Tumor biopsies represent the standard for cancer diagnosis and the primary method for molecular testing to guide the selection of precision therapies. Liquid biopsies, particularly those involving cell-free DNA (cfDNA) from plasma, are rapidly emerging as an important and minimally invasive adjunct to standard tumor biopsies and, in some cases, even a potential alternative approach. Liquid biopsy is becoming a valuable tool for molecular testing, for new insights into tumor heterogeneity, and for cancer detection and monitoring. Here, we review the current and potential clinical applications of cfDNA analysis in patients with cancer (see video).

Liquid Biopsies

Although liquid biopsy has most often referred to the analysis of cfDNA from peripheral blood, this term also encompasses the isolation and analysis of tumor-derived material (e.g., DNA, RNA, or even intact cells) from blood or other bodily fluids (Fig. 1). For example, intact circulating tumor cells intravasate into the bloodstream at low frequency (often <10 circulating tumor cells per milliliter of blood in patients with metastatic cancer). With specialized technology, circulating tumor cells can be detected and isolated from a background of normal blood cells, facilitating molecular analysis and even implantation and growth of these cells in immunocompromised mice. Subcellular particles called exosomes, or extracellular membrane-encased vesicles, are also released by tumor cells into the bloodstream and contain tumor-specific proteins and nucleic acids. Cell-free nucleic acids, including cell-free RNA (which is less stable than DNA) and cfDNA (the focus of this review), are also released into the circulation.

Blood is not the only bodily fluid that can be used for liquid-biopsy approaches. Urine, stool, cerebrospinal fluid (CSF), saliva, pleural fluid, and ascites are all potential sources of tumor-derived material, including cfDNA. Although this review will focus on the analysis of cfDNA from blood, detection and analysis of cfDNA from other bodily fluids may have applications for specific cancer types (e.g., CSF for cancers of the central nervous system) or for the detection of cancers arising in defined organ systems (e.g., stool for colorectal cancer or saliva for head and neck cancers).

Plasma-Derived cfDNA

The term “cfDNA” refers to fragmented DNA found in the noncellular component of the blood, as first reported by Mandel and Metais in 1948. It is thought that cfDNA is released into the bloodstream through apoptosis or necrosis, and cfDNA is typically found as double-stranded fragments of approximately 150 to 200 base pairs in length, corresponding to nucleosome-associated DNA. Molecules of cfDNA are rapidly cleared from the circulation, with a half-life of an hour or less.
The cfDNA from normal cells is found in plasma at low levels in healthy persons (approximately 10 to 15 ng per milliliter, on average), and the level can increase under conditions of tissue stress, including exercise, inflammation, surgery, or tissue injury.

More than 40 years ago, it was observed that patients with cancer have higher overall levels of cfDNA than persons without cancer. In patients with cancer, cfDNA that is released from tumor cells is often referred to as circulating tumor DNA (ctDNA) and constitutes only a portion of the overall cfDNA. The fraction of ctDNA in overall cfDNA in patients with cancer can vary greatly, from less than 0.1% to more than 90%. Although the fraction of ctDNA tends to parallel tumor burden within an individual patient, substantial variability has been observed among patients with the same cancer type, possibly reflecting biologic differences or differences in rates of cell death in the individual tumors. Moreover, patients with different tumor types...
show considerable variation in the frequency of detectable ctDNA. Thus, the detection and analysis of ctDNA amidst a background of normal germline cfDNA presents a considerable challenge.

Isolation and Analysis of cfDNA

Owing to the low levels and short half-life of tumor-derived cfDNA, specialized approaches are needed for both isolation and analysis of cfDNA. Two key issues are the stability of the cfDNA itself and the potential for lysis of normal blood cells, leading to contamination with normal DNA. To limit these effects, when cfDNA is isolated from blood collected in standard phlebotomy tubes, plasma must typically be centrifuged and separated within 1 to 4 hours after collection. This need for rapid processing creates logistic challenges and the potential for preanalytic variability caused by fluctuations of cfDNA concentration and purity due to differences in processing times.14,15 Alternatively, specialized cfDNA collection tubes containing fixatives can stabilize both cfDNA and intact cells for up to 7 to 14 days at room temperature, allowing for easy shipping, storage, and batched or centralized processing.16

The fraction of ctDNA within the background of normal cfDNA in patients with cancer is typically small and highly variable from patient to patient. Thus, ultrasensitive methods are required to detect mutations, copy-number changes, or other alterations that are present in cfDNA at very low variant-allele frequencies (i.e., the percentage of variant alleles present among all alleles, including wild-type alleles) (Fig. 2). For detection of individual point mutations, mutation-specific techniques based on polymerase-chain-reaction (PCR) analysis — such as BEAMing (beads, emulsion, amplification, and magnetics) or droplet digital PCR (ddPCR) analysis — can identify and quantify alterations present at allele frequencies of 0.01% or less in cfDNA.17,18 Next-generation sequencing methods have also been tailored for cfDNA, ranging from whole-genome or whole-exome sequencing to targeted sequencing of a limited gene panel.19-22 However, the sensitivity and specificity of these approaches are limited by the error rate of DNA polymerase and the sequencing reaction. Thus, modified approaches incorporating deep sequencing coverage, molecular barcoding methods (in which individual input template DNA fragments are tagged with a unique nucleotide barcode), and error-suppression algorithms have improved the limits of detection.23-25

Clinical Applications

The ability to analyze tumor-derived DNA from a routine blood draw without the need for an invasive tumor biopsy represents a critical advance with potentially transformative clinical applications (Fig. 3). In particular, the minimally invasive nature of cfDNA analysis provides a means of molecular profiling for tumors that are difficult or unsafe to biopsy and allows a practical means for monitoring tumor DNA serially over time without the risk and potential complications of standard tumor biopsy. In addition, cfDNA analysis may better capture the molecular heterogeneity harbored by multiple distinct clonal populations in a patient’s tumor, as compared with a needle biopsy of a single tumor lesion. Finally, cfDNA analysis offers the potential for tumor detection or monitoring in patients without clinically evident disease.

Diagnosis and Molecular Profiling

Tumor molecular profiling for the selection of therapy has become a fundamental practice in cancer medicine.26 The potential to assess the molecular profile of a patient’s tumor from a simple blood draw, without the need for an invasive biopsy, makes cfDNA analysis an attractive tool. However, a key initial question is whether the mutational profile established through cfDNA testing reliably reproduces the mutational profile derived from a direct tumor biopsy, which remains the standard of care. Early studies, based on small numbers of patient samples, suggested low concordance between DNA alterations detected in tumor and plasma samples from the same patient.27,28 However, the validity of these studies was compromised by the shortcoming that tumor and plasma samples were often not collected at the same time, yielding potential differences due to molecular evolution of the tumor. In addition, many plasma samples were collected at suboptimal times, such as during therapy, when ctDNA levels are at their lowest.

Nonconcordance of key alterations is most often observed in patients with low ctDNA levels, and low levels make detection of alterations more challenging. For example, one recent study compared the performance of two commercial cfDNA sequencing assays on parallel pairs of blood
samples drawn from 40 patients with metastatic prostate cancer. Low concordance rates were observed, raising questions about the reproducibility of cfDNA analysis. However, the study had major limitations, including the facts that at least half the patients had normal levels of prostate-specific antigen and that participants were not restricted to those not receiving therapy. Thus, much of the variability may be attributable to low cfDNA levels at or below the limit of detection of these assays. In addition, the way in which true positive or negative results are scored in patients with low levels of cfDNA remains an important challenge.

Larger and more carefully controlled studies have shown high concordance rates of 80 to 90% between plasma and tissue samples obtained simultaneously, particularly for alterations in key

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**Figure 2. Isolation and Analysis of cfDNA.**

As shown on the left, cfDNA is isolated from plasma after centrifugation of peripheral blood to separate the cellular component of the blood, including white and red cells. Circulating tumor DNA (ctDNA) is found (often at low fractions) among a background of normal germline cfDNA released by normal cells throughout the body. The cfDNA can be analyzed to identify several common DNA-based alterations observed in tumors, including mutations, copy-number alterations, gene fusions, and DNA methylation changes. As shown at the upper right, owing to the frequently low fraction of ctDNA in a background of normal cfDNA, specialized methods for cfDNA analysis have been developed, including sequencing and methods based on polymerase-chain-reaction (PCR) analysis (BEAMing [beads, emulsion, amplification, and magnetics] and droplet digital PCR). Typically, those analyses providing the greatest breadth or nucleotide coverage have lesser sensitivity and require higher fractions of ctDNA in overall cfDNA (percentages in red) for analysis. Conversely, methods that are directed against targeted mutation panels or single mutations offer improved limits of detection. As shown at the lower right, molecular barcoding to tag individual molecules of DNA has allowed next-generation sequencing approaches for cfDNA to achieve better limits of detection, allowing discrimination of true mutations present in the original template DNA molecule (and thus present in all sequencing reads corresponding to a specific molecular barcode) from mutations introduced by polymerase error during the sequencing reaction.
Similarly, analyses of large cfDNA sequencing databases have yielded molecular landscapes that closely match mutation frequencies produced by large-scale tumor tissue sequencing compendia, such as the Cancer Genome Atlas.

Although these data suggest that cfDNA testing could be a potential surrogate for or adjunct to standard-of-care tumor biopsy testing, for now tumor biopsy remains the standard for initial pathological diagnosis and molecular testing. Tumor biopsies allow for histologic interpretation and the assessment of non-DNA-based alterations, such as in the expression of hormone receptors or other proteins, which can be important for diagnosis and treatment decisions. Further-

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**Clinical Application:**

<table>
<thead>
<tr>
<th>Localized Disease</th>
<th>Metastatic Disease</th>
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<tr>
<td>Clinically undetectable</td>
<td>Metastatic disease</td>
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<tr>
<td>Clinically detectable</td>
<td>Treatment response</td>
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<tr>
<td>Minimal residual disease</td>
<td>Disease progression</td>
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</table>

**Surgical resection**

**Systemic therapy 1**

**Systemic therapy 2**

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**Abundance of ctDNA**

- Normal germline cfDNA
- Baseline clonal alteration in ctDNA
- Subclonal resistance alteration A
- Subclonal resistance alteration B

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**Subclonal resistance/alteration A**

**Subclonal resistance/alteration B**

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**Metastatic Disease**

**Localized Disease**

**Residual disease**

**Molecular profiling**

**Response monitoring**

**Identification of resistance mechanisms**

**Monitoring of clonal dynamics**

**Clinical Application:**

- Early detection
- Residual-disease detection
- Molecular profiling
- Response monitoring
- Identification of resistance mechanisms
- Monitoring of clonal dynamics
Figure 3 (facing page). Clinical Applications of cfDNA Analysis.

Analysis of cfDNA has potential applications at multiple points throughout the natural course of cancer development, diagnosis, and treatment. Early-detection methods that screen for evidence of nascent tumors in cfDNA are currently under development. A liquid-biopsy test capable of identifying early-stage cancers in asymptomatic persons may allow cancers to be identified at a stage when they are more likely to be curable. After surgery with curative intent, cfDNA from postoperative plasma drawn during the weeks after surgery can be analyzed for the persistent presence of mutations or other alterations known to exist in the patient’s resected tumor. Since the half-life of cfDNA is very short (an hour or less), any evidence of persistent tumor-derived mutations in cfDNA from postoperative plasma can provide direct evidence of residual disease that may ultimately lead to tumor relapse. Detection of residual disease in cfDNA shortly after surgery may allow patients to be stratified according to risk of recurrence and may offer an opportunity for early intervention to salvage cure. In the context of metastatic disease, clinical sequencing of cfDNA can identify potentially targetable genetic alterations to select precision therapies. Sequencing of cfDNA can identify many of the same target alterations identified by tumor sequencing, which can be particularly useful when insufficient tumor material is available for clinical sequencing. Studies have suggested that ctDNA levels closely parallel overall tumor burden and can be used as an accurate means of monitoring treatment response and the development of resistance. On disease progression, cfDNA analysis has proved effective in identifying emergent genetic alterations that drive therapeutic resistance, which can guide subsequent therapy choice. Owing to the potential for extensive tumor heterogeneity in the context of acquired resistance, cfDNA analysis may identify multiple concurrent resistance alterations residing in distinct tumor metastases that would not be captured by a single tumor biopsy. Analysis of cfDNA has been used to monitor the clonal dynamics of multiple tumor subclones during subsequent therapy, an approach that can provide a molecular basis for mixed clinical responses to therapy.

more, large series have shown that approximately 15% of patients with metastatic cancer may not have sufficient ctDNA levels to allow for mutational profiling from plasma, and these numbers vary depending on tumor type and tumor burden.13,31,33 Although improving the timing of blood draws for cfDNA analysis (e.g., before the initiation of therapy) may increase the yield of cfDNA testing, confidence in the presence or absence of key alterations decreases as the ctDNA fraction approaches the limit of detection of current technologies. This factor must be taken into account when interpreting clinical cfDNA tests.

Nonetheless, cfDNA testing can play an important role in initial molecular testing, particularly for patients in whom standard tumor biopsies yield insufficient material for clinical sequencing, which can occur in as many as 20 to 25% of needle biopsies.30,34 In such circumstances, cfDNA testing for treatment selection is increasingly used as an alternative to repeat invasive biopsy and may reveal actionable mutations that guide treatment decisions in these patients.30,35 Technological advances are likely to allow more rapid and inexpensive testing of cfDNA for relatively rare abnormalities that could identify actionable mutations and predict the likelihood of response (e.g., the presence of microsatellite instability as a predictor of response to T-cell checkpoint inhibition).

For repeat or serial testing after one or more lines of therapy, minimally invasive cfDNA analysis offers many apparent advantages over repeat invasive tumor biopsies, which are less practical, less safe, and less cost-effective. In particular, liquid biopsy may identify emergent genetic alterations driving acquired resistance to therapy that can be targetable with newer-generation therapies.22,36-43 One of the earliest and best examples is the use of cfDNA testing to identify the emergence of the epidermal growth factor receptor (EGFR) T790M gatekeeper mutation after EGFR inhibitor therapy in EGFR-mutated non–small-cell lung cancer. Key studies have shown a high degree of concordance of cfDNA analysis with tissue testing in identifying the presence of the T790M mutation, which responds to third-generation EGFR inhibitors, such as osimertinib.35,44 Indeed, the cobas EGFR Mutation Test can identify T790M and other EGFR driver mutations in plasma and has been approved by the Food and Drug Administration as a companion diagnostic for choosing specific EGFR therapies.35

Tracking of Therapeutic Response

Although ctDNA levels can vary greatly among patients, ctDNA levels in an individual patient over time correlate well with changes in tumor burden and treatment response. The short half-life of cfDNA in circulation (approximately 1 hour) can be advantageous for measuring real-time tumor burden in response to therapy, in contrast to many standard serum tumor markers in current clinical use (e.g., carcinoembryonic antigen [CEA] and cancer antigen 125 [CA-125]), which
have half-lives of days to weeks. Many standard clinical tumor markers also have limited sensitivity and specificity, whereas tumor-specific clonal alterations (i.e., those alterations present in the original tumor clone and thus present in all tumor cells) can be monitored in plasma with high sensitivity and are unique to a patient’s tumor. Some studies have suggested that ctDNA levels may actually increase transiently after the initiation of therapy, as tumor-cell death leads to increased release of ctDNA. However, within 1 to 2 weeks after the initiation of therapy, ctDNA levels drop dramatically in patients who have a response to therapy.

Indeed, some studies have suggested that changes in ctDNA can outperform standard tumor markers in the prediction of treatment response. Moreover, a rise in ctDNA levels may precede radiographic progression by weeks to months. Thus, as technologies for cfDNA monitoring become increasingly available and cost-effective, their potential to detect early evidence of response or progression may become important for clinical management.

**MONITORING OF RESISTANCE AND TUMOR HETEROGENEITY**

The clinical benefit of precision cancer therapies is limited by the eventual emergence of acquired resistance. In general, acquired resistance arises from one or more tumor subclones that harbor preexisting resistance alterations, which emerge under the selective pressure of therapy. Analysis of cfDNA has become an effective tool for the early detection and identification of molecular alterations leading to clinical resistance to therapy. A key advantage of cfDNA is the ability to capture molecular heterogeneity associated with resistance (Fig. 4). Acquired resistance is often characterized by the clonal outgrowth of multiple resistant subclones in an individual patient. These resistant subclones may coexist in the same lesion or in distinct metastatic sites. Thus, a single-lesion tumor biopsy may dramatically underestimate the molecular heterogeneity present, whereas the presence of multiple resistance mechanisms can often be captured by cfDNA, which is shed from tumor cells throughout the body.

Indeed, studies incorporating multiple tumor biopsies or autopsy specimens have shown that multiple unique resistance alterations frequently coexist in different metastatic sites in an individual patient but can be detected collectively in cfDNA from a single plasma sample. A study involving patients with colorectal cancer after anti-EGFR antibody therapy showed that patients may harbor as many as 13 different resistance alterations, as detected in cfDNA, with fewer than 10% of patients having only a single resistance alteration. Studies comparing matched tumor biopsies and cfDNA tests at the time of disease progression have suggested that cfDNA testing may reveal additional alterations not identified by a single tumor biopsy in as many as two thirds of cases. Thus, targeting a single resistance mechanism on the basis of the results of a single tumor biopsy may lead to mixed clinical responses as a result of the outgrowth of distinct resistant subclones not obtained in the biopsy specimen, and cfDNA testing may help guide treatment decisions.

In addition to emerging as an important discovery tool, cfDNA analysis can be applied clinically for the management of therapeutic resistance. The use of cfDNA to identify the presence of the T790M resistance mutation in EGFR-mutated lung cancer was discussed above. Other studies have shown that cfDNA analysis can identify the coexistence of T790M with other resistance alterations, such as MET amplification, and that patients with such coexisting alterations may derive less benefit from subsequent therapy with third-generation EGFR inhibitors, such as osimertinib. Similarly, detection of one or more ESR1 mutations in the cfDNA of patients with estrogen-receptor–positive breast cancer predicted poorer outcome of subsequent hormonal therapy. Other studies show that resistance to various targeted therapies can be observed in cfDNA before the clinical detection of progression by standard imaging or tumor markers.

Analysis of cfDNA has also been used to track the clonal dynamics of distinct resistant subclones during sequential therapy and even after the discontinuation of therapy. Siravegna et al. found that the RAS mutations emerging in patients with colorectal cancer during EGFR blockade can fall to undetectable levels in cfDNA after withdrawal of EGFR-directed therapy, and many of these patients may then benefit from reinstitution of anti-EGFR therapy. Integration of real-time cfDNA analysis into clinical trials and eventually into standard clinical management has the potential to become a valuable tool for precision medicine.
Detection of Postsurgical Residual Disease

One of the most transformative potential applications of cfDNA analysis may be its ability to detect the presence of tumor in patients with no clinically evident disease — for example, as a screening tool in the early detection of new cancers or for detection of relapse after surgery or adjuvant therapy. In general, the primary means by which solid tumors can be cured is surgical resection. If any tumor cells remain postsurgically, they can lead to eventual tumor relapse. In high-risk patients, adjuvant chemotherapy can reduce the risk of relapse. However, it is currently not possible to determine which patients harbor residual disease immediately after surgery and which patients have been cured of their disease. In particular contexts, such as patients with stage II colorectal cancer who have low...
clinical risk and no evidence of nodal or distant metastases, adjuvant therapy is not routinely given, although approximately 15% of these patients may eventually have a recurrence. An effective method for the detection of postoperative residual disease could spare patients who have been cured the need to undergo potentially toxic adjuvant chemotherapy or could identify those patients who have residual disease and might benefit from adjuvant therapy (Fig. 5).

In a landmark study, Diehl et al. found that cfDNA analysis just a few weeks after surgery for colorectal cancer could accurately predict those patients who had residual disease and would eventually relapse.12 First, tumor-specific mutations were identified in each patient by standard sequencing of resected tumor. Next, highly sensitive methods (BEAMing), capable of detecting mutant-allele frequencies as low as 0.01%, were used to determine whether one or more of these tumor-specific mutations persisted in that patient’s plasma when collected approximately 4 weeks after surgery. Detection of even trace levels of these mutations would provide direct evidence of residual tumor cells. In a follow-up study involving 178 patients with stage II colon cancer who did not receive adjuvant chemotherapy, the detection of residual tumor-specific mutations in postoperative plasma was associated with a risk of tumor recurrence that was 18 times as high as that among patients with undetectable ctDNA (P<0.001). In addition, ctDNA markedly outperformed clinical risk factors and a standard tumor marker (CEA) for predicting relapse.52

Similarly, several key studies have shown that the postoperative detection of tumor-specific mutations in cfDNA can predict residual disease and tumor relapse in breast cancer, lung cancer, and pancreatic cancer.53-56 This approach has the potential to become a critical tool in the postoperative management of the care of patients with cancer and is currently being tested in prospective clinical trials that will assess the usefulness of residual postoperative ctDNA detection to guide adjuvant chemotherapy (Fig. 5).

EARLY CANCER DETECTION

The holy grail of liquid-biopsy applications is the potential for early cancer detection through a simple blood test in otherwise healthy, asymptomatic persons. At present, no mature technology exists to achieve that goal, but such an approach would require a highly sensitive method — to detect trace amounts of cfDNA or other material released by precancerous lesions or early-stage cancers — and would also require high specificity to minimize false positive results in the large unaffected population undergoing screening.

Additional challenges complicate this undertaking. First, since many cancer types share common mutations in genes such as KRAS, BRAF, or TP53, localizing a cancer to a specific organ after a positive liquid-biopsy test may be difficult.57 In addition, benign lesions may harbor the same mutations seen in cancer and have the potential to shed cfDNA into the circulation. Indeed, benign nevi may harbor the same BRAF mutations observed in advanced cancers.58 Mutations
Cell-free DNA Analysis and Cancer Treatment

Current standard of care

- Stage II disease and low clinical risk (~10–15% with residual disease)
- No adjuvant chemotherapy for all
- ~10–15% have recurrence and may have benefited from chemotherapy
- Cure by surgery alone
- Residual disease after surgery

Potential cfDNA-guided postoperative management

- Stage II disease and low clinical risk
- No adjuvant chemotherapy
- Active surveillance
- Serial cfDNA monitoring

- Stage III disease (~45% with residual disease)
- Adjuvant chemotherapy for all
- >50% cured by surgery alone; chemotherapy unnecessary
- ~30% have recurrence despite chemotherapy and ~15% cured by chemotherapy

Sequence resected tumor, identify clonal mutations
Detection of tumor-specific mutations in postoperative plasma

- Stage II disease and low clinical risk
- No evidence of residual disease in postoperative cfDNA
- Evidence of residual disease in postoperative cfDNA

- Stage III disease
- No adjuvant chemotherapy
- Active surveillance
- Serial cfDNA monitoring

Adjuvant chemotherapy

~10–15% have recurrence and may have benefited from chemotherapy

Adjuvant chemotherapy

>50% cured by surgery alone; chemotherapy unnecessary

~30% have recurrence despite chemotherapy and ~15% cured by chemotherapy
detectable in cfDNA can also originate from aberrant and often benign clonal populations in the bone marrow, through a process referred to as clonal hematopoiesis of indeterminate potential (CHIP). The frequency of CHIP increases exponentially with age, with a rate of more than 10% among persons older than 70 years of age.\(^9\) CHIP is thus a common source of diverse mutations detectable in cfDNA, posing a major challenge for this approach. For this reason, methods other than simple mutation detection in cfDNA — including tumor-associated viral sequences, such as in tumors associated with Epstein–Barr virus (EBV) infection and human papillomavirus infection, and DNA methylation changes — are being explored for early detection.\(^7,60,61\)

Two recent studies have illustrated the potential of liquid biopsy for the early detection of cancer. Chan et al. used detection of EBV DNA in plasma to screen for nasopharyngeal carcinoma in 20,174 Chinese patients.\(^60\) A total of 309 patients (1.5%) had detectable EBV DNA in plasma, as confirmed on two sequential tests, and 34 of these patients (0.17% of the original population) had nasopharyngeal carcinoma on endoscopic evaluation. Conversely, only 1 of the EBV DNA–negative patients presented with nasopharyngeal carcinoma within a year after screening. Overall, the sensitivity and specificity of this approach were 97.1% and 98.6%, respectively. Recently, Cohen et al. developed a screening method integrating mutation detection in cfDNA with circulating protein markers, termed CancerSEEK.\(^67\) In 1005 patients with nonmetastatic, clinically detectable tumors across eight common tumor types, the test was positive in a median of 70% of patients, with sensitivities ranging from 69 to 98%. Overall specificity was more than 99%, with only 7 of 812 healthy controls testing positive. Moreover, using supervised machine learning, the investigators used the profile of detected mutations and proteins to localize the cancer to its tissue of origin in 83% of cases.

Though these results are encouraging, evaluation in a more representative screening population of asymptomatic patients will be critical. Although further improvement of liquid-biopsy screening approaches and validation in prospective clinical trials are needed, cfDNA analysis as an early-detection tool offers a potentially transformative advance for cancer medicine.

**SUMMARY AND FUTURE DIRECTIONS**

Analysis of cfDNA has rapidly emerged as a technology with many promising clinical applications in oncology. Effective clinical integration of cfDNA analysis will require a careful understanding of the advantages and limitations of this approach for proper interpretation of results to guide clinical decision making. Although further prospective study is needed, cfDNA analysis harbors the potential to have a transformative effect on cancer medicine.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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