. 8 (2002) 3761–3766 PMID: 12473587.
[378] H.M. Müller, A. Widschwendter, H. Fiegl, L. Ivarsson, G. Goebel, E. Perkmann, et al., DNA methylation in serum of breast cancer patients: an independent prognostic marker, Cancer Res. 63 (22) (2003) 7641–7645 PMID: 12473587.
[379] R.A. Zanetti-Dällenbach, S. Schmid, E. Wight, W. Holzgreve, A. Ladewig, S. Hahn, X.Y. Zhong, Levels of circulating cell-free serum DNA in benign and malignant breast lesions, Int. J. Biol. Markers 22 (2) (2007) 95–99 PMID: 17549664.
[380] E. Sunami, A.T. Vu, S.L. Nguyen, A.E. Giuliano, D.S.B. Hoon, Quantification of LINE1 in circulating DNA as a molecular biomarker of breast cancer, Ann. N. Y. Acad. Sci. 1137 (2008) 171–174, https://doi.org/10.1196/annals.1448.011 PMID: 18837943.
[381] R.A. Zanetti-Dällenbach, E. Wight, A.X.C. Fan, O. Lapaire, S. Hahn, W. Holzgreve, et al., Positive correlation of cell-free DNA in plasma/serum in patients with malignant and benign breast disease, Anticancer Res. 28 (2A) (2008) 921–925 PMID: 18507037.
[382] C. Kohler, R. Radpour, Z. Barekati, R. Asadollahi, J. Bitzer, E. Wight, et al., Levels of plasma circulating cell free nuclear and mitochondrial DNA as potential biomarkers for breast tumors, Mol. Cancer 8 (2009), https://doi.org/10.1186/1476-4598-8-105 PMID: 19922604.
[383] M. Zurita, P.C. Lara, R. del Moral, B. Torres, J.L. Linares-Fernández, S.R. Arrabal, et al.,) Hypermethylated 14-3-3-sigma and ESR1 gene promoters in serum as candidate biomarkers for the diagnosis and treatment efficacy of breast cancer metastasis, BMC Cancer 10 (1) (2010) 217, https://doi.org/10.1186/1471-2407-10-217] PMID: 20487521.
[384] B. Gong, J. Xue, J. Yu, H. Li, H. Hu, H. Yen, et al., Cell-free DNA in blood is a potential diagnostic biomarker of breast cancer, Oncol. Lett. 3 (4) (2012) 897–900, https://doi.org/10.3892/ol.2012.576 PMID: 22741014.
[385] H. Schwarzenbach, C. Eichelser, J. Kropidlowski, W. Janni, B. Rack, K. Pantel, Loss of heterozygosity at tumor suppressor genes detectable on fractionated circulating cell-free tumor DNA as indicator of breast cancer progression, Clin. Cancer Res. 18 (20) (2012) 5719–5730, https://doi.org/10.1158/1078-0432.CCR-12-0142] PMID: 23014523.
[386] D.S. Guttery, K. Page, A. Hills, L. Woodley, S.D. Marchese, B. Rghebi, et al., Noninvasive detection of activating estrogen receptor 1 (ESR1) mutations in estrogen receptor-positive metastatic breast cancer, Clin. Chem. 61 (7) (2015) 974–982, https://doi.org/10.1373/clinchem.2015.238717 PMID: 25979954.
[387] R. Maltoni, V. Casadio, S. Ravaioli, F. Foca, M. Tumedei, S. Salvi, et al., Cell-free DNA detected by "liquid biopsy" as a potential prognostic biomarker in early breast cancer, Oncotarget 8 (10) (2017) 16642–16649, https://doi.org/10.18632/oncotarget.15120 PMID: 28186965.
[388] T. Takeshita, Y. Yamamoto, M. Yamamoto-Ibusuki, M. Tomiguchi, A. Sueta, K. Murakami, et al., Analysis of *ESR1* and *PIK3CA* mutations in plasma cell-free DNA from ER-positive breast cancer patients, Oncotarget 8 (32) (2017) 52142–52155, https://doi.org/10.18632/oncotarget.18479 PMID: 28881720.
[389] D. Li, P. Li, J. Wu, J. Yi, Y. Dou, X. Guo, et al., Methylation of NBPF1 as a novel marker for the detection of plasma cell-free DNA of breast cancer patients, Clin. Chim. Acta 484 (2018) 81–86, https://doi.org/10.1016/j.cca.2018.05.030 PMID: 29775621.
[390] T. Takeshita, Y. Yamamoto, M. Yamamoto-Ibusuki, M. Tomiguchi, A. Sueta, K. Murakami, H. Iwase, Clinical significance of plasma cell-free DNA mutations in PIK3CA, AKT1, and ESR1 gene according to treatment lines in ER-positive breast cancer, Mol. Cancer (2018), https://doi.org/10.1186/s12943-018-0808-y PMID: 29482551.
[391] R.R. Zachariah, S. Schmid, N. Buerki, R. Radpour, W. Holzgreve, X. Zhong, Levels of circulating cell-free nuclear and mitochondrial DNA in benign and malignant ovarian tumors, Obstet. Gynecol. 112 (4) (2008) 843–850, https://doi.org/10.1186/s12943-018-0808-y PMID: 29482551.
[392] B. Dobrzycka, S.J. Terlikowski, M. Kinalski, O. Kowalczuk, W. Niklinska, L. Chyczewski, Circulating free DNA and p53 antibodies in plasma of patients with ovarian epithelial cancers, Ann. Oncol. 22 (5) (2011) 1133–1140, https://doi.org/10.1093/annonc/mdq584 PMID: 21098618.
[393] T.E. Liggett, A. Melnikov, Q. Yi, C. Replogle, W. Hu, et al., Distinctive DNA methylation patterns of cell-free plasma DNA in women with malignant ovarian tumors, Gynecol. Oncol. 120 (1) (2011) 113–120, https://doi.org/10.1016/j.ygyno.2010.09.019 PMID: 21056906.
[394] P. Wimberger, C. Roth, K. Pantel, S. Kasimir-Bauer, R. Kimmig, H. Schwarzenbach, Impact of platinum-based chemotherapy on circulating nucleic acid levels, protease activities in blood and disseminated tumor cells in bone marrow of ovarian cancer patients, Int. J. Cancer 128 (11) (2011) 2572–2580, https://doi.org/10.1002/ijc.25602 PMID: 20715113.
[395] J.H. No, K. Kim, K.H. Park, Y.B. Kim, Cell-free DNA level as a prognostic biomarker for epithelial ovarian cancer, Anticancer Res. 32 (8) (2012) 3467–3471 PMID: 22843932.
[396] Q. Zhang, G. Hu, Q. Yang, R. Dong, X. Xie, et al., A multiplex methylation-specific PCR assay for the detection of early-stage ovarian cancer using cell-free serum DNA, Gynecol. Oncol. 130 (1) (2013) 132–139, https://doi.org/10.1016/j.ygyno.2013.04.048 PMID: 23623832.
[397] S. Choudhuri, C. Sharma, A. Banerjee, S. Kumar, L. Kumar, N. Singh, A repertoire of biomarkers helps in detection and assessment of therapeutic response in epithelial ovarian cancer, Mol. Cell. Biochem. 386 (1–2) (2014) 259–269, https://doi.org/10.1007/s11010-013-1863-8 PMID: 24141793.
[398] E. Pereira, O. Camacho-Venegas, S. Anand, R. Sebra, S. Catalina Camacho, et al., Personalized circulating tumor DNA biomarkers dynamically predict treatment response and survival in gynecologic cancers, PLoS One 10 (12) (2015), https://doi.org/10.1371/journal.pone.0145754 PMID: 26717006.
[399] F.R. Harris, I.V. Kovtun, J. Smadbeck, F. Multinu, A. Jatoi, F. Kosari, et al., Quantification of Somatic Chromosomal Rearrangements in Circulating Cell-Free DNA from Ovarian Cancers, Sci. Rep. 6 (1) (2016) 29831, https://doi.org/10.1038/srep29831] PMID: 27436510.
[400] C.A. Parkinson, D. Gale, A.M. Piskorz, H. Biggs, C. Hodgkin, H. Addley, et al., Exploratory analysis of TP53 mutations in circulating tumour DNA as biomarkers of treatment response for patients with relapsed high-grade serous ovarian carcinoma: a retrospective study, PLoS Med. 13 (12) (2016), https://doi.org/10.1371/journal.pmed.1002198 PMID: 27997533.
[401] E.L. Christie, S. Fereday, K. Doig, S. Pattnaik, S.J. Dawson, D.D.L. Bowtell, Reversion of BRCA1/2 germline mutations detected in circulating tumor DNA from patients with high-grade serous ovarian cancer, J. Clin. Oncol. 35 (12) (2017) 1274–1280, https://doi.org/10.1200/JCO.2016.70.4627 PMID: 284149251.
[402] L. Giannopoulou, I. Chebouti, K. Pavlakis, S. Kasimir-Bauer, E.S. Lianidou, RASSF1A promoter methylation in high-grade serous ovarian cancer: a direct comparison study in primary tumors, adjacent morphologically tumor cell-free tissues and paired circulating tumor DNA, Oncotarget 8 (13) (2017) 21429–21443, https://doi.org/10.18632/oncotarget.15249 PMID: 28206954.
[403] B. Weigelt, I. Comino-Méndez, I. de bruijn, L. Tian, J.L. Meisel, et al., Diverse BRCA1 and BRCA2 reversion mutations in circulating cell-free DNA of therapy-resistant breast or ovarian Cancer, Clin. Cancer Res. 23 (21) (2017) 6708–6720, https://doi.org/10.1158/1078-0432.CCR-17-0544] PMID: 28765325.
[404] M. Widschwendter, M. zikan, B. Wahl, H. Lempiainen, T. Paprotka, et al., The potential of circulating tumor DNA methylation analysis for the early detection and management of ovarian cancer, Genome Med. 9 (1) (2017) 116, https://doi.org/10.1186/s13073-017-0500-7 PMID: 29268796.
[405] L. Giannopoulou, S. Mastoraki, P. Buderath, A. Strati, K. Pavlakis, et al., ESR1 methylation in primary tumors and paired circulating tumor DNA of patients with high-grade serous ovarian cancer, Gynecol. Oncol. 150 (2) (2018) 355–360, https://doi.org/10.1016/j.ygyno.2018.05.026 PMID: 29807696.
[406] Y.R. Park, Y.M. Kim, S.W. Lee, H.Y. Lee, G.E. Lee, et al., Optimization to detect TP53 mutations in circulating cell-free tumor DNA from patients with serous epithelial ovarian cancer, Obstet. Gynecol. Sci. 61 (3) (2018) 328, https://doi.org/10.5468/ogs.2018.61.3.328 PMID: 29780774.
[407] C. Jerónimo, H. Usadel, R. Henrique, C. Silva, J. Oliveira, C. Lopes, D. Sidransky, Quantitative GSTP1 hypermethylation in bodily fluids of patients with prostate cancer, Urology 60 (6) (2002) 1131–1135, https://doi.org/10.1016/S0090-

4295(02)01949-0 PMID: 12475696.
[408] J. Reibenwein, D. Pils, P. Horak, B. Tomicek, G. Goldner, N. Worel, et al., Promoter hypermethylation of GSTP1, AR, and14-3 -3 s in serum of prostate Cancer patients and its clinical relevance, Prostate 67 (September 2006) (2007) 427–432, https://doi.org/10.1002/pros PMID: 17192910.
[409] A.V. Cherepanova, S.N. Tamkovich, O.E. Bryzgunova, V.V. Vlassov, P.P. Laktionov, Deoxyribonuclease activity and circulating DNA concentration in blood plasma of patients with prostate tumors, Ann. N. Y. Acad. Sci. 1137 (1) (2008) 218–221, https://doi.org/10.1196/annals.1448.016 PMID: 18837950.
[410] J. Ellinger, K. Haan, L.C. Heukamp, P. Kahl, R. Büttner, S.C. Müller, et al., CpG island hypermethylation in cell-free serum DNA identifies patients with localized prostate cancer, Prostate 68 (1) (2008) 42–49, https://doi.org/10.1002/pros.20651 PMID: 18004747.
[411] E. Sunami, M. Shinozaki, C.S. Higano, R. Wollman, T.B. Dorff, S.J. Tucker, et al., Multimarker circulating DNA assay for assessing blood of prostate cancer patients, Clin. Chem. 55 (3) (2009) 559–567, https://doi.org/10.1373/clinchem.2008.108498 PMID: 19131636.
[412] P.O. Delgado, B.C.A. Alves, F. de Sousa Gehrke, R.K. Kuniyoshi, M.L. Wroclavski, A. Del Giglio, F.L.A. Fonseca, Characterization of cell-free circulating DNA in plasma in patients with prostate cancer, J. Immunother. Emphasis Tumor Immunol. 34 (2) (2013) 983–986, https://doi.org/10.1007/s13277-012-0634-6 PMID: 23269609.
[413] J. Feng, F. Gang, X. Li, T. Jin, H. Houbao, C. Yu, L. Guorong, Plasma cell-free DNA and its DNA integrity as biomarker to distinguish prostate cancer from benign prostatic hyperplasia in patients with increased serum prostate-specific antigen, Int. Urol. Nephrol. 45 (4) (2013) 1023–1028, https://doi.org/10.1007/s11255-013-0491-2 PMID: 23779229.
[414] A.A. Azad, S.V. Volik, A.W. Wyatt, A. Haegert, S. Le Bihan, R.H. Bell, et al., Androgen receptor gene aberrations in circulating cell-free DNA: biomarkers of therapeutic resistance in castration-resistant prostate cancer, Clin. Cancer Res. 21 (10) (2015) 2315–2324, https://doi.org/10.1158/1078-0432.CCR-14-2666 PMID: 25712683.
[415] A. Kienel, D. Porres, A. Heidenreich, D. Pfister, CfDNA as a prognostic marker of response to taxane based chemotherapy in patients with prostate cancer, J. Urol. 194 (4) (2015) 966–971, https://doi.org/10.1016/j.juro.2015.04.055 PMID: 25896555.
[416] A. Romanel, D.G. Tandefelt, V. Conteduca, A. Jayaram, N. Casiraghi, D. Wetterskog, et al., Plasma AR and abiraterone-resistant prostate cancer, Sci. Transl. Med. 7 (312) (2015) 312re10, https://doi.org/10.1126/scitranslmed.aac9511 PMID: 26537258.
[417] E. Schütz, M.R. Akbari, J. Beck, H. Urnovitz, W.W. Zhang, K. Bornemann-Kolatzki, et al., Chromosomal instability in cell-free DNA is a serum biomarker for prostate cancer, Clin. Chem. 61 (1) (2015) 239–248, https://doi.org/10.1373/clinchem.2014.226571 PMID: 25348670.
[418] Y. Xia, C.C. Huang, R. Dittmar, M. Du, Y. Wang, H. Liu, et al., Copy number variations in urine cell free DNA as biomarkers in advanced prostate cancer, Oncotarget 7 (24) (2016) 35818–35831, https://doi.org/10.18632/oncotarget.9027 PMID: 27127882.
[419] J. Goodall, J. Mateo, W. Yuan, H. Mossop, N. Porta, S. Miranda, et al., Circulating cell-free DNA to guide prostate cancer treatment with PARP inhibition, Cancer Disc 7 (9) (2017) 1006–1017, https://doi.org/10.1158/2159-8290.CD-17-0261 PMID: 28450425.
[420] M.A. Schirmer, J. Beck, M. Leu, M. Oellerich, M. Rave-Fränk, P.D. Walson, et al., Cell-free plasma DNA for disease stratification and prognosis in head and neck Cancer, Clin. Chem. 64 (6) (2018) 959–970, https://doi.org/10.1373/clinchem.2017.285668 PMID: 29661793.
[421] C. Pupilli, P. Pinzani, F. Salvianti, B. Fibbi, M. Rossi, L. Petrone, et al., Circulating BRAFV600E in the diagnosis and follow-up of differentiated papillary thyroid carcinoma, J. Clin. Endocrinol. Metab. 98 (8) (2013) 3359–3365, https://doi.org/10.1210/jc.2013-1072 PMID: 23788690.
[422] S. Hu, M. Ewertz, R.P. Tufano, M. Brait, A.L. Carvalho, D. Liu, A.P. Tufaro, et al.,) Detection of serum deoxyribonucleic acid methylation markers: a novel diagnostic tool for thyroid cancer, J. Clin. Endocrinol. Metab. 91 (1) (2006) 98–104, https://doi.org/10.1210/jc.2005-1810 PMID: 16263813.
[423] F. Salvianti, C. Giuliani, L. Petrone, I. Mancini, V. Vezzosi, et al., Integrity and quantity of total cell-free DNA in the diagnosis of thyroid Cancer: correlation with cytological classification, Int. J. Mol. Sci. 18 (7) (2017), https://doi.org/10.3390/ijms18071350 PMID: 28672797.
[424] M. Lupo, R. Guttler, Z. Geck, T.R. Tonozzi, A. Kammesheidt, G.D. Braunstein, Is measurement of circulating tumor DNA of diagnostic use in patients with thyroid nodules? Endocr. Pract. 24 (5) (2018) 453–459, https://doi.org/10.4158/EP-2017-0213 PMID: 29498908.
[425] E. Boysen Hansen, Blood sample monitoring of patients with EGFR mutated lung cancer, Clinical trials.gov, U.S. National Library of Medicine, 2015 Available from: https://clinicaltrials.gov/ct2/show/NCT02284633.
[426] Sidney Kimmel Comprehensive Cancer centre at John Hopkins, Monitoring plasma tumor DNA in early-stage breast cancer, Clinical Trials.gov, U.S. National Library of Medicine, 2016 Available from: https://clinicaltrials.gov/ct2/show/NCT02743910.
[427] K. Wilkinson, plasmaMATCH: A Clinical Trials Aiming to Assess the Safety and Activity of Targeted Treatments in Patients With Advanced Breast Cancer Where the Targetable Mutation Is Identified Through Circulating Tumour DNA Screening, Available from: ISRCTN. International Standard Randomised Controlled Trials Number Registry, 2016, http://www.isrctn.com/ISRCTN16945804.