Clinical relevance of liquid biopsy for cancer screening

Rita Tanos[1,2,3,4], Alain R. Thierry[1,2,3,4]

[1]IRCM, Institut de Recherche en Cancérologie de Montpellier, Montpellier, France; [2]INSERM, U1194, Montpellier, France; [3]Université de Montpellier, Montpellier, France; [4]Institut régional du Cancer de Montpellier, Montpellier, France

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Correspondence to: Alain R. Thierry. INSERM, U1194, Institut de Recherche en Cancérologie de Montpellier, Campus Val d'Aurelle, 208 avenue des Apothicaires, 34298 Montpellier, cedex 5, France. Email: alain.thierry@inserm.fr.

Abstract: Curative therapies for cancer are often successful when it is early detected and treated. Cancer screening aims at detecting cancer at early stages, before symptoms appear, and when the treatment is most likely to be effective. But most cancer types lack well-established biomarkers allowing the identification of the disease. Non-invasive tests, such as liquid biopsy, hold promise for screening people for cancer and could help advance cancer early detection. Transposing liquid biopsy into clinic to improve survival rates of patients with various types of cancers has been attracting much attention, due to the great potential of blood-based biomarkers for early diagnosis. The present review focuses on the clinical relevance of liquid biopsy for cancer screening, and summarizes the different studies conducted on several circulating biomarkers including circulating cell-free DNA (cfDNA), circulating tumor cells (CTCs), circulating microRNAs (miRNAs) and others, for the development of tests for early cancer detection.

Keywords: Cancer; screening; liquid biopsy; early detection; circulating DNA; circulating tumor cell (CTC)

Cancer screening

The progression of cancer to late stages without the appearance of symptoms is one of the main reasons for being among the leading causes of death worldwide. The development of an effective screening test that identifies asymptomatic individuals to assess their likelihood having the disease has a major objective to reduce morbidity or mortality in the screened population by early detection, when treatment is more successful (1). Therefore, an early detection of cancer, before a person shows any signs of illness, would increase the chances of recovery and patients' overall survival and might help reduce cancer-related mortality.

A screening test differs from a diagnostic test by the fact that the second is used when a subject shows signs or symptoms, to determine the presence or absence of a disease, and is usually performed after a positive screening test to establish a definitive diagnosis.

The development of effective screening techniques for early detection does not exist for many types of cancers, and many have not proven effective in reducing cancer mortality. That was due to various reasons, in particular reduced sensitivity and specificity of the tests, inter and intra-tumoral heterogeneity (2-4) or epidemiological factors…

How to evaluate a screening test for cancer

In 1968, the World Health Organization (WHO) published guidelines on the principles and practice of screening for disease, which are often referred to as Wilson's criteria (5). But with the emergence of new genomic technologies, the WHO modified these guidelines in 2008 with the new understanding as follows (6): "*The screening program should respond to a recognized need, and its objectives should be defined*

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at the outset. There should be a defined target population and scientific evidence of the screening program's effectiveness. The program should integrate education, testing, clinical services and program management, along with quality assurance and mechanisms to minimize potential risks of screening. It should ensure informed choice, confidentiality and respect for autonomy, and promote equity and access to screening for the entire target population. The evaluation should be planned from the outset, and the overall benefits of screening should outweigh the harm".

An ideal screening test for cancer would be able to perfectly discriminate between individuals who have or do not have the disease (1). In practice, screening tests could exhibit false positives and false negatives (7). The consequences of these results need to be carefully considered when evaluating the advantages and disadvantages of the test, and so taking into account its benefits on one hand and its risks on the other (8). When a new screening test is developed, it is regularly compared to the gold standard test, the best test available, which usually consists of a diagnostic test considered as definitive, like a biopsy for instance. However, the latter is often invasive, expensive, unpleasant, too late, or impractical to be used widely as a screening test (1). The new test would be less expensive or noninvasive for example. Its validity is translated by a high sensitivity, which is the ability of the test to identify correctly those who have that disease, and a high specificity reflecting its capacity to identify correctly those who do not have the disease (9).

In order to fully evaluate the performance of a screening test, particular attention must be paid to the tested cohorts. Indeed, a blind study, and an association of already diagnosed individuals and populations at risk are a necessity.

Conventional biomarkers and screening tests

Different screening tests are currently being used for various types of cancer. The pap smear test, for example, is used for cervical dysplasia or cervical cancer but it does not show a high sensitivity, however, it has a high specificity (10,11). Mammography is the most common test used for breast cancer screening (12,13), it has played a key role in reducing breast cancer mortality nevertheless it presents a limited sensitivity along with excessive false-positive results and the potential of overdiagnosis. Assays for serum markers, such as the tumor antigen CA 15-3 (cancer antigen 15-3) was also applied in patients with breast cancer, but showed an average sensitivity (55,6% sensitivity for a 98% specificity) (14). As a result, this marker is preferentially used for treatment

response monitoring than screening and early diagnosis. The prostate-specific antigen (PSA) test could help find prostate cancer before symptoms appear (15), although a high PSA level does not always result from the presence of cancer. Low-dose computed tomographic (CT) screening is usually recommended for people with a high risk of developing lung cancer (16) but some cancers might be missed at screening and others might develop between screening and detection. For colorectal cancer (CRC), the two most common serum-based glycoprotein CRC markers, the cancer embryonic antigen (CEA) and the carbohydrate antigen 199 (CA199) are not appropriate for CRC screening due to their low sensitivity and the lack of specificity, especially for early-stage CRC [for CEA: sensitivity of 40.9–51.8% and specificity of 85.2–95% (17-19)] and are more appropriate to be used in monitoring the CRC recurrence or patients' response to surgical or systemic therapy. In addition, stool based screening tests for CRC were developed. The Hemoccult fecal occult blood test (FOBT) has been used for a long time to aid physicians in detecting hidden blood in stool specimen as an early indication of CRC, with a sensitivity varying between 12.9% and 79.4% and a specificity of 86.7–97.7% (20). However, since this test has many drawbacks in CRC screening, the fecal immunochemical test (FIT) is more commonly used in current CRC screening thanks to its low cost with an overall sensitivity of 0.79 and overall specificity of 0.94 (20). Another is the fecal DNA test (21). This multitarget assay detected invasive cancers and adenomas with high-grade dysplasia with 40.8% sensitivity and 94.4% specificity (22). Cologuard is the first commercial Food and Drug Administration (FDA) approved [2014] fecal DNA test presenting a higher sensitivity than the FIT in CRC and polyps but a lower specificity. However, this test is somewhat expensive (23).

What is liquid biopsy?

Upon the National Cancer Institute (NCI) Dictionary of Cancer Terms liquid biopsy is "*A test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood*" (24). To our point of view this definition is rather imperfect and one can be uncomfortable to associate a so complex entity such as cells regrouping molecules, intermolecular associations, organelles, compartmentalization, a closed concentration of factors or enzymes, and programming, which are the smallest unit that

can live on its own and that makes up all living organisms and the tissues of the body, with cellular components such as macromolecules (DNA, RNA or microRNA) either strongly associated with proteins or encapsulated in microvesicles (25). The improbable association of such different biological entities may only rely on their circulating property and on the potential clinical use of the information provided by both biological sources. As indicated in the NCI Dictionary of Cancer Terms: "*A liquid biopsy may be used to help find cancer at an early stage. It may also be used to help plan treatment or to find out how well treatment is working or if cancer has come back. Being able to take multiple samples of blood over time may also help doctors understand what kinds of molecular changes are taking place in a tumor*." Despite incoherence in the term, we will use in this review the term liquid biopsy in accordance with the NCI Dictionary terminology and conventionally in the literature. The term liquid biopsy therefore applies mainly in oncology mirroring the biopsy of the tumor tissue. In addition, this terminology cannot be used for circulating DNAs that are analysed in the field of prenatal diagnosis, severe/acute inflammation (sepsis), transplantation, or sports, for instance.

Liquid biopsies are not limited to the blood, though this is greatly where the research is focused. Urine, saliva or cervical fluid may also be used, as genetic information is also present in these fluids.

Circulating cell-free DNA (cfDNA)

cfDNA has emerged as a potential biomarker especially in cancer and is being widely investigated in translational and clinical research (25-27). It may present the opportunity to diagnose, monitor recurrence, and evaluate response to therapy solely through a non-invasive blood draw. Several efforts are being made in order to assess the potential of this biomarker for early cancer screening and qualitative as well as quantitative cfDNA alterations have been examined (26). But despite intensive research, few cfDNA-based tests have been translated to clinical practice. For instance, conflicting data regarding total nuclear cfDNA concentration made it hard for cfDNA-based tests to be developed and used in clinic: plasma cfDNA concentrations in cancer patients range from a few ng/mL to several thousand ng/mL, which overlaps with the concentration range for healthy individuals (27-29).

A cfDNA-based screening test must be able to distinguish between signals from non-cancer and pre-cancerous processes and the invasive malignancy in order to achieve high clinical sensitivity (30). It would also help if the test could provide information on the tissue origin which might be possible through circulating tumor DNA (ctDNA), given the distinct differences in the patterns of somatic alterations between different tumor types.

Until now, several groups worked on developing tests for the early screening of different types of cancer from a single blood analysis. We listed in *Table 1* the most useful and efficient screening tests. For CRC for example, many groups have studied the screening or diagnostic relevance of different cfDNA parameters, and several reports were focused on the detection of methylated Septin9 in the plasma which was found to be significantly higher in patients with CRC than in patients with no evidence of disease (17), making it a potential biomarker for this type of cancer (33,34). The Epi proColon® (Epigenomics AG Corporation, Berlin, Germany), based on a real-time polymerase chain reaction (PCR) detection of methylated Septin9 from blood, is the only commercially available blood based DNA hypermethylation screening test for CRC (32) and is so far the best among the commercial blood-based cancer detection assays. The test discriminated between patients with CRC and healthy controls with a sensitivity of 75–81% and a specificity of 96–99%. Other studies were intended to evaluate, in the plasma, different hypermethylated DNA promoter regions and genes (37) previously found to be CRC specific. The highest area under the curves (AUCs) achieved were 0.85 for a test combining seven promotors along with age and gender (35). In addition, an age-adjusted panel of four cell-free nucleosomes was developed by Volition and it provided an AUC of 0.97 (0.87 if not age-adjusted) for the discrimination between CRC patients and healthy controls (*Table 1*). It showed high sensitivity for early stages (75 and 86 at 90% specificity for stages I and II, respectively). A second combination of four cf-nucleosome biomarkers provided an AUC of 0.72 for the discrimination of polyps from the healthy group (31).

The diagnostic potential of cfDNA was also examined in other types of cancer (43), and cfDNA levels were studied (38). For instance, quantitative analysis in lung cancer (*Table 1*) showed increased levels of cfDNA in cancer patients than in healthy individuals with approximately a value of 0.88 for areas under the summary receiver operating characteristic curves (44-47). Similar results were observed for breast cancer with 78% sensitivity and 83% specificity (53), and ovarian cancer (54) with a sensitivity of 70% and a specificity of 90%.

Circulating DNA consists not only of nuclear but

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Table 1 cfDNA and cancer screening

Article	Journal/date	Summary/results
Circulating nucleosomes as new blood-based biomarkers for detection of colorectal cancer (31)	*Clinical Epigenetics*, May 2017	❖ The levels of 12 epigenetic cell-free nucleosome epitopes were measured in the sera of 58 individuals referred for endoscopic screening for CRC
		❖ For the discrimination of CRC patients from healthy individuals, they developed an age-adjusted panel of four cell-free nucleosomes. The AUC was 0.97 (0.87 if not age-adjusted) with a high sensitivity at early stages (sensitivity of 75 and 86 at 90% specificity for stages I and II, respectively). A second combination of four cf-nucleosome biomarkers provided an AUC of 0.72 for the discrimination of polyps from the healthy group
Epi proColon® 2.0 CE: A Blood-Based Screening Test for Colorectal Cancer (32)	*Molecular Diagnosis and Therapy*, April 2017	❖ Epi proColon® 2.0 CE consists of the Epi proColon® Plasma Quick Kit (M5-02-001), the Epi proColon® Sensitive PCR Kit (M5-02-002) and the Epi proColon® Control Kit (M5-02-003) → sufficient quantities of reagents for processing up to 32 samples including controls, divided between a maximum of four independent test runs
		❖ It consists of a real-time PCR detection of methylated Septin9 from blood derived DNA in bisulfite converted DNA (bisDNA) from 3.5 mL human plasma samples
		❖ It is the only commercially available blood based DNA hypermethylation screening test for CRC
		❖ The test results do not confirm the presence nor absence of colorectal disease and it must be evaluated together with other clinical parameters, and positive test results should be verified by colonoscopy or sigmoidoscopy
		❖ Epi proColon® 2.0 CE has been determined to have an estimated 95% limit of detection (LoD) of 14 pg/mL (95% CI, 9–19 pg/mL), assessed using the Applied Biosystems 7500 Fast Dx with SDS v1.4
		❖ Across studies, Epi proColon® 2.0 CE discriminated between patients with colorectal cancer and healthy controls with a sensitivity of 75–81%; its specificity for colorectal cancer versus healthy individuals was 96–99%
Performance of a second-generation methylated SEPT9 test in detecting colorectal neoplasm (33)	*Journal of Gastroenterology and Hepatology*, April 2015	❖ Peripheral blood samples of 135 patients with CRC, 169 with adenomatous polyps, 81 with hyperplastic polyps, and 91 healthy controls were taken for SEPT9 testing using Epi proColon 2.0 test
		❖ The sensitivity and specificity of SEPT9 for CRC were 74.8% and 87.4%, respectively, but the sensitivity for advanced adenomas was 27.4%
		❖ SEPT9 was positive in 66.7% of stage I, 82.6% of stage II, 84.1% of stage III, and 100% of stage IV CRCs
		❖ For 177 patients, both SEPT9 and FIT (fecal immunochemical test) were performed and the sensitivity and specificity of FIT for CRC was 58.0% and 82.4%, respectively
		❖ SEPT9 showed better performance in CRC detection than FIT, but similar results were found for advanced adenomas
Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer (34)	*Gut*, February 2014	❖ This study assessed the accuracy of circulating methylated SEPT9 DNA (mSEPT9) for detecting CRC in a screening population
		❖ Blood plasma samples of asymptomatic individuals ≥50 years old scheduled for screening colonoscopy [7,941 men (45%) and women (55%), mean age =60 years] were tested using the first generation of the commercially available Epi proColon Assay
		❖ Results from 53 CRC cases and from 1,457 subjects without CRC showed a standardised sensitivity of 48.2%; for CRC stages I–IV, values were 35.0%, 63.0%, 46.0% and 77.4%, respectively for a 91.5% specificity
		❖ The sensitivity for advanced adenomas was low (11.2%)

Table 1 *(continued)*

Table 1 (*continued*)

Article	Journal/date	Summary/results
Hypermethylated DNA, a circulating biomarker for colorectal cancer detection (35)	*PloS One*, July 2017	❖ The article presents a cross-sectional case-control study of 193 CRC patients and 102 colonoscopy-verified healthy controls
		❖ Thirty DNA promoter regions previously found to be CRC specific were evaluated using methylation specific polymerase chain reaction
		❖ Individual DNA promoter regions could not provide an overall sensitivity above 30% at a reasonable specificity showing that individual hypermethylated DNA promoter regions have limited value as CRC screening markers
		❖ However, the combination of seven hypermethylated promoter regions (ALX4, BMP3, NPTX2, RARB, SDC2, SEPT9, and VIM) along with the covariates sex and age showed an optimism corrected AUC of 0.86 for all stage CRC and 0.85 for early stage CRC. The overall sensitivity was 90.7% at 72.5% specificity using a cut point value of 0.5
Diagnostic and prognostic role of cell-free DNA testing for colorectal cancer patients (36)	*International Journal of Cancer*, January 2017	❖ Using an *ALU*-based Q-PCR method, the presence and integrity of cfDNA was assessed in a large cohort of CRC patients (n=114) in comparison to healthy subjects (n=56) and patients with adenomatous lesions (n=22)
		❖ cfDNA concentration and integrity index were increased in CRC patients, and cfDNA was significantly higher in advanced histopathological stage
		❖ The discriminative capacity between CRC patients on one hand and controls or adenoma patients on the other hand was moderate for the *ALU83* and *ALU244* fragment dosage. For *ALU83* ROC curves' AUCs were 0.7105 and 0.77083, respectively, and *ALU244* showed AUCs of 0.7205 and 0.7636, respectively
		❖ The methylation profile of the promoters of *OSMR* and *SFRP1* genes was also evaluated in the cohort and was compared for 25 CRC patients in matched tissue and plasma. Only three mismatched cases were observed
		❖ A lower methylation quantification was observed in cfDNA than in tissue DNA, but cfDNA methylation frequency was statistically different in controls, adenoma and CRC patients and this frequency increased with the histopathological stage of tumor
		❖ The adenoma and CRC patients' methylated cfDNA showed a higher quantity of *ALU83* and *ALU244*
		❖ An approach combining the detection of *ALU* fragments and cancer type-specific epigenetic alteration, might improve the diagnostic efficiency for CRC
Aberrant Methylation of APC, MGMT, RASSF2A, and Wif-1 Genes in Plasma as a Biomarker for Early Detection of Colorectal Cancer (37)	*Clinical Cancer Research*, October 2009	❖ The study consists of a retrospective analysis of the methylation status of 10 genes in fresh-frozen tissues and corresponding plasma samples from 243 patients with stage I and II sporadic colorectal cancer, 276 healthy individuals, and plasma from 64 colorectal adenoma patients using methylation-specific PCR
		❖ In order to find molecular markers with high sensitivity and specificity, the methylation score (M score) was used
		❖ Of the 243 colorectal cancer tissues, methylation was detected in 18% for *p14*, 34% for *p16*, 27% for *APC*, 34% for *DAPK*, 32% for *HLTF*, 21% for *hMLH1*, 39% for *MGMT*, 24% for *RARβ2*, 58% for *RASSF2A*, and 74% for *Wif-1*
		❖ The plasma analysis for cancerous patients and healthy individuals showed that the M score of any single gene had a sensitivity less than 40% after controlling for age, sex, and tumor location, but in a model including *APC*, *MGMT*, *RASSF2A*, and *Wif-1* genes, the M score had 86.5% sensitivity and 92.1% specificity when 1.6 was used as a cutoff (AUC =0.927) with a positive predictive value of 90.6% and a negative predictive value of 88.8%
		❖ In the plasma of colorectal adenoma patients, the overall M score of the model (*APC*, *MGMT*, *RASSF2A*, and *Wif-1 genes*) was found to show a sensitivity of 74.6% and a specificity of 91.3% (AUC =0.864), when 1.8 was used as the cutoff value, after adjusting for age and sex. The positive predictive value was 71.6% and the negative predictive value was 93.9%

Table 1 (*continued*)

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Table 1 (*continued*)

Article	Journal/date	Summary/results
Liquid Biopsies for Cancer: Coming to a Patient near You (38)	*Journal of Clinical Medicine*, January 2017	❖ Circulating DNA size profiling might distinguish early from late malignancies, that's why it's being examined for inclusion in a screening blood test for cancer
		❖ The detection of tumor-specific DNA methylation through a liquid biopsy is another feasible approach for the development of diagnostic tests for early-stage cancer:
		◆ Differential methylation levels of three promoters, RASSF1A, CALCA, and EP300, in the cell-free plasma could detect ovarian cancer from healthy controls with a sensitivity of 90% and a specificity of 86.7% in a 30-patient cohort study (39)
		◆ The methylation of the promoter region of the thrombomodulin gene (*THBD*) could differentiate colorectal cancer and control blood samples with a sensitivity of 71% and a specificity of 80% (40)
		◆ Several clinical studies have demonstrated the utility of ctDNA-based biomarkers relative to protein biomarkers
		◆ The quantification of ctDNA mutants and the detection of their presence/ absence in colon cancer patients after surgery and chemotherapy proved to be more clinically useful than the cancer embryonic antigen (CEA) test (41)
		◆ In metastatic breast cancer patients, a study demonstrated an improved sensitivity for cancer detection of ctDNA over CA 15-3: of 85% *vs.* 59% (42)
Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies (43)	*Science Translational Medicine*, February 2014	❖ They evaluated the ability of ctDNA to detect tumors in 640 patients with various cancer types using digital polymerase chain reaction-based technologies
		❖ In more than 75% of patients with advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers, ctDNA was detected but in less than 50% of primary brain, renal, prostate, or thyroid cancers
		❖ For patients with localized tumors, ctDNA was detected in 73%, 57%, 48%, and 50% of patients with colorectal cancer, gastroesophageal cancer, pancreatic cancer, and breast adenocarcinoma, respectively
		❖ ctDNA was often present in patients without detectable circulating tumor cells, suggesting that these two biomarkers are distinct entities
		❖ In a separate panel of 206 patients with metastatic colorectal cancers, the sensitivity of ctDNA for the detection of clinically relevant KRAS gene mutations was 87.2% and its specificity was 99.2%
Circulating DNA: diagnostic tool and predictive marker for overall survival of NSCLC patients (44)	*PloS One*, 2012	❖ The study aimed to assess the discriminative capacity and the prognostic value of the amounts of circulating DNA (cDNA) between NSCLC patients and healthy individuals
		❖ Plasma of 309 individuals (104 cancer patients and 205 healthy controls) were analysed and the cDNA levels were assessed through a real-time PCR method targeting the hTERT single copy gene
		❖ Increased cDNA levels in NSCLC patients compared to control individuals were observed and the area under the ROC curve was 0.88 (95% CI, 0.84–0.92; P<0.0001)
		❖ Lower cut-off values increased the sensitivity of the assay but at the cost of specificity and vice versa: with a threshold of 20 ng/mL, there is a probability of illness of 71% when the test is positive (PPV). A DNA cut-off level of >20 ng/mL differentiated between lung cancer patients and controls with a specificity of 83% and sensitivity of 79%
		❖ A decreased overall survival time was observed in patients presenting high cDNA levels, when compared to lower cDNA concentrations

Table 1 (*continued*)

Table 1 *(continued)*

Article	Journal/date	Summary/results
Value of quantitative analysis of circulating cell free DNA as a screening tool for lung cancer: a meta-analysis (45)	*Lung Cancer*, August 2010	❖ It consists of a meta-analysis of 10 studies were including 752 lung cancer patients and 635 healthy controls ❖ Sensitivity, specificity, and other measures of accuracy of circulating DNA assay in the diagnosis of lung cancer were pooled using random-effects models and summary ROC curves were used to summarize overall test performance ❖ For quantitative analysis of circulating cell-free DNA in lung cancer screening, the summary estimates were a sensitivity of 0.80 (95% CI, 0.77–0.83); a specificity of 0.77 (95% CI, 0.74–0.80); a positive likelihood ratio of 4.54 (95% CI, 2.66–7.76); a negative likelihood ratio of 0.28 (95% CI, 0.19–0.40); and a diagnostic odds ratio of 20.33 (95% CI, 10.12–40.86) ❖ The AUC was 0.89 (weighted AUC, 0.88), indicating a high level of overall accuracy
The diagnostic value of circulating cell free DNA quantification in non-small cell lung cancer: A systematic review with meta-analysis (46)	*Lung Cancer*, October 2016	❖ The diagnostic value of cfDNA quantification for non-small cell lung cancer (NSCLC) was estimated in 15 studies with a total of 1,193 patients with lung cancer and 1,059 controls ❖ Pooled results showed 81% sensitivity (95% CI, 76–84%); 85% specificity (95% CI, 77–91%); 23.87 diagnostic odds ratio (95% CI, 13.37–42.61); and 0.89 for areas under the summary receiver operating characteristic curves (95% CI, 0.86–0.92)
The Emerging Role of "Liquid Biopsies," Circulating Tumor Cells, and Circulating Cell-Free Tumor DNA in Lung Cancer Diagnosis and Identification of Resistance Mutations (47)	*Current Oncology Reports*, January 2017	❖ Endorsed screening strategies including low-dose CT scans have a low sensitivity and high false positive rates of >90% as well as low adoption as a practiced standard of care ❖ The significant difference in DNA concentration in the serum/plasma of lung cancer patients with healthy controls or patients with benign diseases opened up the possibility of the use of this biomarker in screening assays (48-51)
Identification of Circulating Tumor DNA for the Early Detection of Small-cell Lung Cancer (52)	*EBioMedecine*, August 2016	❖ Plasma of 51 small cell lung cancer (SCLC) and 123 controls were assessed for the presence of TP53 mutations ❖ Thirty-one TP53 mutations were detected in the cfDNA of 49% SCLC patients (35.7% early-stage and 54.1% late-stage) and 18 mutations in 11.4% of non-cancer controls ❖ The results were replicated in an independent series of 102 non-cancer controls and showed a comparable proportion of TP53 mutated samples (10.8%) which suggests that somatic mutations occur in cfDNA among individuals without cancer diagnosis and causes a serious challenge for ctDNA screening tests development ❖ Allelic fractions of the TP53 mutations were significantly higher in cases than in controls (P=0.0004)
Value of circulating cell-free DNA analysis as a diagnostic tool for breast cancer: a meta-analysis (53)	*Oncotarget*, February 2017	❖ The objective of this study was to systematically evaluate the diagnostic value of cfDNA for breast cancer ❖ Twenty-five studies with relevant diagnostic screening were included with 15 quantitative analysis studies and 10 qualitative analysis studies and a total number of 1,705 histologically diagnosed breast cancer patients, 1,079 healthy controls, and 234 patients with benign breast diseases ❖ The mean sensitivity, specificity and AUC of the Summary ROC plots for 24 studies that distinguished breast cancer patients from healthy controls were 0.70, 0.87, and 0.9314, yielding a DOR of 32.31 ❖ For the 14 quantitative analyses of cfDNA for breast cancer diagnosis, the estimates of sensitivity and specificity were 0.78 and 0.83, respectively. The value for PLR was 4.83, and NLR was 0.22. The DOR value was 24.40 and AUC 0.9116 ❖ The 10 qualitative studies produced 0.50, 0.98, 0.9919, and 68.45, sensitivity, specificity, AUC and DOR respectively ❖ The specificity, sensitivity, AUC and DOR for eight studies that distinguished malignant breast cancer from benign diseases were 0.75, 0.79, 0.8213, and 9.49

Table 1 *(continued)*

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Table 1 *(continued)*

Article	Journal/date	Summary/results
Circulating Cell Free DNA as the Diagnostic Marker for Ovarian Cancer: A Systematic Review and Meta-Analysis (54)	*PloS One*, June 2016	❖ A meta-analysis of nine diagnostic studies published from 2001 to 2014, including 462 ovarian cancer patients and 407 controls was conducted
		❖ The summary estimates for quantitative analysis of circulating cfDNA in ovarian cancer screening showed a sensitivity of 0.70, a specificity of 0.90, a positive likelihood ratio of 6.60, a negative likelihood ratio of 0.34, a diagnostic odds ratio of 26.05, and an AUC of 0.89
Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers (55)	*Proceedings of the National Academy of Sciences*, September 2017	❖ The objective of this study was to combine blood tests for KRAS gene mutations with carefully thresholded protein biomarkers to determine whether the combination of these markers was superior to any single marker
		❖ Two hundred and twenty-one patients with resectable pancreatic ductal adenocarcinomas and 182 control patients without known cancer were tested
		❖ KRAS mutations were detected in the plasma of 30% of the patients, and with a 100% concordance with the mutations found in the patient's primary tumor
		❖ The combination of KRAS with four thresholded protein biomarkers (CA19-9, CEA, HGF, and OPN) increased the sensitivity to 64%. Only one of the 182 plasma samples from the control cohort was positive for any of the DNA or protein biomarkers (99.5% specificity)
Direct detection of early-stage cancers using circulating tumor DNA (56)	*Science Translational Medicine*, August 2017	❖ They developed an approach called targeted error correction sequencing (TEC-Seq) that allows ultrasensitive direct evaluation of sequence changes in circulating cell-free DNA using massively parallel sequencing, and used it to examine 58 cancer-related genes encompassing 81 kb
		❖ The analysis of plasma from 44 healthy individuals identified genomic changes related to clonal hematopoiesis in 16% of asymptomatic individuals but no alterations in driver genes related to solid cancers were found
		❖ Plasma samples from 194 patients with breast cancer (n=45), colorectal cancer (n=42), lung cancer (n=65), and ovarian cancer (n=42) were analyzed and the concentration of cfDNA in plasma from cancer patients (12 ng/mL) was significantly higher than that observed in healthy individuals (average of 7 ng/mL; P=0.001)
		❖ The evaluation of 200 patients with colorectal, breast, lung, or ovarian cancer detected somatic mutations in the plasma of 71%, 59%, 59%, and 68%, respectively, of patients with stage I or II disease
		❖ Of the 194 patients analyzed, more than 3/4 of colorectal cancer patients, 2/3 of ovarian cancer patients, and most of the lung and breast cancer patients had detectable alterations in driver genes
		❖ The analysis of mutations in the circulation revealed high concordance with alterations in the tumors of these patients

Table 1 *(continued)*

Table 1 (continued)

Article	Journal/date	Summary/results
Next-Generation Sequencing of Circulating Tumor DNA for Early Cancer Detection (30)	*Cell*, February 2017	❖ For successful cancer screening, a platform that provides direct, sensitive, and specific measures of cancer and its attributes is needed
		❖ The fraction of tumors that shed detectable levels of ctDNA, by tumor type and stage, is not well studied. The studies to date have small numbers of samples and use a variety of measurement techniques that are often not comparable
		❖ ctDNA detection has the potential to be more specific to the presence of the tumor than other measurements of proteins and metabolites. However, the implementation of such a test would be technically challenging, since many genes would have to be simultaneously queried for alterations in order to cover enough of the known diversity in cancer genomes
		❖ While next-generation DNA sequencing technology does enable high degrees of target multiplexing, the depth of sequencing would also have to be very high to sample enough ctDNA molecules to reliably measure them in a background of mostly non-tumor-derived cfDNA
		❖ To achieve high clinical specificity, a ctDNA-based screening test must be capable of distinguishing between the background signal originating from such non-cancer or pre-cancerous processes and the invasive malignancy of real interest
		❖ An ideal non-invasive screening test would also provide information on the tissue of origin to streamline the downstream workup, including imaging and tissue diagnosis. This information may be possible through ctDNA, given the distinct differences in the patterns of somatic alterations between different tumor types, at least at a population level
		❖ GRAIL is a recently created company, formed to develop ctDNA-based cancer screening tests. It is conducting a 10,000-plus subject study, called the Circulating Cell-Free Genome Atlas (CCGA) to create a reference library of the cancer mutations in the blood for the most common cancers and the background mutations found in matched healthy subjects. It will be the largest database on mutations found in the blood of cancer patients. It intends to apply a next-generation sequencing approach, combining sequencing depth and breadth of genomic coverage, as well as machine learning, to develop models based on cell-free DNA for the accurate classification of subjects with and without cancer
		❖ The Cancer Moonshot Initiative recently announced the Blood Profiling Atlas Project, which also aims to compile data on cancer signals in the blood

CRC, colorectal cancer; AUC, area under the curve; PCR, polymerase chain reaction; Q-PCR, quantitative polymerase chain reaction; ROC, receiver operating characteristic; DOR, diagnostic odds ratio; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

mitochondrial DNA (mtDNA). Other studies have been published on the clinical significance of mtDNA levels and integrity in the peripheral blood in different types of cancer (*Table 2*) such as lung (57,58), breast (59), colorectal (60,61), non-Hodgkin lymphoma (62), and others (63-70). At this time, published data are discordant and it is impossible to draw any conclusion. The lack of pre-analytical and analytical studies on circulating cell-free mtDNA could explain in part this discordance, since it is poorly characterized and little is known about its structural properties.

Circulating tumor cells (CTCs)

The discovery of cells released in the bloodstream or escaping from the tumor is of primary importance and has led to intense research for about 20 years. CTCs are incredibly hard to isolate and do not always indicate genetically cancerous cells. The value of CTCs in diagnosing different types of cancers has been also assessed in several studies (*Table 3*) (71,72). In lung cancer for example (78), Tanaka *et al.* showed that CTC enumerations

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Table 2 Mitochondrial DNA and cancer screening

Article	Journal/date	Summary/results
Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study (57)	*Carcinogenesis*, May 2010	❖ The association of mtDNA copy number and lung cancer risk was assessed in 227 prospectively collected cases and 227 matched controls
		❖ There was evidence that the risk of lung cancer increased in a dose-dependent manner with mtDNA copy number (ptrend 5 0.008)
		❖ The association between mtDNA copy number and lung cancer risk was evident among heavy smokers (≥20 cigarettes per day), but not light smokers (<20 cigarettes per day), however, the interaction between mtDNA copy number and smoking was not significant
Pre-diagnostic leukocyte mitochondrial DNA copy number and risk of lung cancer (58)	*Oncotarget*, March 2016	❖ This study consists of a prospective investigation, using a Q-PCR based assay, of the relationship between mitochondrial DNA copy number (mtCN) and the risk of lung cancer in 463 case-control pairs from the the Nurses' Health Study (NHS) (285 cases and 285 controls) and the Health Professionals Follow-Up Study (HPFS) (178 cases and 178 controls)
		❖ Current heavy smokers (>24 cigarettes/day) had significantly lower mtCN compared with never smokers (P=0.05)
		❖ No overall association was observed between mtCN and lung cancer risk
		❖ Compared to the high log_mtCN group, the risk of lung cancer was 1.29 (95% CI, 0.89–1.87) for the median group, and 1.11 (95% CI, 0.75–1.64) for the low group
		❖ Among current smokers, compared to participants with high levels of log_mtCN, those with median levels had a significantly higher risk of lung cancer (OR =2.09; 95% CI, 1.12–3.90), but not those with low levels (OR =1.37; 95% CI, 0.75–2.48)
		❖ The interaction between mtCN and smoking status on lung cancer risk was not significant
Mitochondrial DNA Copy Number Is Associated with Breast Cancer Risk (59)	*PloS One*, June 2013	❖ The association between mtDNA copy number in peripheral blood and breast cancer risk was studied in 183 breast cancer cases with pre-diagnostic blood samples and 529 individually matched controls
		❖ The relative quantification of mtDNA copy number to nuclear DNA, was positively associated with breast cancer risk overall (P for trend =0.01)
		❖ Relative mtDNA copy number was associated with breast cancer risk only among those women from whom a blood sample was collected within 3 years of breast cancer diagnosis
		❖ No association was observed between mtDNA copy number and breast cancer risk among women who donated a blood sample ≥3 years prior to breast cancer diagnosis (P for trend =0.41)
		❖ MtDNA copy number was negatively correlated with time to breast cancer diagnosis (r=20.15; P=0.048)
Mitochondrial Copy Number Is Associated with Colorectal Cancer Risk (60)	*Cancer Epidemiology, Biomarkers & Prevention*, September 2012	❖ The association between mtDNA copy number in peripheral blood and colorectal cancer risk was studied in 422 colorectal cancer cases (168 cases with pre-diagnostic blood and 254 cases with post-diagnostic blood) and 874 controls who were free of colorectal cancer
		❖ After measuring the relative mtDNA to nuclear DNA copy number using real-time PCR, a U-shaped relationship between the relative mtDNA copy number and colorectal cancer risk was observed
		❖ Compared with the 2nd quartile, the OR (95% CI) for subjects in the lowest and highest quartiles of relative mtDNA copy numbers were 1.81 (1.13–2.89) and 3.40 (2.15–5.36), respectively (P curvilinearity <0.0001)
		❖ This U-shaped relationship was present in both men and women, similar for colon cancer and rectal cancer, and independent of the timing of blood draw with regard to cancer diagnosis

Table 2 *(continued)*

Table 2 (*continued*)

Article	Journal/date	Summary/results
Association between mitochondrial DNA content in leukocytes and colorectal cancer risk (61)	*Cancer*, July 2011	❖ mtDNA content was measured in peripheral blood lymphocytes of 320 CRC patients and 320 controls by Q-PCR ❖ mtDNA content was significantly higher in cancer patients than in controls, and high mtDNA content was associated with a significantly increased CRC risk
A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma (62)	*Blood*, November 2008	❖ mtDNA copy number was analysed in peripheral white blood cells of 104 males with non-Hodgkin lymphoma (NHL) and 104 control ❖ The results showed that a dose-response relationship exists between mtDNA copy number and NHL risk, with the most pronounced effect for chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma subtype (SLL)
Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients (63)	*Clinical Chimica Acta*, January 2016	❖ CfDNA was extracted from EDTA plasma of healthy people (n=40), non-metastatic (n=145) and metastatic (n=84) clear cell renal cell cancer (RCC) patients using the QIAamp Circulating Nucleic Acid Kit ❖ Genomic and mitochondrial cfDNA concentrations were determined using qPCR of different cfDNA fragments (67–306 bp) (target: *APP* for nuclear DNA) ❖ Genomic cfDNA fragments of APP with 67 bp (*APP-1*) and 180 bp (*APP-2*) as well as of Alu sequences with 79 bp (*SINE-1*) and 248 bp (*SINE-2*) were not different between the controls and non-metastatic RCC patients, but metastatic RCC patients showed lower concentrations of the long 306 bp *APP-3* fragment compared to the controls ❖ Significantly higher concentrations of the short *APP-1* in comparison to *APP*-2 and *APP*-3 were found in the RCC groups ❖ Increased mitochondrial cfDNA concentrations in metastatic RCC in comparison to controls and non-metastatic RCC were observed with a decreased integrity index ❖ The cfDNA integrity indices decreased from controls to metastatic patients ❖ An AUC >0.75 was observed for predicting recurrence-free survival and overall survival with concordance indices >0.80
Circulating Mitochondrial DNA Level, a Noninvasive Biomarker for the Early Detection of Gastric Cancer (64)	*Cancer Epidemiology, Biomarkers & Prevention*, November 2014	❖ MtDNA in peripheral leukocytes of 28 patients with non-atrophic gastritis (NAG), 74 patients with gastric cancer, and 48 matched asymptomatic controls was measured by quantitative real-time PCR assay. In parallel, the serologic level of IL8 was determined ❖ Mean mtDNA level was higher in patients with gastric cancer (P=0.0095) than in controls, with values >8.46 significantly associated with gastric cancer (OR =3.93) ❖ Three ranges of mtDNA values were identified: interval I <2.0, interval II 2.0–20, interval III >20 ❖ Interval I included mainly NAG cases, and few gastric cancer samples and interval III corresponded almost exclusively to patients with gastric cancer. All controls fell in interval II, together with some NAG and gastric cancer cases ❖ IL8 levels were significantly higher in patients with gastric cancer (P<0.05), with levels > 50 pg/mL observed exclusively in patients with gastric cancer, allowing to distinguish them within interval II ❖ mMtDNA results validated in a second cohort of patients: mtDNA was significantly higher in gastric cancer than in patients with preneoplasia

Table 2 (*continued*)

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Table 2 (*continued*)

Article	Journal/date	Summary/results
Cell-free Circulating Mitochondrial DNA in the Serum: A Potential Non-invasive Biomarker for Ewing's Sarcoma (65)	*Archives of Medical Research*, July 2012	❖ ccf-mtDNA copy number in serum samples obtained from 25 patients with Ewing's sarcoma (EWS) as well as 20 age-matched individuals were detected by quantitative real-time PCR assays using mtDNA 16s RNA-specific primers to amplify a 79-bp fragment
		❖ The quantification of ccf nuclear DNA was determined by amplifying a 97-bp fragment of the house-keeping gene *GAPDH*
		❖ Levels of ccf-mtDNA in the serum of EWS patients were significantly lower than in healthy controls with a sensitivity =76.1%, a specificity =68.4% and an AUC =0.708
		❖ Serum levels of ccf-mtDNA were associated with the status of tumor metastasis
Cell-free circulating mitochondrial DNA content and risk of hepatocellular carcinoma in patients with chronic HBV infection (66)	*Scientific Reports*, April 2016	❖ This study aimed to determine circulating mtDNA content in serum samples from 116 HBV related hepatocellular carcinoma (HCC) cases and 232 frequency-matched cancer-free HBV controls, and evaluate the retrospective association between mtDNA content and HCC risk
		❖ The relative mtDNA content was measured by qRT-PCR in which the ratio of the copy number for mitochondrial ND1 gene to the copy of a human single copy gene 36B4 was used to determine the relative mtDNA content
		❖ HCC cases had significantly lower circulating mtDNA content than controls (1.06 versus 2.47, P=0.000017)
		❖ Patients with a lower level of serum mtDNA content (≤2.47) exhibited a significantly increased HCC risk with a crude OR of 2.22 (95% CI, 1.39–3.56; P=0.00087) in univariate analysis and an adjusted OR of 2.19 (95% CI, 1.28–3.72, P=0.004) in multivariate analysis adjusting for age, gender, smoking status, drinking status, family history of cancer, and cirrhosis, compared to those with a higher mtDNA content (>2.47)
		❖ Using the patients with the highest level of mtDNA content as reference, patients with lower levels of mtDNA content showed significantly increased HCC risk in a dose-dependent manner in both univariate and multivariate analyses (P for trend =0.00016, and 0.001, respectively)
		❖ When mtDNA content was added to multivariate analysis adjusting for age, gender, smoking status, drinking status, family history of cancer, and cirrhosis, the AUC of the ROC curve significantly increased from 0.7133 to 0.7511 (P=0.046)
		❖ The AUC was 0.8020 in the multivariate model including demographic variables plus AFP, and the AUC significantly increased to 0.8498 after adding mtDNA to the model (P=0.032). Similar results were found in the models including each of the liver enzymes which showed that mtDNA provided additional diagnostic value when jointly used with AFP or common liver enzymes
A prospective study of mitochondrial DNA copy number and the risk of prostate cancer (67)	*Cancer Causes & Control*, June 2017	❖ Seven hundred and ninety-three cases and 790 men control were assessed to evaluate the association between pre-diagnosis mtDNA copy number, measured in peripheral blood leukocytes, and the risk of prostate cancer (PCa)
		❖ Overall, no significant difference of the median mtDNA copy number between cases and controls
		❖ When the results were stratified by disease aggressiveness, a positive association was found with increasing mtDNA copy number for non-aggressive disease (OR =1.29, P=0.044) but not aggressive PCa (OR =1.02, P=0.933), though a Wald test for heterogeneity of the coefficients for mtDNA copy number was not statistically significant (P=0.334)
		❖ Among controls, higher mitochondrial DNA copy number was associated with an increased PSA level (P=0.014)
		❖ Increasing mitochondrial DNA copy number was associated with an increased risk of non-aggressive prostate cancer with high (≥4.0 ng/mL) PSA at diagnosis (OR =1.32, P=0.037), but not low PSA at diagnosis (OR =1.16, P=0.527)

Table 2 (*continued*)

Table 2 (*continued*)

Article	Journal/date	Summary/results
Lower mitochondrial DNA copy number in peripheral blood leukocytes increases the risk of endometrial cancer (68)	*Molecular Carcinogenesis*, June 2016	❖ MtDNA copy number was measured in peripheral blood leukocytes (PBLs) from 139 endometrial cancer patients and 139 age-matched controls to determine the association of mtDNA copy number with the risk of endometrial cancer
		❖ The normalized mtDNA copy number was significantly lower in endometrial cancer cases (median, 0.84; range, 0.24–2.00) than in controls (median, 1.06; range, 0.64–1.96) (P<0.001)
		❖ Dichotomized into high and low groups based on the median mtDNA copy number value in the controls, individuals with low mtDNA copy number had a significantly increased risk of endometrial cancer (adjusted OR, 5.59; 95% CI, 3.05–10.25; P<0.001) compared to those with high mtDNA copy number
Circulating mitochondrial DNA in the serum of patients with testicular germ cell cancer as a novel noninvasive diagnostic biomarker (69)	*BJU International*, July 2009	❖ The diagnostic and prognostic value of the quantification and the integrity of cell-free mtDNA was studied in the serum of 74 patients with testicular cancer and 35 healthy individuals
		❖ mtDNA levels were significantly higher in cancer patients than in controls with a distinguishing sensitivity of 59.5%, a specificity of 94.3% and an AUC of 0.787
		❖ No difference in the mtDNA integrity was observed between patients and healthy individuals
Circulating mitochondrial DNA in serum: a universal diagnostic biomarker for patients with urological malignancies (70)	*Urologic Oncology: Seminars and Original Investigations*, July 2012	❖ The serum of 84 bladder cancer, 33 renal cell carcinoma, 23 prostate cancer patient and 79 healthy individual, was analyzed for cell-free circulating mtDNA levels and integrity
		❖ The results showed a significant increase in circulating mtDNA levels in cancer patients compared to healthy controls (84% sensitivity and 97% specificity)
		❖ mtDNA integrity was increased in renal cell carcinoma and bladder cancer compared to healthy individuals and prostate cancer patients
		❖ A correlation was also observed between mtDNA integrity and pathological stage in renal cell cancer on one hand, and tumor grade in bladder cancer on the other hand

Q-PCR, quantitative polymerase chain reaction; CRC, colorectal cancer; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio; AUC, area under the curve; PSA, prostate-specific antigen.

had an inadequate discriminating potential between patients with lung cancer and nonmalignant disease [AUC =0.598 (P=0.122)] (73). But on the other hand, other groups showed that a CTC count of more than 25 had a high sensitivity (89%) and specificity (100%) for the differentiation between benign and malignant disease (74), and a cut-off threshold of 8.7 folate receptor-positive-CTC units between the control group and patients with lung cancer presented an AUC of 0.7956 (sensitivity =77.7% and specificity =89.5%) (75). CTCs were also detected in patients with chronic obstructive pulmonary disease (COPD) (*Table 3*), a risk factor for lung cancer, without clinically detected lung cancer (76), in addition CTCs number was higher in patients with stage IV NSCLC compared with patients with stage IIIB (77).

While technology to capture and profile CTCs has advanced rapidly, the complexity and the weak analytical signal may limit clinical utility relative to ctDNA-based methods (38). Initial studies, such as that performed by Diaz *et al.*, suggest that when both ctDNA and CTCs were present, ctDNA fragments outnumbered CTCs by 50 to 1 (79) providing a much higher analytical signal. Nonetheless, CTCs do not have the disadvantage of the necessity to measure very small amounts of mutated fragments in the plasma due to the important release of wild type cfDNA in some patients whose tumors are invaded by a tumor microenvironment in a large proportion (>90% of the cells). In a recent trial of lung cancer patients, ctDNA outperformed CTCs for detection of the KRAS mutation, revealing sensitivities of 96% and 52%, respectively (80). Very recently, there has been a certain enthusiasm for single cell analysis which might be of importance for screening, since this approach is technically feasible. The results

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Table 3 CTCs and cancer screening

Article	Journal/date	Summary/results
Circulating tumour cells as a biomarker for diagnosis and staging in pancreatic cancer (71)	*British Journal of Cancer*, June 2016	❖ Blood was collected prospectively from 100 pre-treated patients [28 with non-adenocarcinoma diagnosis and 72 with pancreatic ductal adenocarcinoma (PDAC)], and samples were evaluated for the presence and number of CTCs using the microfluidic NanoVelcro CTC chip
		❖ KRAS mutation analysis was used to compare the CTCs with primary tumor tissue
		❖ In five patients tested: 100% concordance for KRAS mutation subtype between primary tumor and CTCs
		❖ The presence of CTCs was observed in 54/72 patients with confirmed PDAC with a sensitivity =75.0%, a specificity =96.4% and an AUC =0.867)
		❖ Using a cut-off of ≥3, CTCs in 4 ml of blood were able to discriminate between local/regional and metastatic disease (AUC =0.885)
The prognostic and diagnostic value of circulating tumor cells in bladder cancer and upper tract urothelial carcinoma: a meta-analysis of 30 published studies (72)	*Oncotarget*, June 2017	❖ Based on the published results of 30 different studies with a total of 2,161 urothelial cancer patients, the prognostic and diagnostic value of CTCs in urothelial cancer was assessed
		❖ Concerning the diagnostic accuracy of CTC detection, the overall sensitivity and specificity were 0.35 and 0.97 respectively with significant heterogeneity (I^2=89.40% and 89.71%), with a pooled positive likelihood ratio (PLR) and negative likelihood ratio (NLR) of 11.2 and 0.67 respectively. The diagnostic odds ratio (DOR) was 17 and the summary ROC curve (sROC) for the included studies which reflects the global summary of test's performance showed an AUC of 0.70, so a moderate accuracy of the diagnostic test
Circulating tumor cell as a diagnostic marker in primary lung cancer (73)	*Clinical Cancer Research*, November 2009	❖ The role of CTC counts in the discernment between primary lung cancer and nonmalignant diseases was examined in a cohort of 150 patients clinically suspected to have or with a diagnosis of primary lung cancer (125 primary lung cancer and 25 with nonmalignant disease)
		❖ Thirty point six percent of lung cancer patients and 12% of patients with nonmalignant disease had detectable CTCs
		❖ CTC enumerations were higher in lung cancer patients, but ROC curve analysis demonstrated an inadequate potential of the CTC counts to discriminate between patients with lung cancer and nonmalignant disease [AUC =0.598 (P=0.122)]
Circulating tumor cells in diagnosing lung cancer: clinical and morphologic analysis (74)	*The Annals of Thoracic Surgery*, June 2015	❖ CTCs were evaluated from potential lung cancer patients to predict the malignancy of lung lesions
		❖ CTCs were isolated by size method from peripheral blood of 77 patients with malignant (n=60) and benign (n=17) lung lesions. They were morphologically classified as cells with malignant feature, cells with uncertain malignant feature, and cells with benign feature, then statistically correlated with clinicocytopathologic characteristics of corresponding lung lesion
		❖ A CTC count of >25 had high sensitivity and specificity for the differentiation between benign and malignant disease (sensitivity =89% and specificity =100%)
		❖ Isolated CTCs shared similar histology and morphological features (72%) with biopsy samples
		❖ In tested stage I patients (42%), the numbers of CTCs correlated with tumor size (P=0.001)

Table 3 *(continued)*

Table 3 *(continued)*

Article	Journal/date	Summary/results
Clinical Significance of Folate Receptor-positive Circulating Tumor Cells Detected by Ligand-targeted Polymerase Chain Reaction in Lung Cancer (75)	*Journal of Cancer*, 2017	❖ Folate receptor (FR)-positive circulating tumor cells (FR±CTCs) were detected by a novel ligand-targeted polymerase chain reaction (LT-PCR) detection technique ❖ FR±CTC levels of patients with lung cancer were significantly higher than controls (patients with benign lung diseases and healthy controls) ❖ A cut-off threshold of 8.7 CTC units was established between control group and patients with lung cancer with an AUC =0.7956, a sensitivity =77.7% and a specificity =89.5% ❖ Compared with established clinical biomarkers [CEA, cytokeratin 19 fragment (CYFRA21-1), and neuron-specific enolase (NSE)], FR±CTC showed the highest diagnostic efficiency (highest AUC), so a combination of FR±CTC, CEA, NSE, and CYFRA21-1 could significantly improve the diagnostic efficacy in differentiating patients with lung cancer from benign lung disease
"Sentinel" circulating tumor cells allow early diagnosis of lung cancer in patients with chronic obstructive pulmonary disease (76)	*PloS One*, October 2014	❖ Chronic obstructive pulmonary disease (COPD) is a risk factor for lung cancer; ❖ This study aimed to examine the presence of CTCs, in complement to CT-scan, in COPD patients without clinically detectable lung cancer as a first step to identify a new marker for early lung cancer diagnosis ❖ The presence of CTCs was examined by an ISET filtration-enrichment technique, for 245 subjects without cancer, including 168 (68.6%) COPD patients, and 77 subjects without COPD (31.4%), including 42 control smokers and 35 non-smoking healthy individuals ❖ The presence of CTCs in 5 out of 168 COPD patients predicted the appearance of lung nodules 1–4 years after initial detection of CTCs ❖ No CTCs were detected in control smoking and non-smoking healthy individuals ❖ CTCs can be detected in patients with COPD without clinically detectable lung cancer ❖ Monitoring "sentinel" CTC-positive COPD patients may allow early diagnosis of lung cancer
Evaluation and Prognostic Significance of Circulating Tumor Cells in Patients With Non–Small-Cell Lung Cancer (77)	*Journal of Clinical Oncology*, April 2011	❖ The detection as well as the prognostic significance of CTCs were assessed in 101 stage III or IV NSCLC patients ❖ The CTCs number was higher in patients with stage IV NSCLC (n=60; range, 0–146) compared with patients with stage IIIB (n=27; range, 0–3) or IIIA disease (n=14; no CTCs detected) ❖ In univariate analysis, progression-free survival was 6.8 *vs.* 2.4 months with P<0.001, and overall survival (OS) was 8.1 *vs.* 4.3 months with P<0.001 for patients with fewer than five CTCs compared with five or more CTCs before chemotherapy, respectively ❖ In multivariate analysis, CTC number was the strongest predictor of OS [hazard ratio (HR), 7.92; 95% CI, 2.85–22.01; P<0.001], and the point estimate of the HR was increased with incorporation of a second CTC sample that was taken after one cycle of chemotherapy (HR, 15.65; 95% CI, 3.63–67.53; P<0.001)

CTC, circulating tumor cell; AUC, area under the curve; NSCLC, non-small cell lung cancer.

on this subject are, for the moment, little discussed or not convincing (81). In addition to the paucity of CTCs' number in blood, one of the major drawbacks of CTC analysis would be the necessity of an immediate processing (within a half day), while it would be up to 5 days for cfDNA analysis with full blood stabilizing tubes (*Table 3*).

Nevertheless, CTCs are more related to the liquid biopsy terminology and intrinsically more powerful since determination of cellular markers may be combined to the genetic information in the same blood sample, and they are rather of relevance for real-time diagnosis of cancer progression.

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Other molecular circulating biomarkers

Other circulating biomarkers were investigated for the early detection of cancer (*Table 4*). The diagnostic relevance of circulating cell-free microRNAs (miRNAs) was studied in the blood of patients with different types of cancer (96). A study showed that tumor-associated circulating miRNAs are elevated in the blood of breast cancer patients and associated with tumor progression (82). A multivariable signature of nine circulating miRNAs was validated and it provided a high discrimination between breast cancer patients and healthy controls with a corresponding AUC of 0.665 (83). Other circulating miRNA signatures were identified for the early diagnosis of lung malignancies (84).

Another study suggested, for breast cancer, that the presence of circulating cancer-associated macrophage like cells might have a utility as a screening tool and may differentiate patients with malignant disease, benign breast conditions, and healthy individuals (92). Tumor educated platelets (TEPs) are another studied circulating biomarker and it was shown that TEPS mRNA profiles can be used to distinguish between healthy donors and cancer patients (93,94) (*Table 4*).

New avenues for molecular cancer screening tests based on cfDNA analysis

Genetic alteration profile

Based on the assumption that early-detection coupled with early treatment would be key to saving lives, liquid biopsies also have the potential to allow physicians to identify patients whose tumors have specific mutations in the least invasive way possible. Several attempts were made towards this goal especially with the use of sophisticated Next-Gen sequencing methods applied on circulating DNA. Thus, the group of Velculescu recently evaluated this strategy on 138 patients with early tumors and it successfully identified the early-stage cancer in more than half of the patients using targeted error correction sequencing (TEC-Seq) (56). cfDNA analysis was used to detect the return of cancers after treatment. Authors noted that 58 genes are typically associated with breast, lung, ovarian cancer and CRC. Of the 138 cancers, they could detect 86 stage I and stage II cancers. The genes were sequenced in 100 patients and 82 of them showed the same mutations in blood samples as well as in the tumor tissue samples. None of 44 tested healthy patients as control group have cancer-derived mutations. The limitations of the study/technological strategy is the difficulty

in identifying the rare DNA from cancers and in showing up results from other types of genetic alterations or mutations that a person is born with or develops during his life.

Another study showed that TP53 mutations were also detected in the plasma of 49% small cell lung cancer (SCLC) patients with significantly higher allelic fractions in cases than in controls (52).

Virus genome detection

Dennis Lo's group very recently described an elegant study in which the strategy is to detect the Epstein-Barr virus which is involved in most nasopharyngeal cancer cases, and to hunt for viral DNA that tumors shed into the blood in large quantities, rather than rare bits of cancer cells themselves (97). Viral DNA was found in 1,112 or 5.6% of a cohort of 20,000 men. Of those, 309 also had the DNA on confirmatory tests a month later; and, 34 turned out to have cancer following endoscopy and MRI examinations. More cases were found at the earliest stage. Only one person who tested negative on screening developed nasopharyngeal cancer within a year. Clearly this approach is promising and prescription appears warranted.

Circulating DNA fragmentation

Our team first observed that (I) shorter circulating DNA molecules were more abundant in the plasma of CRC patients relative to healthy individuals; (II) the quantity of short circulating DNA fragments <145 bp is directly correlated with ctDNA concentration (98); (III) and that mutant cfDNA derived from malignant cells is highly fragmented compared to non-mutant cfDNA (99). Optimal detection by quantitative PCR (Q-PCR) of ctDNA is obtained with amplicons <100 bp (100) and Atomic Force Microscopy analysis showed that cfDNA fragments from cancer patient plasma are mostly averaging 135 bp (28). High discrimination between stage IV CRCs and healthy individuals was reported when targeting a short amplicon (63 bp) (28). In another report, we revealed that mutant cfDNA fragment proportion was much higher than non-mutant cfDNA below 145 bp size range (99). These observations were later confirmed by Leszinski *et al.*, who showed that DNA integrity was significantly higher in patients with CRC when compared with healthy controls and with individuals with benign colorectal diseases (P=0.005 and 0.006, respectively) (101); and by Jiang *et al.* using massively parallel sequencing to study the size profiles

Table 4 Other molecular circulating biomarkers for cancer screening

Article	Journal/date	Summary/results
Circulating microRNAs as blood-based markers for patients with primary and metastatic breast cancer (82)	*Breast Cancer Research*, November 2010	❖ In the serum of 59 primary breast cancer patients, 30 metastatic patients and 29 healthy women, the relative concentration of total RNA and of breast cancer-associated miR10b, miR141 and miR155 were measured
		❖ The relative concentrations of total RNA (P=0.0001) and miR155 (P=0.0001) in serum significantly discriminated primary breast cancer patients from healthy women
		❖ miR10b (P=0.005), miR34a (P=0.001) and miR155 (P=0.008) allowed the discrimination between metastatic patients and healthy controls
		❖ The presence of metastases correlated with the levels of total RNA (P=0.0001), miR10b (P=0.01), miR34a (P=0.003) and miR155 (P=0.002)
		❖ For patients with primary breast cancer, individuals with an advanced tumor stage (pT3 to 4) had significantly more total RNA (P=0.0001) and miR34a (P=0.01) in their blood than patients at early tumor stages (pT1 to 2)
Novel circulating microRNA signature as a potential non-invasive multi-marker test in ER-positive early-stage breast cancer: A case control study (83)	*Molecular Oncology*, July 2014	❖ Serum from 48 patients with ER-positive early-stage breast cancer obtained at diagnosis (24 lymph node-positive and 24 lymph node-negative) and 24 age-matched healthy controls underwent Global miRNA analysis using LNA-based quantitative real-time PCR (qRT-PCR)
		❖ A signature of miRNAs was subsequently validated in an independent set of 111 serum samples from 60 patients with early-stage breast cancer and 51 healthy controls and further tested for reproducibility in three independent data sets from the GEO Database
		❖ A multivariable signature, that provided considerable discrimination between breast cancer patients and healthy controls with P=0.012 and a corresponding AUC =0.665, was identified. It consisted of nine miRNAs (miR-15a, miR-18a, miR-107, miR-133a, miR-139-5p, miR-143, miR-145, miR-365, and miR-425)
		❖ No association between miRNA expression and tumor grade, tumor size, menopausal or lymph node status was observed
		❖ The signature was also successfully validated in a previously published independent data set of circulating miRNAs in early-stage breast cancer (P=0.024)
Circulating epigenetic biomarkers in lung malignancies: From early diagnosis to therapy (84)	*Lung Cancer*, May 2017	❖ The hypermethylation of tumor suppressor genes is frequently observed in cancers, and such epigenetic changes are potential markers for detecting and monitoring tumors
		❖ The presence of methylated DNA in the serum or plasma of patients was revealed for various types of malignancy, including lung cancer (29,85)
		❖ Methylated tumor suppressor genes, such as *p16INK4A*, *RARB2*, and *RASSF1A* were found in the blood of lung cancer patients (86-88)
		❖ A profile of 10-serum miRNAs (miR-20a, miR-24, miR-25, mir-145, miR-152, miR-199a-5p, miR-221, miR-222, miR-223, miR-320) has been identified by analyzing serum miRNAs from a sample set including 400 NSCLC cases and 220 controls (89)
		❖ The miR-183 family (miR-96, miR-182, and miR-183), a group of onco miRs, has been found to be overexpressed in lung tumors and serum of NSCLC patients (90)
		❖ Based on the use of miRNAratios, a mirRNA signature was developed with a potential for general clinical use. A miRNA signature classified (MSC) algorithm was defined by using 24-miRNA ratios, for prediction, diagnosis, and prognosis of lung cancer. It was able to reduce false-positive rate of low-dose computer tomography (LDCT), thus improving the efficacy of LC screening (91)

Table 4 (*continued*)

Table 4 (continued)

Article	Journal/date	Summary/results
Circulating Cancer-Associated Macrophage-Like Cells Differentiate Malignant Breast Cancer and Benign Breast Conditions (92)	*Cancer Epidemiology, Biomarkers & Prevention*, July 2016	The article consists of two related but separate studies: ❖ In the first study, circulating cancer-associated macrophage-like cells (CAML) were isolated from blood samples of patients with known malignant disease (n=41) using CellSieve microfilters. The prevalence and specificity were compared against 16 healthy volunteers ❖ A follow-up double-blind pilot study was conducted on 41 women undergoing core-needle biopsy to diagnose suspicious breast masses. CAMLs were found in 93% of known malignant patients (average 19.4 cell/sample), but none in the healthy controls. In subjects undergoing core biopsy for initial diagnosis, CAMLs were found in 88% of subjects with invasive carcinoma and 26% with benign breast conditions ◆ Comparing subjects with benign conditions (n=19, excluding the high-risk noninvasive lesions) to those with invasive carcinoma (n=17) results in an ROC curve with an AUC of 0.78 (95% CI, 0.63–0.92), with a threshold of 1CAML as a positive finding, a sensitivity =88%, a specificity =74%, a PPV =75%, and a NPV =88% ◆ The results showed that all breast cancer subtypes based on ER, PR, and HER2 status produce detectable levels of CAMLs and that there is not a pronounced effect of tumor stage or nodal status on the presence of these cells ◆ These preliminary pilot studies suggest that the presence of CAMLs may differentiate patients with malignant disease, benign breast conditions, and healthy individuals, and therefore have a utility as a screening tool
RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics (93)	*Cancer Cell*, November 2015	❖ MRNA sequencing of 283 platelet samples [healthy donors (n=55) and both treated and untreated patients with early, localized (n=39) or advanced, metastatic cancer (n=189)] was conducted to determine the diagnostic potential of Tumor-educated blood platelets (TEPs) ❖ The study includes six tumor types: non-small cell lung carcinoma (NSCLC, n=60), colorectal cancer (CRC, n=41), glioblastoma (GBM, n=39), pancreatic cancer (PAAD, n=35), hepatobiliary cancer (HBC, n=14), and breast cancer (BrCa, n=39) ❖ Tumor-educated platelets (TEPs) are implicated as central players in the systemic and local responses to tumor growth, thereby altering their RNA profile ❖ MRNA profiles of tumor-educated platelets are distinct from platelets of healthy individuals, and they were able to distinguish 228 patients with localized and metastasized tumors from 55 healthy individuals with a 96% accuracy ❖ Across six different tumor types, the location of the primary tumor was correctly identified with 71% accuracy ❖ MET or HER2-positive, and mutant KRAS, EGFR, or PIK3CA tumors were accurately distinguished using surrogate TEP mRNA profiles

Table 4 (continued)

Table 4 (*continued*)

Article	Journal/date	Summary/results
Platelet RNA signatures for the detection of cancer (94)	*Cancer and Metastasis Reviews*, July 2017	❖ TEP (tumor-educated platelets): Tumor-associated biomolecules are transferred to platelets resulting in their "education". External stimuli, such as activation of platelet surface receptors and lipopolysaccharide-mediated platelet activation, induce specific splicing of pre-messenger RNAs (mRNAs) in circulating TEPs. TEPs may also undergo queue-specific splice events in response to signals released by cancer cells and the tumor microenvironment such as by stromal and immune cells
		❖ Platelet mRNA profiles can be used to distinguish between healthy donors and cancer patients
		❖ Platelets can sequester extracellular vesicles from cancer cells harboring tumor-specific RNA
		❖ EGFRvIII, a deletion mutant of the epidermal growth factor receptor (EGFR), is such a specific tumor RNA which is considered to be present in 30% of glioblastoma tumors. Traces of this very malignant tumor of the central nervous system could be detected by RT-PCR of platelets from these patients. The EGFRvIII RNA transcript was detected with a sensitivity of 80% (4 out of 5 EGFRvIII-positive tumors were detected), and a specificity of 96% (25 out of 26 EGFRvIII-negative tumors were scored as negative). In addition, microarray analysis discovered an RNA signature that could distinguish between glioblastoma patients (n=8) and healthy controls (n=12) (95)
		❖ mRNA sequencing of tumor-educated platelets distinguishes cancer patients from healthy individuals with 96% accuracy (93)

RNA, ribonucleic acid; ER, estrogen-receptor; LNA, locked nucleic acid; GEO, Gene Expression Omnibus; AUC, area under the curve; NSCLC, non-small cell lung cancer; NPV, negative predictive value; PPV, positive predictive value; PR, progesterone receptor; LC, lung cancer.

of hepatocellular carcinoma patient plasma DNA samples at a single-base resolution in a genome-wide manner (102). Based upon these observations, various DNA integrity indexes were evaluated with various efficacies in discriminating healthy and cancer patients due to the lack of readily standard operating procedures (69,70), and sufficient tested patient number (98,99). Only the recent study of Tanos *et al.* reported statistically evaluated screening power of a specific DNA integrity index as determined by a Q-PCR method (103).

This screening strategy is based upon a differential between cfDNA structure deriving from malignant and healthy cells rather than on the cfDNA sequence. This would lead to easier implementation, and to lower screening test cost. Works on circulating DNA size profiling are ongoing to set Q-PCR and sequencing approaches toward its inclusion in a screening blood test for cancer.

A test based on circulating mtDNA: the MiTest

We previously showed that the amount of cfDNA may be a discriminatory factor between healthy subjects and CRC patients (28) and the receiver operating characteristic (ROC) curve analysis revealed an AUC of 0.91 (*Figure 1*). We also showed that quantifying and associating circulating mtDNA and nuclear DNA content enables to distinguish cancer subjects from healthy individuals (103). We determined an index based on the detection of particular sequences in the nuclear and mitochondrial genomes. When applied to cell culture supernatant, a significant difference was observed between normal and cancer cell lines (*Figure 2A*), and in plasma, this index is statistically lower in CRC patients than that of healthy subjects (*Figure 2B,C,D*). Our findings suggest that the MiTest consists of a powerful screening test for early cancer detection, and studies are ongoing to combine this marker with other parameters in order to increase the discriminative potential of the test in large cohorts of patients and healthy individuals.

Conclusions

Scientists discovered that tumors shed cells and nucleic acids into the blood circulation more than a century and 70 years ago, respectively (104,105). These molecules and cells were more recently found to reveal some of the same information

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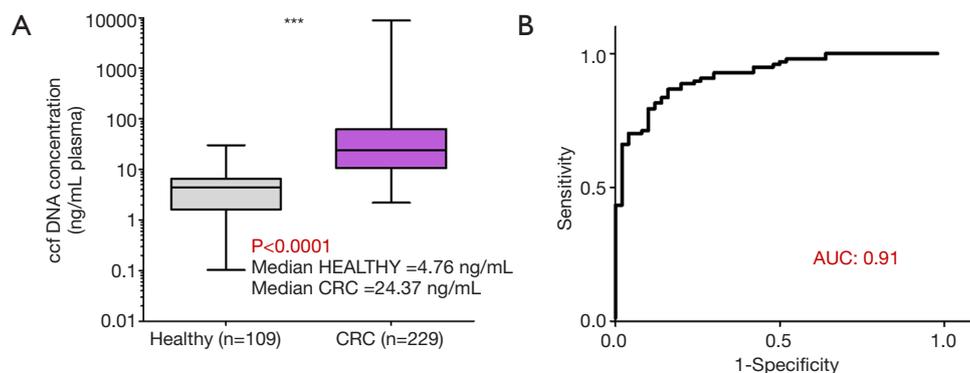
Figure 1 Diagnostic performance of cfDNA concentration in discriminating healthy individuals and cancer patients. (A) Comparison of the quantification of plasma cfDNA (in ng/mL) from healthy individuals (n=109) and colorectal cancer (CRC) patients (stage I–II, n=15; stage III, n=25; stage IV, n=189; and total CRC, n=229). The concentration observed in CRC patients is significantly greater than those of healthy individuals (P<0.0001); (B) diagnosis predictive capacity of total cfDNA concentration to distinguish plasma from CRC patients and healthy subjects. ROC curve representation deriving from the univariate logistic analysis corresponding to the total cfDNA (AUC =0.91) (28). ***, P value <0.0001. cfDNA, circulating cell-free DNA; ROC, receiver operating characteristic; AUC, area under the curve.
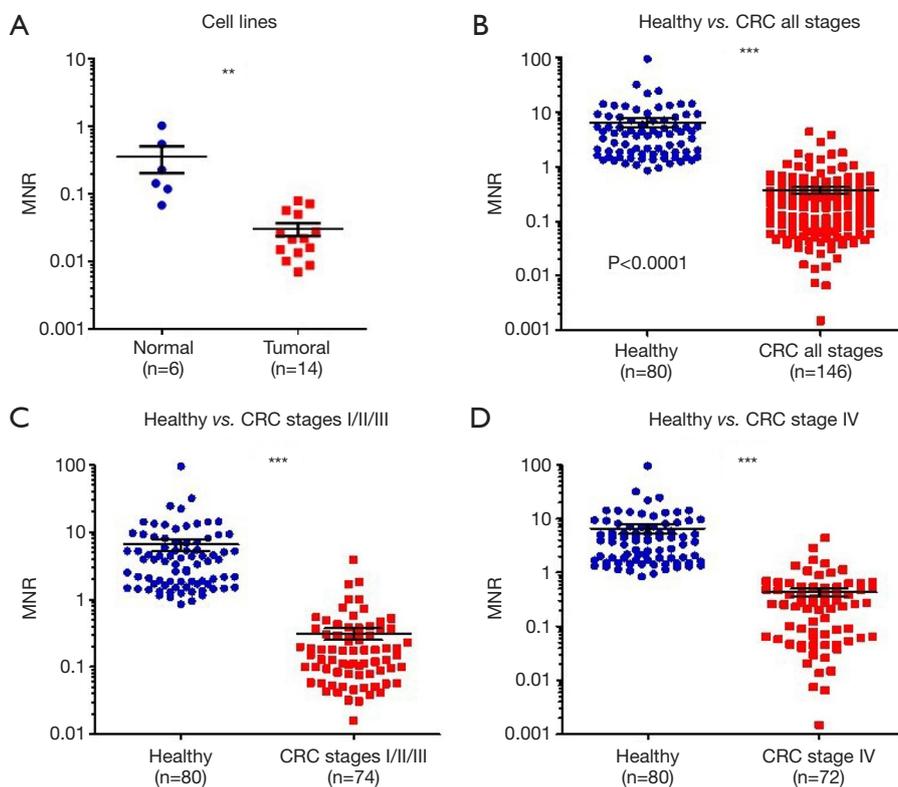
Figure 2 Screening capacity of the MNR test (103). (A) Comparison between the MNR in the supernatant of various tumor cell lines (n=14) and normal cell lines (n=6); (B) dot plot of the MNR in the blood of healthy individuals (n=80) and in CRC patients from stages I to IV (n=146); (C) dot plot of the MNR in blood of healthy individuals (n=80) and in stage I/II/III CRC patients (n=74); (D) dot plot of the MNR in blood of healthy individuals (n=80) and in stage IV CRC patients (n=72). **, P value <0.01; ***, P value <0.001. CRC, colorectal cancer; MNR, multi-normalized ratio.

that tissue biopsies provide (25,106,107). As termed here, liquid biopsy research has expanded in the last decade, generating a rapidly growing area of interest in oncology. Both academic and industry researchers from diverse areas of expertise are working on many fronts to develop, refine, and establish clinical uses for liquid biopsy tests (108).

The minimally invasive nature of liquid biopsy for malignancy without the delay, cost, and risk associated with tissue biopsy, potentially at a microscopic stage before radiologic detectability are promising advantages for cancer screening (38). Several circulating biomarkers are being investigated, from cfDNA, CTCs, circulating miRNAs and others, for the development of tests for early cancer detection. Exosomes, containing certain proteins and nucleic acids, could also be a source of multiple markers of malignancy which the analysis might be promising for the development of screening methods (109,110). But a few of these biomarkers were validated towards clinical practice. cfDNA of nuclear and mitochondrial origin seems to have an advantage in cancer screening compared to other biomarkers by showing better efficiency, and at this time, it appears to possess the characteristics to be more rapidly implemented. Combining various analysis from blood sample such as the detection by sequencing of selected mutations and genes and of protein biomarkers might be an attractive approach as very recently reported by Cohen *et al.* (111). While high specificity level (99%) and an overall AUC of 0.91 were observed, their data showed a moderate sensitivity (varying from 30% to 99% upon cancer types), and the cost of this multi-parametric analysis could hinder its routine use as a massive screening test. This approach should, at least, be considered for populations at risk or for specific malignant diseases.

It is to be feared or hoped that the worldwide use of a screening test will be distinguished in two ways: (I) with a test approved by public health administrations and reimbursed, followed by a statistically long and rigorous study; and (II) privately/individually (e.g., pregnancy test) with a moderate level of performance or evaluation proposed in the near future.

Standardization of the pre-analytical parameters and better knowledge on the exact origin and structure of cfDNA would provide the additional step for the implementation of its analysis. Advancement on sophisticated Q-PCR methods or Next-Gen sequencing will inevitably improve reliability of the analytical performance of the future tests. As indicated by Dennis Lo of The Chinese University of Hong Kong: "*We are brick by brick putting that technology into place*". Looking forward,

we may consider that liquid biopsies could add a new dimension to the cancer screening and diagnosis role of the primary care physician prior to oncology referral (38). At least, investigation of liquid biopsy screening power in tandem with other tests, such as a magnetic resonance imaging (MRI) is warranted. We envision that liquid biopsy tests may be used to screen for early-stage cancer in high-risk individuals, such as those with hereditary cancer syndromes. Nevertheless, it is crucial to further investigate these emerging biomarkers. In addition, a combined use of multiple markers may be a way to achieve more significance in early cancer detection, and increase the sensitivity and specificity of the tests. The years to come seem to be exciting, while universal screening, that constitutes the "holy grail" in oncology, appears to be accessible.

Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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References

1. Maxim LD, Niebo R, Utell MJ. Screening tests: a review with examples. Inhal Toxicol 2014;26:811-28.
2. Parsons DW, Jones S, Zhang X, et al. An Integrated Genomic Analysis of Human Glioblastoma Multiforme. Science 2008;321:1807-12.
3. Navin N, Kendall J, Troge J, et al. Tumour evolution inferred by single-cell sequencing. Nature 2011;472:90-4.
4. Boesch M, Zeimet AG, Reimer D, et al. The side population of ovarian cancer cells defines a heterogeneous compartment exhibiting stem cell characteristics. Oncotarget 2014;5:7027-39.
5. Wilson JM, Jungner G. Principles and practice of screening for disease. Geneva: World Health Organization, 1968.
6. Andermann A, Blancquaert I, Beauchamp S, et al. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ 2008;86:317-9.
7. Petticrew MP, Sowden AJ, Lister-Sharp D, et al. False-negative results in screening programmes: systematic review of impact and implications. Health Technol Assess 2000;4:1-120.
8. PubMed Health. Benefits and risks of screening tests. 2016. Available online: https://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0072602
9. Hakama M, Coleman MP, Alexe DM, et al. Cancer screening: Evidence and practice in Europe 2008. Eur J Cancer 2008;44:1404-13.
10. Arbyn M, Sankaranarayanan R, Muwonge R, et al. Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. Int J Cancer 2008;123:153-60.
11. Mayrand MH, Duarte-Franco E, Rodrigues I, et al. Human Papillomavirus DNA versus Papanicolaou Screening Tests for Cervical Cancer. N Engl J Med 2007;357:1579-88.
12. Friedewald SM, Rafferty EA, Rose SL, et al. Breast Cancer Screening Using Tomosynthesis in Combination With Digital Mammography. JAMA 2014;311:2499-507.
13. Rafferty EA, Park JM, Philpotts LE, et al. Assessing Radiologist Performance Using Combined Digital Mammography and Breast Tomosynthesis Compared with Digital Mammography Alone: Results of a Multicenter, Multireader Trial. Radiology 2013;266:104-13.
14. Stieber P, Nagel D, Blankenburg I, et al. Diagnostic efficacy of CA 15-3 and CEA in the early detection of metastatic breast cancer—A retrospective analysis of kinetics on 743 breast cancer patients. Clin Chim Acta 2015;448:228-31.
15. Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of Prostate-Specific Antigen in Serum as a Screening Test for Prostate Cancer. N Engl J Med 1991;324:1156-61.
16. Horeweg N, Scholten ET, de Jong PA, et al. Detection of lung cancer through low-dose CT screening (NELSON): a prespecified analysis of screening test performance and interval cancers. Lancet Oncol 2014;15:1342-50.
17. Tóth K, Wasserkort R, Sipos F, et al. Detection of Methylated Septin 9 in Tissue and Plasma of Colorectal Patients with Neoplasia and the Relationship to the Amount of Circulating Cell-Free DNA. PLoS One 2014;9:e115415.
18. Wild N, Andres H, Rollinger W, et al. A Combination of Serum Markers for the Early Detection of Colorectal Cancer. Clin Cancer Res 2010;16:6111-21.
19. Chen JS, Chen KT, Fan WC, et al. Combined analysis of survivin autoantibody and carcinoembryonic antigen biomarkers for improved detection of colorectal cancer. Clin Chem Lab Med 2010;48:719-25.
20. Song LL, Li YM. Current noninvasive tests for colorectal cancer screening: An overview of colorectal cancer screening tests. World J Gastrointest Oncol 2016;8:793-800.
21. Dhaliwal A, Vlachostergios PJ, Oikonomou KG, et al. Fecal DNA testing for colorectal cancer screening: Molecular targets and perspectives. World J Gastrointest Oncol 2015;7:178-83.
22. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Fecal DNA versus Fecal Occult Blood for Colorectal-Cancer Screening in an Average-Risk Population. N Engl J Med 2004;351:2704-14.
23. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget Stool DNA Testing for Colorectal-Cancer Screening. N Engl J Med 2014;370:1287-97.
24. National Cancer Institute. NCI Dictionary of Cancer Terms. Available online: https://www.cancer.gov/publications/dictionaries/cancer-terms
25. Thierry AR, El Messaoudi S, Gahan PB, et al. Origins, structures, and functions of circulating DNA in oncology. Cancer Metastasis Rev 2016;35:347-76.

26. Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer—A survey. Biochim Biophys Acta 2007;1775:181-232.

27. Perakis S, Auer M, Belic J, et al. Chapter Three - Advances in Circulating Tumor DNA Analysis. In: Makowski GS. Advances in Clinical Chemistry. Philadelphia: Elsevier, 2017:73-153.

28. Mouliere F, El Messaoudi S, Pang D, et al. Multi-marker analysis of circulating cell-free DNA toward personalized medicine for colorectal cancer. Mol Oncol 2014;8:927-41.

29. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011;11:426-37.

30. Aravanis AM, Lee M, Klausner RD. Next-Generation Sequencing of Circulating Tumor DNA for Early Cancer Detection. Cell 2017;168:571-4.

31. Rahier JF, Druez A, Faugeras L, et al. Circulating nucleosomes as new blood-based biomarkers for detection of colorectal cancer. Clin Epigenetics 2017;9:53.

32. Lamb YN, Dhillon S. Epi proColon® 2.0 CE: A Blood-Based Screening Test for Colorectal Cancer. Mol Diagn Ther 2017;21:225-32.

33. Jin P, Kang Q, Wang X, et al. Performance of a second-generation methylated SEPT9 test in detecting colorectal neoplasm. J Gastroenterol Hepatol 2015;30:830-3.

34. Church TR, Wandell M, Lofton-Day C, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. Gut 2014;63:317-25.

35. Rasmussen SL, Krarup HB, Sunesen KG, et al. Hypermethylated DNA, a circulating biomarker for colorectal cancer detection. PLoS One 2017;12:e0180809.

36. Bedin C, Enzo MV, Del Bianco P, et al. Diagnostic and prognostic role of cell-free DNA testing for colorectal cancer patients. Int J Cancer 2017;140:1888-98.

37. Lee BB, Lee EJ, Jung EH, et al. Aberrant Methylation of APC, MGMT, RASSF2A, and Wif-1 Genes in Plasma as a Biomarker for Early Detection of Colorectal Cancer. Clin Cancer Res 2009;15:6185-91.

38. Krishnamurthy N, Spencer E, Torkamani A, et al. Liquid Biopsies for Cancer: Coming to a Patient near You. J Clin Med 2017;6:3.

39. Liggett TE, Melnikov A, Yi Q, et al. Distinctive DNA methylation patterns of cell-free plasma DNA in women with malignant ovarian tumors. Gynecol Oncol 2011;120:113-20.

40. Lange CP, Campan M, Hinoue T, et al. Genome-Scale Discovery of DNA-Methylation Biomarkers for Blood-Based Detection of Colorectal Cancer. PLoS One

2012;7:e50266.

41. Diehl F, Li M, Dressman D, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci U S A 2005;102:16368-73.

42. Shaw JA, Stebbing J. Circulating free DNA in the management of breast cancer. Ann Transl Med 2014;2:3.

43. Bettegowda C, Sausen M, Leary RJ, et al. Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. Sci Transl Med 2014;6:224ra24.

44. Catarino R, Coelho A, Araújo A, et al. Circulating DNA: Diagnostic Tool and Predictive Marker for Overall Survival of NSCLC Patients. PLoS One 2012;7:e38559.

45. Zhang R, Shao F, Wu X, et al. Value of quantitative analysis of circulating cell free DNA as a screening tool for lung cancer: A meta-analysis. Lung Cancer 2010;69:225-31.

46. Jiang T, Zhai C, Su C, et al. The diagnostic value of circulating cell free DNA quantification in non-small cell lung cancer: A systematic review with meta-analysis. Lung Cancer 2016;100:63-70.

47. Esposito A, Criscitiello C, Trapani D, et al. 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 2017;19:1.

48. Gautschi O, Bigosch C, Huegli B, et al. Circulating Deoxyribonucleic Acid As Prognostic Marker in Non–Small-Cell Lung Cancer Patients Undergoing Chemotherapy. J Clin Oncol 2004;22:4157-64.

49. Sozzi G, Conte D, Mariani L, et al. Analysis of Circulating Tumor DNA in Plasma at Diagnosis and during Follow-Up of Lung Cancer Patients. Cancer Res 2001;61:4675-8.

50. Sozzi G, Conte D, Leon M, et al. Quantification of Free Circulating DNA As a Diagnostic Marker in Lung Cancer. J Clin Oncol 2003;21:3902-8.

51. Paci M, Maramotti S, Bellesia E, et al. Circulating plasma DNA as diagnostic biomarker in non-small cell lung cancer. Lung Cancer 2009;64:92-7.

52. Fernandez-Cuesta L, Perdomo S, Avogbe PH, et al. Identification of Circulating Tumor DNA for the Early Detection of Small-cell Lung Cancer. EBioMedicine 2016;10:117-23.

53. Lin Z, Neiswender J, Fang B, et al. Value of circulating cell-free DNA analysis as a diagnostic tool for breast cancer: a meta-analysis. Oncotarget 2017;8:26625-36.

54. Zhou Q, Li W, Leng B, et al. Circulating Cell Free DNA as the Diagnostic Marker for Ovarian Cancer: A Systematic Review and Meta-Analysis. PLoS One

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2016;11:e0155495.

55. Cohen JD, Javed AA, Thoburn C, et al. Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers. Proc Natl Acad Sci 2017;114:10202-7.

56. Phallen J, Sausen M, Adleff V, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med 2017;9. pii: eaan2415.

57. Hosgood HD, Liu CS, Rothman N, et al. Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study. Carcinogenesis 2010;31:847-9.

58. Meng S, De Vivo I, Liang L, et al. Pre-diagnostic leukocyte mitochondrial DNA copy number and risk of lung cancer. Oncotarget 2016;7:27307-12.

59. Thyagarajan B, Wang R, Nelson H, et al. Mitochondrial DNA Copy Number Is Associated with Breast Cancer Risk. PLoS One 2013;8:e65968.

60. Thyagarajan B, Wang R, Barcelo H, et al. Mitochondrial Copy Number is Associated with Colorectal Cancer Risk. Cancer Epidemiol Biomarkers Prev 2012;21:1574-81.

61. Qu F, Liu X, Zhou F, et al. Association between mitochondrial DNA content in leukocytes and colorectal cancer risk. Cancer 2011;117:3148-55.

62. Lan Q, Lim U, Liu CS, et al. A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. Blood 2008;112:4247-9.

63. Lu H, Busch J, Jung M, et al. Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients. Clin Chim Acta 2016;452:109-19.

64. Fernandes J, Michel V, Camorlinga-Ponce M, et al. Circulating Mitochondrial DNA Level, a Noninvasive Biomarker for the Early Detection of Gastric Cancer. Cancer Epidemiol Biomarkers Prev 2014;23:2430-8.

65. Yu M, Wan YF, Zou QH. Cell-free Circulating Mitochondrial DNA in the Serum: A Potential Non-invasive Biomarker for Ewing's Sarcoma. Arch Med Res 2012;43:389-94.

66. Li L, Hann HW, Wan S, et al. Cell-free circulating mitochondrial DNA content and risk of hepatocellular carcinoma in patients with chronic HBV infection. Sci Rep 2016;6:23992.

67. Moore A, Lan Q, Hofmann JN, et al. A prospective study of mitochondrial DNA copy number and the risk of prostate cancer. Cancer Causes Control 2017;28:529-38.

68. Sun Y, Zhang L, Ho SS, et al. Lower mitochondrial DNA copy number in peripheral blood leukocytes increases the risk of endometrial cancer. Mol Carcinog 2016;55:1111-7.

69. Ellinger J, Albers P, Müller SC, et al. Circulating mitochondrial DNA in the serum of patients with testicular germ cell cancer as a novel noninvasive diagnostic biomarker. BJU Int 2009;104:48-52.

70. Ellinger J, Müller DC, Müller SC, et al. Circulating mitochondrial DNA in serum: A universal diagnostic biomarker for patients with urological malignances. Urol Oncol 2012;30:509-15.

71. Ankeny JS, Court CM, Hou S, et al. Circulating tumour cells as a biomarker for diagnosis and staging in pancreatic cancer. Br J Cancer 2016;114:1367-75.

72. Zhang Z, Fan W, Deng Q, et al. The prognostic and diagnostic value of circulating tumor cells in bladder cancer and upper tract urothelial carcinoma: a meta-analysis of 30 published studies. Oncotarget 2017;8:59527-38.

73. Tanaka F, Yoneda K, Kondo N, et al. Circulating Tumor Cell as a Diagnostic Marker in Primary Lung Cancer. Clin Cancer Res 2009;15:6980-6.

74. Fiorelli A, Accardo M, Carelli E, et al. Circulating Tumor Cells in Diagnosing Lung Cancer: Clinical and Morphologic Analysis. Ann Thorac Surg 2015;99:1899-905.

75. Wang L, Wu C, Qiao L, et al. Clinical Significance of Folate Receptor-positive Circulating Tumor Cells Detected by Ligand-targeted Polymerase Chain Reaction in Lung Cancer. J Cancer 2017;8:104-10.

76. Ilie M, Hofman V, Long-Mira E, et al. "Sentinel" Circulating Tumor Cells Allow Early Diagnosis of Lung Cancer in Patients with Chronic Obstructive Pulmonary Disease. PLoS One 2014;9:e111597.

77. Krebs MG, Sloane R, Priest L, et al. Evaluation and Prognostic Significance of Circulating Tumor Cells in Patients With Non–Small-Cell Lung Cancer. J Clin Oncol 2011;29:1556-63.

78. Hofman P. Liquid biopsy for early detection of lung cancer. Curr Opin Oncol 2017;29:73-8.

79. Diaz LA, Bardelli A. Liquid Biopsies: Genotyping Circulating Tumor DNA. J Clin Oncol 2014;32:579-86.

80. Freidin MB, Freydina DV, Leung M, et al. Circulating Tumor DNA Outperforms Circulating Tumor Cells for KRAS Mutation Detection in Thoracic Malignancies. Clin Chem 2015;61:1299-304.

81. Alix-Panabières C, Pantel K. Characterization of single circulating tumor cells. FEBS Lett 2017;591:2241-50.

82. Roth C, Rack B, Müller V, et al. Circulating microRNAs as blood-based markers for patients with primary and metastatic breast cancer. Breast Cancer Res 2010;12:R90.

83. Kodahl AR, Lyng MB, Binder H, et al. Novel circulating microRNA signature as a potential non-invasive multi-marker test in ER-positive early-stage breast cancer: A case control study. Mol Oncol 2014;8:874-83.

84. Tomasetti M, Amati M, Neuzil J, et al. Circulating epigenetic biomarkers in lung malignancies: From early diagnosis to therapy. Lung Cancer 2017;107:65-72.

85. Jiang T, Ren S, Zhou C. Role of circulating-tumor DNA analysis in non-small cell lung cancer. Lung Cancer 2015;90:128-34.

86. Nikolaidis G, Raji OY, Markopoulou S, et al. DNA Methylation Biomarkers Offer Improved Diagnostic Efficiency in Lung Cancer. Cancer Res 2012;72:5692-701.

87. Bearzatto A, Conte D, Frattini M, et al. p16INK4A Hypermethylation Detected by Fluorescent Methylation-specific PCR in Plasmas from Non-Small Cell Lung Cancer. Clin Cancer Res 2002;8:3782-7.

88. Ponomaryova AA, Rykova EY, Cherdyntseva NV, et al. Potentialities of aberrantly methylated circulating DNA for diagnostics and post-treatment follow-up of lung cancer patients. Lung Cancer 2013;81:397-403.

89. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257-61.

90. Castro D, Moreira M, Gouveia AM, et al. MicroRNAs in lung cancer. Oncotarget 2017;8:81679-85.

91. Sozzi G, Boeri M, Rossi M, et al. Clinical Utility of a Plasma-Based miRNA Signature Classifier Within Computed Tomography Lung Cancer Screening: A Correlative MILD Trial Study. J Clin Oncol 2014;32:768-73.

92. Adams DL, Adams DK, Alpaugh RK, et al. Circulating Cancer-Associated Macrophage-Like Cells Differentiate Malignant Breast Cancer and Benign Breast Conditions. Cancer Epidemiol Biomarkers Prev 2016;25:1037-42.

93. Best MG, Sol N, Kooi I, et al. RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics. Cancer Cell 2015;28:666-76.

94. Sol N, Wurdinger T. Platelet RNA signatures for the detection of cancer. Cancer Metastasis Rev 2017;36:263-72.

95. Nilsson RJ, Balaj L, Hulleman E, et al. Blood platelets contain tumor-derived RNA biomarkers. Blood 2011;118:3680-3.

96. Schwarzenbach H. Diagnostic relevance of circulating cell-free and exosomal microRNAs and long non-coding RNAs in blood of cancer patients. LaboratoriumsMedizin. 2016;40:345-53.

97. Chan KC, Woo JK, King A, et al. Analysis of Plasma Epstein–Barr Virus DNA to Screen for Nasopharyngeal Cancer. N Engl J Med 2017;377:513-22.

98. Mouliere F, Robert B, Peyrotte EA, et al. High Fragmentation Characterizes Tumour-Derived Circulating

DNA. PLoS One 2011;6:e23418.

99. Mouliere F, El Messaoudi S, Gongora C, et al. Circulating Cell-Free DNA from Colorectal Cancer Patients May Reveal High KRAS or BRAF Mutation Load. Transl Oncol 2013;6:319-28.

100. Thierry AR, Molina F. Analytical methods for cell free nucleic acids and applications, WO/2012/028746. 2012. Available online: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2012028746&recNum=281&docAn=EP2011065333&queryString=(%2520&

101. Leszinski G, Lehner J, Gezer U, et al. Increased DNA Integrity in Colorectal Cancer. In Vivo 2014;28:299-303.

102. Jiang P, Chan CWM, Chan KC, et al. Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. Proc Natl Acad Sci 2015;112:E1317-25.

103. Thierry A, El Messaoudi S. Methods for screening a subject for cancer, WO/2016/063122. 2016. Available online: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2016063122

104. Mandel P, Metais P. Les acides nucléiques du plasma sanguin chez l'homme. C R Seances Soc Biol Fil 1948;142:241-3.

105. Ashworth TR. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Australas Med J 1869;14:146-9.

106. Stroun M, Anker P, Lyautey J, et al. Isolation and characterization of DNA from the plasma of cancer patients. Eur J Cancer Clin Oncol 1987;23:707-12.

107. Leon SA, Shapiro B, Sklaroff DM, et al. Free DNA in the Serum of Cancer Patients and the Effect of Therapy. Cancer Res 1977;37:646-50.

108. National Cancer Institute. Liquid Biopsy: Using Tumor DNA in Blood to Aid Cancer Care. 2017.

109. Vlassov AV, Magdaleno S, Setterquist R, et al. Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta 2012;1820:940-8.

110. Wang M, Ji S, Shao G, et al. Effect of exosome biomarkers for diagnosis and prognosis of breast cancer patients. Clin Transl Oncol 2017. [Epub ahead of print].

111. Cohen JD, Li L, Wang Y, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018;359:926-30.