Radiology

REVIEWS AND COMMENTARY • REVIEW

Liquid Biopsy for Cancer: Review and Implications for the Radiologist

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Imaging and image-guided procedures play an imperative role in the screening, diagnosis, and surveillance of cancer. Although emerging imaging techniques now enable more precise molecular characterization of tumors, multigenetic tumor profiling for targeted therapeutic selection remains limited to direct tissue acquisition. Even in the context of targeted therapy, tumors adapt to acquire resistance. This necessitates serial monitoring, traditionally through tissue acquisition, to identify the molecular mechanism of resistance and to guide second-line therapy. An alternative to tissue acquisition is the collection of circulating tumor markers such as cell-free nucleic acids and circulating tumor cells in the peripheral blood. This noninvasive diagnostic approach is referred to as the liquid biopsy. The liquid biopsy is currently used clinically for therapeutic guidance when tissue acquisition is impossible or when the specimen is inadequate. It is also being studied in the context of screening, diagnosis, and surveillance. As cancer treatment continues to move toward a focus on precision medicine, this developing technology may alter and/or augment the role of imaging in the management of cancer. This review aims to outline the use of liquid biopsy in cancer and its potential impact on diagnostic imaging and image-guided procedures.

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Learning Objectives:

After reading the article and taking the test, the reader will be able to:

- Identify the major biomarkers and analysis methods used for liquid biopsy
- Describe the current applications of liquid biopsy in the clinical care of cancer patients
- Explain the future implications of blood-based biomarkers for cancer when used in conjunction with imaging and image-guided procedures

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Radiologists play a pivotal role in cancer care through imaging for the purposes of screening, diagnosis, treatment planning, and surveillance. Additionally, image-guided tissue biopsies performed by radiologists allow for more specific diagnosis and the ability to molecularly characterize tumor cells. Radiologists are also involved in cancer treatment as interventional radiologists deliver minimally invasive local-regional therapies and nuclear medicine physicians administer radionuclide therapy. As discussed by Hricak (1), there are many exciting developing advancements in the field of radiology in the context of precision oncology. Although characterizing physiologic and molecular features of tumors through imaging has traditionally been limited to enhancement characteristics and metabolism, deeper tumor characterization is now possible through molecular imaging for treatment selection, dose finding, and data acquisition on tumor metabolism through nuclear medicine and MR spectroscopy. These innovations, combined with the integration of artificial intelligence and the emerging fields of theranostics and radiomics, are just a few of the ways that radiologists are expanding their contributions and adding value to the care of the patient with cancer (1,2).

The emergence of precision medicine and personalized cancer treatment based on genetic analysis of tumor tissue over the past few decades has resulted in more specific treatment options. While tumor cell receptors, driver mutations, and treatment-susceptible alterations allow for tailored treatment selection, cancers continue to adapt through genetic and epigenetic alterations and acquire resistance to therapy (3). Intratumor heterogeneity

Abbreviations

CTC = circulating tumor cell, ctDNA = circulating tumor DNA, EGFR = epidermal growth factor receptor, FDA = Food and Drug Administration, NGS = next-generation sequencing

Summary

Understanding the utility of liquid biopsy is necessary to prepare for the challenges that will arise in the current diagnostic workflow of many cancers and to anticipate the additional opportunities radiologists may have to use this technology.

Essentials

- Liquid biopsy is a noninvasive diagnostic approach involving the isolation of circulating tumor markers such as cell-free nucleic acids and circulating tumor cells from peripheral blood.
- The tumor microenvironment hosts growing and apoptotic cancer cells that release biomarkers into the circulation, which can be collected for the purpose of analyzing tumor biology.
- Circulating biomarkers including circulating tumor DNA and circulating tumor cells can serve as noninvasive tests for screening, diagnosis, prognosis, and therapy guidance for many solid tumors. A variety of analysis methods are being developed to detect and characterize these markers.
- Improving technology and decreasing costs could allow liquid biopsy to play a complementary role to medical imaging for cancer diagnosis and staging. Percutaneous biopsy with image guidance is anticipated to decrease in overall volume because liquid biopsy has the potential to garner some of the same information regarding the tumor landscape while eliminating the risk of major complications.
- Radiologists may have opportunities to use liquid biopsy techniques as a means of treatment selection and postprocedural management for patients with cancer undergoing local-regional therapies.

and clonal heterogeneity contribute to these mechanisms, which necessitate continuously improving methods of tumor profiling (4).

The shifting paradigm toward serially monitoring tumor molecular characteristics for the purpose of more precisely guiding therapy provides an opportunity for new diagnostic studies to query this information. The tumor microenvironment hosts transforming cancer cells that can migrate into the bloodstream and release biomarkers into the circulation. These cells and cellular components can be collected for the purpose of analyzing tumor biology in the form of a liquid biopsy. Liquid biopsy refers to the noninvasive diagnostic approach involving the isolation and analysis of circulating tumor markers from peripheral blood. This technology could supplement existing clinical tools by improving detection of recurrent cancer, monitoring treatment, and guiding therapy.

As cancer treatment continues to move toward a focus on targeted precision medicine, liquid biopsy will substantially impact the diagnosis and management of these diseases. It is imperative for radiologists to be aware of this technology and its potential impact on clinical practice. This review aims to summarize the principles and applications of liquid biopsy in solid tumors and postulate its impact on diagnostic imaging and interventional procedures.

Tumor Microenvironment

The tumor microenvironment is composed of the supportive stromal and cellular elements surrounding a solid tumor (5). The stromal components include the extracellular matrix, blood vessels, macromolecules, and signaling factors. Signaling factors may be generated by active secretion during the lifetime of the cell, passive release during cell death, or by recruitment from native tissues. The cellular component involves the primary tumor cells and tumor-associated cells (fibroblasts, endothelial cells, and immunologic cells) generated by tumor shedding and recruitment from native tissues. These elements can enter the bloodstream and promote oncogenesis, including local tumor growth, metastasis, and immune modulation. Major mechanisms through which this is achieved include programmed tumor signaling; hypoxia and acidosis from tumor overgrowth and metabolism driving genetic instability, progression, and cell migration; and enhanced permeability of tumor microvasculature facilitating leakage of elements from the microenvironment into the bloodstream (5).

An important factor in the process of tumor invasion and metastasis is epithelial-to-mesenchymal transition. This process involves the transition of the epithelial cell to a mesenchymal cell phenotype, leading to an enhanced ability to migrate, invade, and develop resistance to cell death (6). Loss of epithelial cell polarity, loss of cell-cell adherence, and cytoskeletal remodeling contributes to the migratory potential of cells (7). Once cells migrate to an optimal site, they can reverse epithelial-to-mesenchymal transition and re-express epithelial markers, leading to metastasis and colonization of the new site (7) (Fig 1).

During the process of cell migration, cellular components containing genetic information are released into the bloodstream. Sampling of these biomarkers from the blood provides a noninvasive method to analyze tumor biology and may serve as an alternative to tissue acquisition.

Liquid Biopsy Biomarkers and Analytic Techniques

Growing and apoptotic cancer cells have been shown to release specific biomarkers into the circulation that can be collected in a venous blood sample. Most studied among these are short DNA fragments of cell-free DNA called circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) (8,9). Analysis of these tumor biomarkers could serve as a largely noninvasive test for screening, diagnosis, prognosis, and therapy guidance for many tumor types. (It is worth noting that because liquid biopsy requires needle puncture of a peripheral vein, it is not a completely noninvasive method; however, it is generally referred to as noninvasive in the same way diagnostic imaging requiring intravenous contrast is). Liquid biopsy generally involves obtaining blood from a peripheral venous site such as the median cubital vein (Fig 2).

Tests require a relatively small amount of blood. For example, CellSearch (Menarini Silicon Biosystems; Huntington Valley, Pa) requires a 7.5-mL sample of whole blood whereas

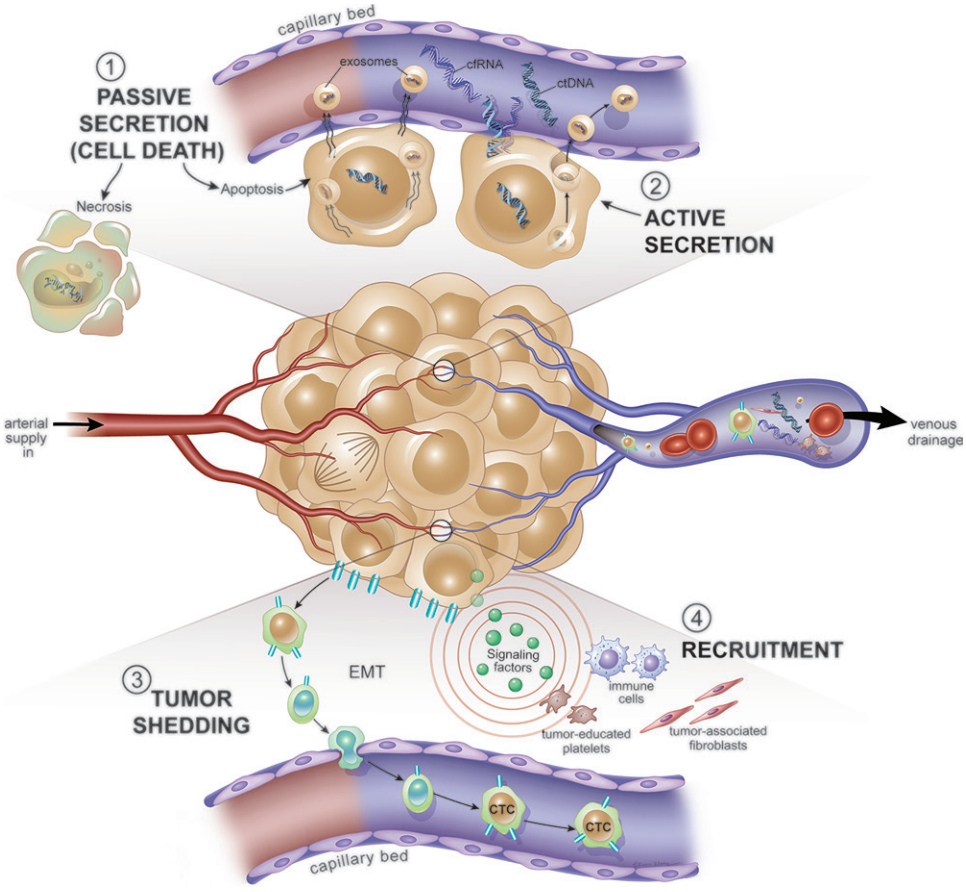

Figure 1: Illustration shows mechanisms of translocation of tumor cells and cellular components into bloodstream. Noncellular tumor components may enter bloodstream through *(1)* passive secretion during cell death by apoptosis or necrosis or *(2)* active secretion. *(3)* Epithelial-mesenchymal transition leads to enhanced ability for tumor cells to migrate. ***(4)*** Recruitment from native tissues also contributes to release of these cells and factors into bloodstream. cfRNA = cell-free RNA, CTC = circulating tumor cell, ctDNA = circulating tumor DNA, EMT = epithelial-to-mesenchymal transition cell.

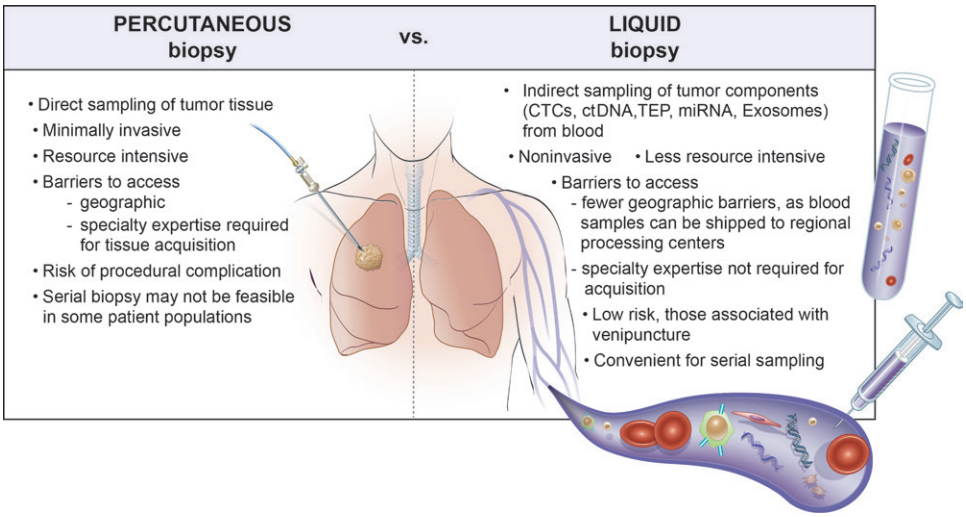

Figure 2: Illustration shows comparison of percutaneous biopsy versus liquid biopsy characteristics. CTC = circulating tumor cell, ctDNA = circulating tumor DNA, miRNA = microRNA, TEP = tumor-educated platelet.

FoundationOne (Foundation Medicine, Cambridge, Mass) liquid biopsy requires two 8.5-mL tubes of blood. After collection, processing is extremely variable. Some systems are benchtop apparatuses that can be purchased for a facility, whereas others require the sample be sent to a central processing facility. Refer to Table 1 and Table 2 for further details.

MicroRNA, tumor-educated platelets, and tumor-derived exosomes are additional biomarkers that are promising but remain early in development. Of note, certain biomarkers including ctDNA, microRNA, and tumor-derived exosomes can be found in bodily fluids other than blood, including saliva, pleural fluid, urine, and cerebral spinal fluid. A summary of the biomarkers for liquid biopsy and their current use can be found in Table 3.

Circulating Tumor DNA

Circulating cell-free DNA can be found in plasma as well as other body fluids. Levels can be elevated in a variety of settings including cancer, infection, and inflammatory conditions. In the setting of cancer, a portion of the cell-free DNA contains tumor-specific characteristics and is termed *ctDNA*, which is often distinguished from normal cell-free DNA by the presence of mutations concordant with the tumor of origin (10). The half-life of ctDNA has been found to be around 2 hours (11). This necessitates rapid processing or preservation of samples but also makes ctDNA a useful dynamic marker of tumor status.

Techniques to analyze ctDNA include several polymerase chain reaction–based methods and next-generation sequencing (NGS). An epidermal growth factor receptor (*EGFR*) mutation test using real-time polymerase chain reaction for ctDNA is U.S. Food and Drug Administration (FDA) approved for determining tumor sensitivity or resistance to tyrosine kinase inhibitors in non–small cell lung cancer (12). Presently, the FDA has approved three treatments

Table 1: Techniques for the Analysis of Circulating Tumor DNA

Technique	Description	Advantages	Disadvantages	Clinical Use
Next-generation sequencing	DNA is fragmented, ligated with custom adapter sequences, and covalently linked to a solid silicon surface. After amplification, the linked clusters of DNA are optically read, resulting in millions of simultaneous sequencing reactions. Finally, these smaller sequences are bioinformatically combined into a complete genomic sequence	High throughput; low cost per sequencing after initial implementation	Analysis is complex; high cost to implement	FoundationOne Liquid (Foundation Medicine, Cambridge, Mass) was recently granted Breakthrough Device designation by the FDA
Real-time PCR	DNA is amplified by repeated cycles of high-temperature denaturation; polymerization with primers, nucleotides, and heat-stable DNA polymerase; and finally, reannealing	Low cost; readily available and simple to implement	Low sensitivity; analysis is specific to primer-enclosed regions of DNA sequence	Cobas *EGFR* Mutation Test v2 (Roche Molecular Diagnostics, Pleasonton, Calif) is a real-time PCR test that is FDA approved for testing of circulating tumor DNA for the presence of *T790M* mutation used to detect sensitivity or resistance to tyrosine kinase inhibitors in non–small cell lung cancer
Digital droplet PCR	DNA is emulsified into droplets before being amplified by PCR in thousands of parallel reactions. Target sequence may then be analyzed by a selected method	High sensitivity and specificity; able to analyze multiple samples simultaneously	Analysis is specific to primer-enclosed regions of DNA sequence	Currently not used clinically
Scorpion ARMS	Specific mutated sequences of DNA are selectively amplified by targeted primer binding. Detection of amplification is carried out by using bifunctional molecules containing a PCR primer covalently linked to a probe. Probe contains both a fluorophore and quencher that are separated during PCR, leading to an increase in fluorescence from the reaction tube	High sensitivity; low cost	Analysis is limited to probe-specific DNA sequence	Currently not used clinically
PNA-LNA PCR	DNA fragments are amplified by PCR in the presence of a PNA clamp that leads to preferential amplification of mutant sequences. Sequences are subsequently detected by a fluorescent primer that incorporates LNAs to increase specificity	High sensitivity; low cost	Analysis is specific to primer-enclosed regions of DNA sequence	Currently not used clinically

Note.—ARMS = amplification refractory mutation system, FDA = Food and Drug Administration, LNA = locked nucleic acid, PCR = polymerase chain reaction, PNA = peptide nucleic acid.

on the basis of tumor molecular characteristics regardless of the primary site of the tumor (tumor agnostic). These include cases with deficient mismatch repair enzymes and tumors harboring *RET* and *TRK* gene alterations, both found in a small proportion of cancers (13). Cell-free DNA assays allow clinicians to evaluate patients for such actionable mutations at any time point during the disease course.

A variety of detection and analysis methods are being developed for ctDNA. A summary of some of these methods can be found in Table 1.

Circulating Tumor Cells

CTCs are rare cells that are shed from tumors into circulation and are found at concentrations of 0 to over 3000 cells per milliliter of blood in patients with metastatic disease (14). The half-life of CTCs is also very short and has been estimated to be between 1–2.4 hours (15). Polymerase chain reaction–based methods, as well as several purification methods to isolate these rare cells from blood, have been investigated. CTC technology has FDA approval for use in the prognostication of metastatic colorectal, breast,

Table 2: Techniques for the Isolation and Detection of CTCs

Technique	Description	Advantages	Disadvantages	Clinical Use
CellSearch (Menarini Silicon Biosystems; Huntington Valley, Pa)	Semiautomated immunomagnetic enrichment and staining system. Centrifuged cells from a 7.5-mL sample of whole blood are mixed with magnetic iron nanoparticles coated with anti-EpCAM antibody. Following immunomagnetic separation, the enriched epithelial cells are stained with fluorescence-labeled antibodies against cytokeratins and leukocyte common antigen CD45; cell nuclei are stained with DAPI fluorescence DNA dye. EpCAM and cells displaying a combination of DAPI+/cytokeratins+/CD45 are thus selected as a CTC. Final step involves trained personnel confirming fluorescent imaging	Well validated; good reproducibility	May miss EMT cells; low sensitivity, low specificity	FDA approved for prognostication in metastatic breast cancer, colorectal cancer, and prostate cancer
ISET systems (includes Rarecells [Paris, France] and ScreenCell [Westford, Mass])	Isolation by filtration technique. Blood samples are diluted with red cell lysis buffer and placed into wells of a filter module. Gentle suction is used to pull the smaller peripheral blood leukocytes through a 7.5- or 8-μm polycarbonate microfiltration platform. The remaining unfiltered cells include the larger tumor cells that can then be further processed with immunohistochemistry and molecular-genetic analyses	Higher sensitivity than CellSearch; nonantigen dependent; isolates circulating tumor microemboli and EMT cells; cells remain intact	Labor-intensive; low specificity; early in development	Currently not used clinically
MagSweeper	Automated immunomagnetic isolation system. 4.5-μm magnetic beads coated with anti-EpCAM antibody are mixed with a sample of blood cells. A 6-mm magnetic rod covered with an ultrathin plastic sheath is robotically swept through a well containing the immunomagnetically labeled sample. Epithelial cells are captured without damage as the rod gently sweeps through the solution. After several rounds of a wash-release-recapture process to remove contaminating cells, the now-purified cells can be further analyzed or frozen for later use	High recovery and purity; cells remain intact	May miss EMT cells; early in development	Currently not used clinically
Microfluidics (includes CTC-Chip, Herringbone-Chip, and CTC-iCHIP)	Immunocytometric isolation method. Microfluidic devices equipped with channels of varying shapes and internal walls coated with anti-EpCAM antibodies are used to capture CTCs as blood is pumped through the channels. Newer prototypes such as CTC-iCHIP use a combination of magnetophoresis and flow dynamics to isolate CTCs as they flow through the device	Higher sensitivity than CellSearch; isolates EMT cells; cells remain intact	Challenging to scale up for industrial use; early in development	Currently not used clinically
Real-time PCR	Indirect method of detection that works by measuring levels of epithelial- or lung-specific mRNA transcripts in cell lysates through reverse-transcription PCR. RNA is isolated, converted to cDNA by a reverse-transcriptase reaction, and then used in a real-time PCR reaction. The levels of target gene expression produced by PCR can be used to estimate CTC levels	High sensitivity	Indirect; difficult to reproduce; unable to accurately assess cell number, morphology, or characteristics because cells are not left intact	Currently not used clinically

Note.—CTC = circulating tumor cell, DAPI = 4',6-diamidino-2-phenylindole, EMT = epithelial-to-mesenchymal transition cells, EpCAM = epithelial cell adhesion molecule, FDA = Food and Drug Administration, ISET = isolation by size of epithelial tumors cells, PCR = polymerase chain reaction.

Table 3: Biomarkers for Liquid Biopsy

Biomarker	Description	Role	Advantages	Disadvantages	Clinical Use
CTCs	Rare, intact tumor cells from solid tumors that have been shed into blood or lymphatic vessels	Cell counts or cell contents (DNA, RNA, and proteins) are used to gain information regarding prognosis, treatment response, treatment selection, and early recurrence detection	Broad utility	Gathering adequate number of intact cells is challenging; analysis methods are still under development	CellSearch (Menarini Silicon Biosystems; Huntington Valley, Pa) analysis of CTCs is FDA approved for prognostication in metastatic breast cancer, colorectal cancer, and prostate cancer
Circulating tumor DNA	Freely circulating single- or double-stranded DNA, shed by either living or dying tumor cells into the blood	Mutations of DNA and DNA methylation patterns used for screening and diagnosis, monitoring and prognosis, prediction of therapy response, and targeted therapy	Well-developed analysis techniques	Rapidly degraded in plasma with short half-life	FDA approved for determining tumor sensitivity or resistance to tyrosine kinase inhibitors in non–small cell lung cancer through Cobas *EGFR* Mutation Test v2 (Roche Molecular Diagnostics, Pleasanton, Calif); FoundationOne Liquid (Foundation Medicine, Cambridge, Mass) was recently granted Breakthrough Device designation by the FDA
MicroRNA	Short, stable, noncoding RNA gene products made up of 19–25 nucleotides	Upregulation or downregulation of expression of specific microRNAs has been shown to be a potential marker for screening, diagnosis, prognosis, and targeted therapy in lung cancer	Stable in blood (compared with other nucleic acids); potentially detectable in multiple body fluids (plasma, serum, urine, saliva)	Early in development; microRNA profiles in lung cancer have been inconsistent from study to study	Currently not used clinically
Tumor-educated platelets	Anucleate fragments of megakaryocytes; tumor cells interact with these fragments by activating surface receptors, which alters expression of platelet cytokines and mRNA	Analysis of the mRNA profiles of tumor-educated platelets may provide important information for lung cancer screening, diagnosis, and treatment selection	Highly stable in blood; abundant; potential use for multiple cancer–type screening	Early in development	Currently not used clinically
Tumor-derived exosomes	Membrane vesicles 50–100-nm in size that are products of endocytosis; transfer information (through contained DNA, RNA, and proteins) from donor to recipient cells	Levels of circulating exosomes and analysis of their contents (RNA and microRNA) may provide information for diagnosis, prognosis, and prediction of response to treatment	Can be found in blood, ascites, and pleural fluid	Early in development	Currently not used clinically

Note.—CTC = circulating tumor cell, FDA = Food and Drug Administration.

and prostate cancer (16–18). Several isolation and analysis methods are being developed for CTCs and a summary of some of these can be found in Table 2.

Imaging and Liquid Biopsy in Selected Cancers

Radiologists play an integral role in the management of virtually every cancer type. Here we discuss the current role of imaging and

the developing complementary role of liquid biopsy by using the examples of lung cancer, colorectal cancer, and pancreatic cancer.

Lung Cancer

Radiologists play an important role in lung cancer screening, staging, surgical and therapeutic planning, and treatment response monitoring. In the last decade, the United States has adopted low-dose CT for lung cancer screening in smokers with high risk (19,20). After detection, TNM staging can be accomplished with various studies including CT, PET, integrated PET/CT, and image-guided tissue biopsy for confirmation (21). The addition of MRI is helpful when brain metastasis, adrenal metastasis, mediastinal invasion, chest wall invasion, or spinal cord invasion is suspected (22).

Higher levels of ctDNA have been shown to correlate with worse prognosis for lung cancer (23). Levels of ctDNA have also been shown to correlate with tumor volume as measured with CT and PET/CT, to help distinguish between residual disease and treatment-related changes at imaging, and to provide earlier response assessment than radiographic approaches (24). Perhaps the greatest utility of ctDNA for lung cancer is identifying driver mutations such as *EGFR*, *ALK*, and *ROS1* mutations. In patients with lung cancer, ctDNA analysis has been shown to detect mutations with relatively high accuracy (25). Higher numbers of *EGFR* mutations have been observed in advanced disease and are correlated with worse survival (26). More importantly, mutations in *EGFR* confer sensitivity to tyrosine kinase inhibitors and are thus essential for treatment selection. Currently, the Cobas *EGFR* Mutation Test v2 (Roche Molecular Diagnostics, Pleasanton, Calif) real-time polymerase chain reaction–based assay is FDA approved to detect *T790M EGFR* mutations in ctDNA for tyrosine kinase inhibitor treatment resistance in non–small cell lung cancer. For this indication, the test has demonstrated a sensitivity of 73% and specificity of 67% (27). The T790 mutation is of particular importance because it is the most common mechanism of resistance to *EGFR* tyrosine kinase inhibitors. Higher levels of T790 mutation before initiation of treatment with tyrosine kinase inhibitors is correlated with worse prognosis (28), and it is found in over 50% of patients who progress on first-line tyrosine kinase inhibitors (29). NGS of ctDNA has been studied as a way to identify oncogenic driver mutations in patients with advanced lung cancer. In one recent study (30), this method showed high concordance with tumor tissue NGS and led to patients being matched to targeted therapy in 21.9% (46 of 210) of cases. The main advantage to using plasma NGS in this study was the median turnaround time of the test of 9 days (range, 4–22 days) for plasma NGS versus a median of 20 days (range, 13–69 days) for tissue NGS (30). This shorter turnaround time allows patients to undergo targeted therapy sooner while still confirming the liquid biopsy result with tissue acquisition, if desired. FoundationOne Liquid and Guardant360 (Guardant Health, Redwood City, Calif) are additional available NGS tests with similar applications (31–33). In a recent study using the Guardant360 test in patients with previously untreated metastatic non–small cell lung cancer, the overall clinical sensitivity of cell-free DNA relative to tissue was 80% (48 of 60) for detection of eight guideline-recommended biomarkers (*EGFR* mutations, *ALK* fusions, *ROS1* fusions, $BRAF^{V600E}$ mutation, *RET* fusions, *MET* amplification and *MET* exon 14 skipping variants, and *ERBB2* [human epidermal growth factor receptor 2, or *HER2*] mutations). For FDA-approved treatment targets (*EGFR*, *ALK*, *ROS1*, *BRAF*), concordance was greater than 98.2% with 100% positive predictive value for cell-free DNA versus tissue in 34 of 34 patients (32). In a study of an earlier version of FoundationOne Liquid called FoundationACT (34), detection of gene mutations covered by both tissue and plasma assays were compared in temporally matched blood and tissue samples. For the genes covered by both assays, a total of 68 reportable genomic alterations were detected in 33 tissue samples, of which 75.0% (51 of 68) were also detected in temporally matched cell-free DNA. Conversely, 75.0% (51 of 68) of genomic alterations detected in cell-free DNA were also detected in tissue.

Higher CTC counts correlate with poor prognosis in lung cancer and higher CTC concentrations are seen in advanced stages (35,36). The genetic material within CTCs can be analyzed to detect driver mutations such as *ALK* gene rearrangement and to detect driver mutations in *EGFR* (37,38). A useful application of this is to monitor treatment response to crizotinib in *ALK*-positive non–small cell lung cancer (39). Similar to ctDNA, CTCs can be used to monitor tyrosine kinase inhibitor resistance by development of *T790M* mutations (38). CTC counts in circulation have been shown to decline significantly after treatment initiation and correlate with radiographic response by measuring tumor volume (38).

Colorectal Cancer

For colorectal cancer, radiologists are involved in screening, staging, and treatment planning. Systematic reviews of screening studies have shown similar diagnostic yield with both CT colonography and colonoscopy for detection (40). However, because colonoscopy permits immediate removal or biopsy of any lesions, it remains the reference standard for screening and investigation of suspected colorectal cancer. Beyond screening for colon cancer, the clinical role of imaging includes identifying distant metastases by using CT, PET/CT, and MRI. Radiologists play an essential role in the local-regional staging of rectal cancer (41). Surgical planning and need for neoadjuvant chemotherapy and radiation is guided by radiologic evaluation with transrectal US and MRI.

Both ctDNA and CTCs have a potential role in several aspects of colorectal cancer including prognosis, treatment selection, treatment response, and early recurrence detection. To detect the presence of specific tumor mutations that were previously only detectable by obtaining tissue biopsies, ctDNA can be used. Although quantification of ctDNA alone has prognostic value, characterizing tumor mutations that determine treatment sensitivity patterns is a primary focus of research. Tissue biopsy of primary and metastatic lesions in colorectal cancer is routinely used to determine the presence of *RAS* mutations to predict resistance to EGFR-targeted monoclonal antibodies and poor prognosis (42). A second significant mutation is of *BRAF*, which has been shown to be a poor prognostic factor when detected in colorectal cancer (43), and ctDNA detected in plasma has been investigated as an alternative source of detecting both of these

mutations with a mutation concordance level between tissue and plasma ranging from 72%–96% for *KRAS* and 87%–100% for *BRAF* (44–47). Microsatellite instability and tumor mutational burden are additional useful predictive biomarkers. These tumor characteristics indicate patients can clinically benefit from cancer immunotherapy agents such as pembrolizumab, which is FDA approved for unresectable or metastatic solid tumors with mismatch repair deficiency or microsatellite instability (48). Microsatellite instability testing is typically performed with tumor tissue testing by using polymerase chain reaction. However, NGS analysis of ctDNA has also been shown to be highly sensitive for this purpose (49).

A challenge in colorectal cancer therapy is the development of resistance to treatment with mutations of *RAS* after initiation of treatment with anti-EGFR antibody agents. Tumor heterogeneity and evolution over time need to be considered but multisite serial tissue biopsy is not always feasible. Studies have shown ctDNA profiles can accurately detect these mutations in the plasma prior to radiologic progression (50,51). Additionally, ctDNA characterization allows for detection of resistance mechanisms occurring in separate metastatic lesions simultaneously and for assessment of resistance overcoming therapies (52). An emerging role of ctDNA in colorectal cancer involves developing a mechanism to identify patients at high risk for recurrence through detection of molecular residual disease. This can be accomplished by monitoring patients for mutation-positive ctDNA following curative resection of colorectal cancer (53,54).

CTC counts in the peripheral blood by using CellSearch analysis have been shown to be an independent prognostic factor of poor progression-free survival and overall survival (16). CellSearch has since been given FDA approval for prognostication in colorectal cancer and can be used clinically alongside imaging findings. In a study by Cohen et al (16) of patients with metastatic colorectal cancer, CTCs had an overall sensitivity of 27% (95% confidence interval: 17%, 39%) and specificity of 93% (95% confidence interval: 89%, 96%) for progressive disease or death. For nonmetastatic colorectal cancer, postoperative presence of CTCs in the blood is also associated with worse overall survival (55). Tissue biopsy in colorectal cancer is routinely used to determine the presence of *KRAS* mutations to predict resistance to EGFR-targeted monoclonal antibodies and a poor prognosis (42). Concordance rate in *KRAS* mutation status between CTCs and primary tumors has been found to be 50%–77% in various studies (56,57). This variability in concordance could be explained by intratumor heterogeneity or metastatic lesions with various mutation statuses (58). CTC-derived xenografts could also play a role in treatment selection because they can allow testing with drug sensitivity assays, potentially predicting patients' response to multiple agents in a relatively short period (59). Many CTC collection methods have diminished sensitivity for epithelial-to-mesenchymal transition cells (Table 2). To address this deficiency, systems such as CanPatrol (SurExam, Guangzhou, China), which screens for both epithelial and mesenchymal cell markers, have been developed. Counts using this more sensitive method correlate with clinical stage, lymph node involvement, and metastases (60).

Pancreatic Cancer

Imaging of the pancreas plays a key role in the characterization of pancreatic focal lesions and in the staging, surgical planning, and treatment monitoring of pancreatic cancer. Diagnosis of pancreatic cancer relies on clinical symptoms followed by imaging with US, CT, MRI, PET, and endoscopic US. CT is the imaging modality of choice for evaluation of pancreatic cancer, although US, endoscopic US, contrast material–enhanced US, and MRI with MR cholangiopancreatography provide complementary information. Endoscopic US is the most sensitive modality for the early detection of lesions; it allows relatively easy access to the pancreas for tissue diagnosis by using fine-needle aspiration and contributes to tumor staging. MRI with MR cholangiopancreatography and PET scanning can also have a successful role as a secondary imaging modality under special circumstances when CT and endoscopic US are not diagnostic (61).

The potential role of ctDNA in several aspects of pancreatic cancer includes screening, prognosis, treatment selection, and detection of recurrence. Some small studies have demonstrated that ctDNA can be detected in a majority of patients with metastatic pancreatic cancer (44). A study of combinations of tumor-specific circulating proteins and cell-free DNA mutations in the peripheral blood for the early detection of potentially resectable pancreatic cancers (62) demonstrated a sensitivity of approximately 75% and high specificity. Although molecular analysis is not always performed in the diagnostic evaluation for pancreatic masses, certain mutations in pancreatic cancer may have prognostic and therapeutic significance. Potentially useful mutations include activating mutations of *KRAS* and inactivating mutations of *CDKN2A, TP53, SMAD4,* and *BRCA2* (63). *KRAS* mutations are of particular importance due to the likelihood that they are an early oncogenic event and the fact that they are found in around 90% of pancreatic adenocarcinomas, making them an ideal target for detecting presence of ctDNA (64). Multiple reports have shown concordance between ctDNA mutations and matched tumor mutations detected in tissue samples (65,66). The frequency of *KRAS* mutations combined with high concordance could theoretically lead to ctDNA-based screening for pancreatic cancer.

While the majority of patients present with unresectable disease, ctDNA has a potential role in monitoring those who do undergo surgical resection. In a study by Sausen et al (67), digital polymerase chain reaction was used to analyze ctDNA in the plasma of 51 patients with pancreatic cancer after surgery. Use of ctDNA helped to detect recurrence 6.5 months earlier than did evidence at CT imaging (recurrence was detected at an average of 3.1 months after surgery by using ctDNA compared with 9.6 months by using CT imaging). Clinically actionable mutations are limited with respect to FDA-approved agents for pancreatic cancer. Actionable genetic alterations, however, were identified in over a third of the patients for which FDA-approved agents exist for alternate indications (67). One such example is amplification of the HER2/neu tyrosine kinase *ERBB2,* for which trastuzumab is commonly used in breast cancer. As with colorectal cancer, microsatellite instability status is also of potential significance

in pancreatic cancer, which can be monitored with NGS of ctDNA (49). Microsatellite instability in pancreatic cancer is not as well characterized as with colorectal cancer. However, it is being investigated as a biomarker to predict response to immunotherapy, surgery, and radiation therapy (68,69).

Early studies of CTC detection in pancreatic cancer have mostly involved CTC capture with antibodies to epithelial cell surface antigens and relatively low rates of CTC detection (5%–50%) (70–72). One barrier to the use of CTCs in pancreatic cancer is the predilection of this cancer for epithelial-to-mesenchymal transition over other cancers, resulting in limited sensitivity of systems such as CellSearch. Studies have shown CTC detection with CellSearch in only 5% of patients before treatment initiation (71). Studies involving methods using epithelial-to-mesenchymal transition cell-sensitive techniques show more promise as possible screening or surveillance tools. One study by Khoja et al (73) comparing a filter-based method to CellSearch showed higher CTC detection rates (93% vs 40%, respectively).

Use of CTCs to monitor therapy response has not been extensively investigated for pancreatic cancer but a study in xenograft mouse models of pancreatic cancer showed a correlation between CTC count detected with microfluidic chip methods and tumor size (74). Additionally, Ren et al (75) examined CTC count in response to 5-fluorouracil chemotherapy in a group of 41 patients with advanced disease showing a reduction in the number of patients positive for CTC during therapy (75).

Other Cancers

While the discussion of the diseases above serves to demonstrate how liquid biopsy for cancer is being investigated, it is far from comprehensive. There are many other cancers such as prostate and breast cancer for which the role of imaging is likely to be influenced by liquid biopsy. In breast cancer, for example, radiology is involved in screening mammography, tissue biopsy, and disease monitoring (76,77). There is little data on liquid biopsy as a screening tool in breast cancer, but CancerSEEK (Thrive Earlier Detection, Cambridge, Mass) is one assay that has been tested for this application (see "Advantages and Disadvantages of Liquid Biopsy" section) (62). Aside from screening, CTC enumeration in breast cancer is a fairly well-established and FDA-approved prognostic factor in metastatic disease (17,78). Also being investigated is the use of ctDNA to asses tumor mutational status, to assess early treatment response, and to monitor patients for minimal residual disease (79–83). Early results are promising, but more data are needed before these monitoring tools are routinely integrated into the clinical setting.

Future Implications for Radiology

Implications for Surveillance Imaging

As liquid biopsy continues to evolve, so will the role of imaging in the diagnosis and posttreatment surveillance of cancer. Improving technology and decreasing costs could allow liquid biopsy to serve as a highly sensitive and specific tool for the diagnosis and treatment of cancer in the near future. The development of screening protocols in certain patient populations for specific cancers could lead to earlier detection of disease. Although the volume of imaging for screening purposes (such as with low-dose CT in lung cancer) may decrease, the utilization of diagnostic imaging for further localizing disease will remain constant or increase. Whereas liquid biopsy offers information regarding the presence and mutational status of disease, anatomic staging—an important prognostic factor—cannot be queried with liquid biopsy. Thus, imaging will continue to have a critical role in anatomic staging and treatment planning with liquid biopsy acting in a complementary role, resulting in more precise treatment selection based on tumor mutational status and earlier detection of disease with screening protocols.

Early clinical studies have demonstrated that liquid biopsy can detect molecular residual disease and disease that is not yet evident at imaging. It is conceivable that patients with early-stage disease could be monitored following surgery or other local-regional therapy with liquid biopsy to evaluate for residual disease prior to detectability with imaging. Large clinical studies will be needed to determine if routine imaging surveillance in the subset of patients without evidence of molecular residual disease can be substituted with serial monitoring by using liquid biopsy. Alternatively, patients with evidence of molecular residual disease may require advanced imaging studies such as PET/CT to maximize the sensitivity for the detection of residual or metastatic disease.

Implications for Tissue-based Diagnosis

Conversely, as this technology develops, percutaneous biopsy with image guidance is anticipated to decrease in overall volume. At the authors' institution, liquid biopsy is being used for mutation testing in cases where tissue biopsy is inadequate or not possible (see "Case Example: Liquid Biopsy in Practice" section) but is not used for any other indications. It is feasible that eventually, liquid biopsy will replace the need for tissue diagnosis in many cases where mutation status is being investigated, with biopsy being reserved for specific situations. These tests have the potential to garner significant oncologic information regarding the tumor landscape within a patient while minimizing the need for serial biopsy and thus the risk of associated complications. CT-guided percutaneous lung biopsy is one such procedure with relatively high complication rates that could be reduced or replaced by liquid biopsy. Pneumothorax is the most common complication with an occurrence rate of 17%–26.6%, and pneumothorax requiring chest tube placement occurring from 1% to 14.2% of the time (84). Pulmonary hemorrhage is the second most common complication with a rate of 4%–27%, and hemoptysis occurring in around 4% of patients (84). Air embolism and tumor seeding are two extremely rare but serious complications of lung biopsy that occur in less than 0.1% of cases (85). Although these complications are rarely life threatening, they can result in additional interventions, patient discomfort, and anxiety.

At this time, the utility of liquid biopsy for cancer screening and in the initial diagnosis of cancer remains speculative and large clinical trials will be required to determine if liquid biopsy can supplant tissue-based methods.

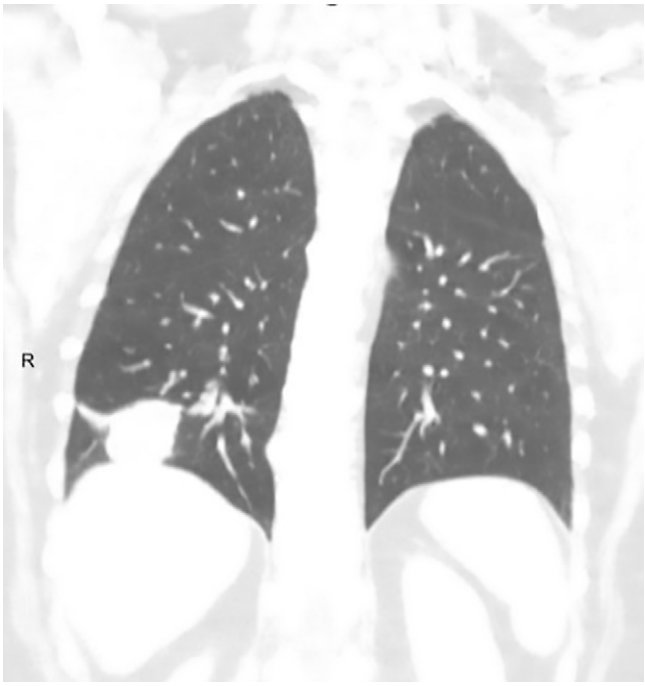

Figure 3: Chest CT in a patient with metastatic colorectal cancer demonstrates right lower lobe pulmonary mass. CT-guided biopsy confirmed mass was of colorectal origin, but scant biopsy material limited genetic characterization. Liquid biopsy detected no mutation in *KRAS* gene and patient was determined to be good candidate for anti-epidermal growth factor receptor–directed therapy.

Case Example: Liquid Biopsy in Practice

A 49-year-old woman with a history of stage IIIB colon cancer 5 years ago presents to her primary care physician with a chief complaint of worsening wheezing when lying down for the past 4 months. After her original diagnosis of colon cancer, she underwent surgical resection followed by 6 months of adjuvant chemotherapy with folinic acid, fluorouracil, and oxaliplatin, or FOLFOX. She was subsequently followed by her oncologist for 2 years but was later lost to follow-up. At the current presentation, her primary care physician orders a chest radiograph that demonstrates a large concerning lesion in her right lung. A subsequent CT of the chest shows a large right lower lobe pulmonary mass invading the right lower lobe bronchus and possibly the pulmonary segmental arteries. Her serum carcinoembryonic antigen level is not elevated above reference range. The remainder of the staging work-up is negative (Fig 3).

A CT-guided biopsy of the lung lesion confirms metastatic colon cancer. The tumor is positive for CK20 and CDX2 and negative for TTF-1 and CK7. The material obtained during biopsy is scant and hence *KRAS* mutational status cannot be determined. This information would be helpful to the treating oncologist in determining candidacy for anti-EGFR–directed therapy, because patients with *KRAS* mutations do not benefit from anti-EGFR therapy.

The patient is disinterested in a repeat biopsy and hence liquid biopsy with NGS of ctDNA is ordered. The result is positive for *APC*, *NOTCH1,* and *TP53* mutations and negative for *KRAS* mutation. She is thus determined to be a candidate for anti-EGFR–directed therapy without the need for repeat lung biopsy.

New Opportunities for Interventional Radiology

Current methods for liquid biopsy rely predominantly on the systemic distribution of ctDNA or CTCs in the peripheral circulation from which blood samples are obtained. There may be advantages to more targeted sampling methods of blood such as tumor draining veins, resulting in more specific localization of molecular residual disease. Venous access and blood sampling would likely have a lower risk of complications such as tumor seeding relative to percutaneous needle biopsy of tumor tissue. There are data from patients with early-stage lung cancer to support the use of selective venous sampling to improve diagnostic yield. A study by Reddy et al (86) compared peripheral blood samples to intraoperative pulmonary vein samples in patients with lung cancer resection and demonstrated a greater than 100-fold increase in the yield of CTCs in the pulmonary vein sample relative to the peripheral blood sample (86). In a rodent model, the use of pulsed focused US has been shown to stimulate the release of intracellular biomarkers, including microRNA, into the circulation (87). This method applied to clinical scenarios could result in increased quantity of biomarker yield for the purpose of guiding patient care.

Additional opportunities for interventional radiologists to use this technology lie in treatment selection and postprocedural treatment of patients with cancer. Interventional radiology plays an integral role in delivering local-regional therapies, such as ablation and embolization, for patients with both primary and metastatic disease. Treatment response monitoring and clinical follow-up may involve CT, MRI, PET, and change in tumor markers. For instance, α-fetoprotein, carcinoembryonic antigen, carbohydrate antigen 19–9 (or CA 19–9), microsatellite instability, and mutational status are all tumor markers that have been used to prognosticate and postprocedurally follow patients. As such markers have limited sensitivity and specificity, monitoring CTCs and ctDNA may serve as a helpful additional tool for interventional radiologists to monitor patients before and after procedures. Assessment of mutational status in tumor tissue has been shown to predict response to some minimally invasive therapies including ablation and embolization (88–90) and extending this concept to ctDNA assessment could be helpful for patient selection. Microsatellite instability and mutational status have historically required tissue acquisition through surgical or percutaneous biopsy. These characteristics can now be evaluated with ctDNA, which may serve as a helpful tool in the multidisciplinary treatment planning of patients with cancer.

Advantages and Disadvantages of Liquid Biopsy

When compared with other clinical tools for cancer care, liquid biopsy with ctDNA and CTCs has several advantages. Compared with tissue biopsy with percutaneous or endoscopic techniques, liquid biopsy has the advantage of being a noninvasive test with minimal complications. Liquid biopsy also serves as a method of overcoming intratumor heterogeneity (51,91). The limitations of both endoscopic and percutaneous biopsy methods include size-dependent visual resolution, limited needle access, user-dependent error, potential radiation exposure, invasive complications (hemorrhage, infection, tumor seeding),

and tissue sampling error. Analyses with ctDNA and CTC are not susceptible to these limitations. As demonstrated by Yoon et al (92), repetitive tissue sampling is possible and can provide satisfactory results; however, in this study the specimen was adequate for mutational analysis in only 80% of patients. This finding is similar to 74% of biopsy specimens collected for research purposes meeting quality control criteria at the National Cancer Institute Developmental Therapeutics Clinic (93). Serial blood draws with ctDNA or CTC analysis allow for a more practical means of monitoring temporal tumor progression. Liquid biopsy is also less resource intensive when compared with percutaneous tissue biopsy. Because a venous blood sample can be taken at virtually any health care facility, many patients with limited access to biopsy services would be able to undergo necessary diagnostic testing.

Compared with serum protein–based markers such as α-fetoprotein, carcinoembryonic antigen, and CA 19–9, liquid biopsy is a more versatile and specific tool for clinical monitoring. Although CA 19–9 for pancreatic cancer can be valuable when used alongside other diagnostic methods, sensitivity and specificity in predicting malignant versus benign pancreatic disease is limited (47% and 88%, respectively, in one recent meta-analysis [94]). Studies have shown that quantification of ctDNA *KRAS* mutations may serve as a complementary marker to CA 19–9, especially for the 10%–15% of patients who do not produce high levels of CA 19–9 due to lack of expression of Lewis body antigen (95,96). When used in conjunction, these complementary blood tests could lead to a more sensitive and specific method for patient monitoring. Carcinoembryonic antigen and CA 19–9 have both been used for detection and monitoring in patients with colorectal cancer, with elevated levels being associated with a poor prognosis. These biomarkers, however, are nonspecific and may be elevated due to unrelated conditions (97,98). When compared with these nonspecific blood markers, ctDNA and CTC analysis have the advantage of being tumor specific and may allow for more accurate detection and monitoring in patients with high risk.

Liquid biopsy also has several disadvantages. Whereas certain genetic and/or epigenetic profiles or cell surface markers garnered from liquid biopsies may point to a specific cancer type (62,99), it cannot comprehensively localize disease. Imaging is still vital for localization of primary and metastatic sites. Another disadvantage is cost. Although liquid biopsy is less resource intensive at the time of acquisition, initial equipment setup and processing can be expensive. It is important to note that there are significant variations in levels of biomarkers depending on cancer type, cancer stage, and tumor burden (24,44,100). Thus, the initial utility for liquid biopsy may be greater with metastatic disease versus localized tumors. Many clinically significant mutations can be assessed for through sequencing of CTC DNA and ctDNA; however, not every gene covered by tissue genetic sequencing has a liquid biopsy analog.

Sensitivity and specificity of liquid biopsy assays will need to be optimized before each test is ready for wide-based applications such as screening. Significant progress is being made in this area. As mentioned previously, one blood test called CancerSEEK (a combined protein biomarker and ctDNA assay) was recently studied as a screening tool for eight different cancer types (ovary, liver, stomach, pancreas, esophagus, colorectal, lung, and breast) and had a median sensitivity of 70% ($P < .001$, one-sided binomial test) and ranged from 98% in ovarian cancers to 33% in breast cancers. At this sensitivity, the specificity was greater than 99%, with only seven of the 812 individuals without known cancers scoring positive (62). Radiology screening programs have demonstrated mortality benefit at the population level. Breast cancer screening with mammography reduces breast cancer–related mortality by at least 20% as demonstrated with pooled estimates from randomized clinical trials (76,77). Similarly, lung cancer–related mortality has been reduced with low-dose CT for lung cancer screening (19,20). Clinical trials involving imaging and other screening tests paired with blood biomarkers will be needed to determine if there is benefit to such applications.

In the era in which cytotoxic chemotherapy and surgical resection were the mainstays of cancer treatment, tissue biopsy fulfilled the need for pathologic diagnosis. As repetitive genetic testing plays an increasing role in the diagnosis and treatment of solid tumors and as cancer treatment continues to move toward a focus on targeted precision medicine, liquid biopsy has the potential to become an adjunct or alternative to tissue biopsies and protein-based markers.

Conclusion

More clinical data are needed with regard to liquid biopsy biomarkers and analytic techniques in cancer, especially given the relatively low blood concentrations of these markers. Current technology detects only focused gene expression and tumor activity at a clinical level, but improvement of these methods to broad multiorgan cancer detection with more extensive tumor analysis is on the horizon (62). Emerging technologies will likely have high costs, but as the industry evolves, liquid biopsy may reach a cost-effective medium. Understanding the utility of liquid biopsy is necessary to prepare for the challenges that will arise in the current diagnostic workflow of cancer and to anticipate the additional opportunities radiologists may have to use this technology.

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References

1. Hricak H. 2016 New Horizons Lecture: Beyond Imaging-Radiology of Tomorrow. Radiology 2018;286(3):764–775.
2. Hensley CT, Faubert B, Yuan Q, et al. Metabolic Heterogeneity in Human Lung Tumors. Cell 2016;164(4):681–694.
3. Huang M, Shen A, Ding J, Geng M. Molecularly targeted cancer therapy: some lessons from the past decade. Trends Pharmacol Sci 2014;35(1):41–50.
4. Turner NC, Reis-Filho JS. Genetic heterogeneity and cancer drug resistance. Lancet Oncol 2012;13(4):e178–e185.
5. Spill F, Reynolds DS, Kamm RD, Zaman MH. Impact of the physical microenvironment on tumor progression and metastasis. Curr Opin Biotechnol 2016;40:41–48.
6. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119(6):1420–1428.
7. Jing Y, Han Z, Zhang S, Liu Y, Wei L. Epithelial-Mesenchymal Transition in tumor microenvironment. Cell Biosci 2011;1(1):29.
8. Thierry AR, El Messaoudi S, Gahan PB, Anker P, Stroun M. Origins, structures, and functions of circulating DNA in oncology. Cancer Metastasis Rev 2016;35(3):347–376.
9. Chen L, Bode AM, Dong Z. Circulating Tumor Cells: Moving Biological Insights into Detection. Theranostics 2017;7(10):2606–2619.
10. Diehl F, Schmidt K, Choti MA, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008;14(9):985–990.
11. Tie J, Semira C, Gibbs P. Circulating tumor DNA as a biomarker to guide therapy in post-operative locally advanced rectal cancer: the best option? Expert Rev Mol Diagn 2018;18(1):1–3.
12. Sumanasuriya S, Lambros MB, de Bono JS. Application of Liquid Biopsies in Cancer Targeted Therapy. Clin Pharmacol Ther 2017;102(5):745–747.
13. Drilon A, Nagasubramanian R, Blake JF, et al. A Next-Generation TRK Kinase Inhibitor Overcomes Acquired Resistance to Prior TRK Kinase Inhibition in Patients with TRK Fusion-Positive Solid Tumors. Cancer Discov 2017;7(9):963–972.
14. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 2004;10(20):6897–6904.
15. Meng S, Tripathy D, Frenkel EP, et al. Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 2004;10(24):8152–8162.
16. Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008;26(19):3213–3221.
17. Cristofanilli M, Hayes DF, Budd GT, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005;23(7):1420–1430.
18. de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 2008;14(19):6302–6309.
19. Moyer VA; U.S. Preventive Services Task Force. Screening for lung cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2014;160(5):330–338.
20. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365(5):395–409.
21. Detterbeck FC, Boffa DJ, Kim AW, Tanoue LT. The Eighth Edition Lung Cancer Stage Classification. Chest 2017;151(1):193–203.
22. Hochhegger B, Marchiori E, Sedlaczek O, et al. MRI in lung cancer: a pictorial essay. Br J Radiol 2011;84(1003):661–668.
23. Sozzi G, Roz L, Conte D, et al. Plasma DNA quantification in lung cancer computed tomography screening: five-year results of a prospective study. Am J Respir Crit Care Med 2009;179(1):69–74.
24. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014;20(5):548–554.
25. Mlika M, Dziri C, Zorgati MM, Ben Khelil M, Mezni F. Liquid Biopsy as Surrogate to Tissue in Lung Cancer for Molecular Profiling: A Meta-Analysis. Curr Respir Med Rev 2018;14(1):48–60.
26. Alegre E, Fusco JP, Restituto P, et al. Total and mutated EGFR quantification in cell-free DNA from non-small cell lung cancer patients detects tumor heterogeneity and presents prognostic value. Tumour Biol 2016;37(10):13687–13694.
27. Thress KS, Brant R, Carr TH, et al. EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. Lung Cancer 2015;90(3):509–515.
28. Wang Z, Chen R, Wang S, et al. Quantification and dynamic monitoring of EGFR T790M in plasma cell-free DNA by digital PCR for prognosis of EGFR-TKI treatment in advanced NSCLC. PLoS One 2014;9(11):e110780.
29. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17(5):1169–1180.
30. Sabari JK, Offin M, Stephens D, et al. A Prospective Study of Circulating Tumor DNA to Guide Matched Targeted Therapy in Lung Cancers. J Natl Cancer Inst 2018 Nov 28 [Epub ahead of print].
31. Zhou C, Yuan Z, Ma W, et al. Clinical utility of tumor genomic profiling in patients with high plasma circulating tumor DNA burden or metabolically active tumors. J Hematol Oncol 2018;11(1):129.
32. Leighl NB, Page RD, Raymond VM, et al. Clinical Utility of Comprehensive Cell-free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-small Cell Lung Cancer. Clin Cancer Res 2019;25(15):4691–4700.
33. Aggarwal C, Thompson JC, Black TA, et al. Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non-Small Cell Lung Cancer. JAMA Oncol 2019;5(2):173–180.
34. Clark TA, Chung JH, Kennedy M, et al. Analytical Validation of a Hybrid Capture-Based Next-Generation Sequencing Clinical Assay for Genomic Profiling of Cell-Free Circulating Tumor DNA. J Mol Diagn 2018;20(5):686–702.
35. Hou JM, Krebs M, Ward T, et al. Circulating tumor cells as a window on metastasis biology in lung cancer. Am J Pathol 2011;178(3):989–996.
36. Tanaka F, Yoneda K, Kondo N, et al. Circulating tumor cell as a diagnostic marker in primary lung cancer. Clin Cancer Res 2009;15(22):6980–6986.
37. Pailler E, Adam J, Barthélémy A, et al. Detection of circulating tumor cells harboring a unique ALK rearrangement in ALK-positive non-small-cell lung cancer. J Clin Oncol 2013;31(18):2273–2281.
38. Maheswaran S, Sequist LV, Nagrath S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 2008;359(4):366–377.
39. Aieta M, Facchinetti A, De Faveri S, et al. Monitoring and Characterization of Circulating Tumor Cells (CTCs) in a Patient With EML4-ALK-Positive Non-Small Cell Lung Cancer (NSCLC). Clin Lung Cancer 2016;17(5):e173–e177.
40. Duarte RB, Bernardo WM, Sakai CM, et al. Computed tomography colonography versus colonoscopy for the diagnosis of colorectal cancer: a systematic review and meta-analysis. Ther Clin Risk Manag 2018;14:349–360.
41. Jhaveri KS, Hosseini-Nik H. MRI of Rectal Cancer: An Overview and Update on Recent Advances. AJR Am J Roentgenol 2015;205(1):W42–W55.
42. Douillard JY, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013;369(11):1023–1034.
43. Taieb J, Zaanan A, Le Malicot K, et al. Prognostic Effect of BRAF and KRAS Mutations in Patients With Stage III Colon Cancer Treated With Leucovorin, Fluorouracil, and Oxaliplatin With or Without Cetuximab: A Post Hoc Analysis of the PETACC-8 Trial. JAMA Oncol 2016;2(5):643–653.
44. 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(224):224ra24.
45. Taly V, Pekin D, Benhaim L, et al. Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from the plasma of colorectal cancer patients. Clin Chem 2013;59(12):1722–1731.
46. Thierry AR, Mouliere F, El Messaoudi S, et al. Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. Nat Med 2014;20(4):430–435.
47. Thierry AR, El Messaoudi S, Mollevi C, et al. Clinical utility of circulating DNA analysis for rapid detection of actionable mutations to select metastatic colorectal patients for anti-EGFR treatment. Ann Oncol 2017;28(9):2149–2159.
48. O'Neil BH, Wallmark JM, Lorente D, et al. Safety and antitumor activity of the anti-PD-1 antibody pembrolizumab in patients with advanced colorectal carcinoma. PLoS One 2017;12(12):e0189848.
49. Deng A, Yang J, Lang J, et al. Monitoring microsatellite instability (MSI) in circulating tumor DNA by next-generation DNA-seq. J Clin Oncol 2018;36(15_suppl):12025.
50. Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012;486(7404):532–536.
51. Diaz LA Jr, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012;486(7404):537–540.
52. Russo M, Siravegna G, Blaszkowsky LS, et al. Tumor Heterogeneity and Lesion-Specific Response to Targeted Therapy in Colorectal Cancer. Cancer Discov 2016;6(2):147–153.
53. Ryan BM, Lefort F, McManus R, et al. A prospective study of circulating mutant KRAS2 in the serum of patients with colorectal neoplasia: strong prognostic indicator in postoperative follow up. Gut 2003;52(1):101–108.

54. Tie J, Wang Y, Tomasetti C, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016;8(346):346ra92.
55. Bork U, Rahbari NN, Schölch S, et al. Circulating tumour cells and outcome in non-metastatic colorectal cancer: a prospective study. Br J Cancer 2015;112(8):1306–1313.
56. Denis JA, Patroni A, Guillerm E, et al. Droplet digital PCR of circulating tumor cells from colorectal cancer patients can predict KRAS mutations before surgery. Mol Oncol 2016;10(8):1221–1231.
57. Fabbri F, Carloni S, Zoli W, et al. Detection and recovery of circulating colon cancer cells using a dielectrophoresis-based device: KRAS mutation status in pure CTCs. Cancer Lett 2013;335(1):225–231.
58. Gasch C, Bauernhofer T, Pichler M, et al. Heterogeneity of epidermal growth factor receptor status and mutations of KRAS/PIK3CA in circulating tumor cells of patients with colorectal cancer. Clin Chem 2013;59(1):252–260.
59. Grillet F, Bayet E, Villeronce O, et al. Circulating tumour cells from patients with colorectal cancer have cancer stem cell hallmarks in *ex vivo* culture. Gut 2017;66(10):1802–1810.
60. Zhao R, Cai Z, Li S, et al. Expression and clinical relevance of epithelial and mesenchymal markers in circulating tumor cells from colorectal cancer. Oncotarget 2017;8(6):9293–9302.
61. Lee ES, Lee JM. Imaging diagnosis of pancreatic cancer: a state-of-the-art review. World J Gastroenterol 2014;20(24):7864–7877.
62. Cohen JD, Li L, Wang Y, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018;359(6378):926–930.
63. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012;491(7424):399–405.
64. Riva F, Dronov OI, Khomenko DI, et al. Clinical applications of circulating tumor DNA and circulating tumor cells in pancreatic cancer. Mol Oncol 2016;10(3):481–493.
65. Douillard JY, Ostoros G, Cobo M, et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol 2014;9(9):1345–1353.
66. Kinugasa H, Nouso K, Miyahara K, et al. Detection of K-ras gene mutation by liquid biopsy in patients with pancreatic cancer. Cancer 2015;121(13):2271–2280.
67. Sausen M, Phallen J, Adleff V, et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. Nat Commun 2015;6(1):7686.
68. Eatrides JM, Coppola D, Al Diffalha S, Kim RD, Springett GM, Mahipal A. Microsatellite instability in pancreatic cancer. J Clin Oncol 2016;34(15_suppl):e15753.
69. Hu ZI, Shia J, Stadler ZK, et al. Evaluating Mismatch Repair Deficiency in Pancreatic Adenocarcinoma: Challenges and Recommendations. Clin Cancer Res 2018;24(6):1326–1336.
70. Rhim AD, Mirek ET, Aiello NM, et al. EMT and dissemination precede pancreatic tumor formation. Cell 2012;148(1-2):349–361.
71. Bidard FC, Huguet F, Louvet C, et al. Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. Ann Oncol 2013;24(8):2057–2061.
72. de Albuquerque A, Kubisch I, Breier G, et al. Multimarker gene analysis of circulating tumor cells in pancreatic cancer patients: a feasibility study. Oncology 2012;82(1):3–10.
73. Khoja L, Backen A, Sloane R, et al. A pilot study to explore circulating tumour cells in pancreatic cancer as a novel biomarker. Br J Cancer 2012;106(3):508–516.
74. Torphy RJ, Tignanelli CJ, Kamande JW, et al. Circulating tumor cells as a biomarker of response to treatment in patient-derived xenograft mouse models of pancreatic adenocarcinoma. PLoS One 2014;9(2):e89474.
75. Ren C, Han C, Zhang J, et al. Detection of apoptotic circulating tumor cells in advanced pancreatic cancer following 5-fluorouracil chemotherapy. Cancer Biol Ther 2011;12(8):700–706.
76. Oeffinger KC, Fontham ET, Etzioni R, et al. Breast Cancer Screening for Women at Average Risk: 2015 Guideline Update From the American Cancer Society. JAMA 2015;314(15):1599–1614.
77. Niell BL, Freer PE, Weinfurtner RJ, Arleo EK, Drukteinis JS. Screening for Breast Cancer. Radiol Clin North Am 2017;55(6):1145–1162.
78. Riethdorf S, Fritsche H, Müller V, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res 2007;13(3):920–928.
79. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 2016;17(4):425–439.
80. Fribbens C, O'Leary B, Kilburn L, et al. Plasma ESR1 Mutations and the Treatment of Estrogen Receptor-Positive Advanced Breast Cancer. J Clin Oncol 2016;34(25):2961–2968.
81. Hrebien S, Citi V, Garcia-Murillas I, et al. Early ctDNA dynamics as a surrogate for progression free survival in advanced breast cancer in the BEECH trial. Ann Oncol 2019;30(6):945–952.
82. Juric D, Ciruelos E, Rubovszky G, et al. Abstract GS3-08: Alpelisib + fulvestrant for advanced breast cancer: Subgroup analyses from the phase III SOLAR-1 trial. Cancer Res 2019;79(4 Supplement):GS308.
83. Garcia-Murillas I, Schiavon G, Weigelt B, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015;7(302):302ra133.
84. Winokur RS, Pua BB, Sullivan BW, Madoff DC. Percutaneous lung biopsy: technique, efficacy, and complications. Semin Intervent Radiol 2013;30(2):121–127.
85. Tomiyama N, Yasuhara Y, Nakajima Y, et al. CT-guided needle biopsy of lung lesions: a survey of severe complication based on 9783 biopsies in Japan. Eur J Radiol 2006;59(1):60–64.
86. Reddy RM, Murlidhar V, Zhao L, et al. Pulmonary venous blood sampling significantly increases the yield of circulating tumor cells in early-stage lung cancer. J Thorac Cardiovasc Surg 2016;151(3):852–858.
87. Chevillet JR, Khokhlova TD, Giraldez MD, et al. Release of Cell-free MicroRNA Tumor Biomarkers into the Blood Circulation with Pulsed Focused Ultrasound: A Noninvasive, Anatomically Localized, Molecular Liquid Biopsy. Radiology 2017;283(1):158–167.
88. Ziv E, Erinjeri JP, Yarmohammadi H, et al. Lung Adenocarcinoma: Predictive Value of KRAS Mutation Status in Assessing Local Recurrence in Patients Undergoing Image-guided Ablation. Radiology 2017;282(1):251–258.
89. Ziv E, Bergen M, Yarmohammadi H, et al. PI3K pathway mutations are associated with longer time to local progression after radioembolization of colorectal liver metastases. Oncotarget 2017;8(14):23529–23538.
90. Gaba RC, Groth JV, Parvinian A, Guzman G, Casadaban LC. Gene expression in hepatocellular carcinoma: pilot study of potential transarterial chemoembolization response biomarkers. J Vasc Interv Radiol 2015;26(5):723–732.
91. Murtaza M, Dawson SJ, Tsui DW, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 2013;497(7447):108–112.
92. Yoon HJ, Lee HY, Lee KS, et al. Repeat biopsy for mutational analysis of non-small cell lung cancers resistant to previous chemotherapy: adequacy and complications. Radiology 2012;265(3):939–948.
93. Ferry-Galow KV, Datta V, Makhlouf HR, et al. What Can Be Done to Improve Research Biopsy Quality in Oncology Clinical Trials? J Oncol Pract 2018 Oct 4:JOP1800092 [Epub ahead of print].
94. Cao S, Hu Y, Gao X, Liao Q, Zhao Y. Serum Carbohydrate Antigen 19-9 in Differential Diagnosis of Benign and Malignant Pancreatic Cystic Neoplasms: A Meta-Analysis. PLoS One 2016;11(11):e0166406.
95. Chen I, Raymond VM, Geis JA, et al. Ultrasensitive plasma ctDNA *KRAS* assay for detection, prognosis, and assessment of therapeutic response in patients with unresectable pancreatic ductal adenocarcinoma. Oncotarget 2017;8(58):97769–97786.
96. Tempero MA, Uchida E, Takasaki H, Burnett DA, Steplewski Z, Pour PM. Relationship of carbohydrate antigen 19-9 and Lewis antigens in pancreatic cancer. Cancer Res 1987;47(20):5501–5503.
97. Stiksma J, Grootendorst DC, van der Linden PW. CA 19-9 as a marker in addition to CEA to monitor colorectal cancer. Clin Colorectal Cancer 2014;13(4):239–244.
98. Litvak A, Cercek A, Segal N, et al. False-positive elevations of carcinoembryonic antigen in patients with a history of resected colorectal cancer. J Natl Compr Canc Netw 2014;12(6):907–913.
99. Lehmann-Werman R, Neiman D, Zemmour H, et al. Identification of tissue-specific cell death using methylation patterns of circulating DNA. Proc Natl Acad Sci U S A 2016;113(13):E1826–E1834.
100. Lanman RB, Mortimer SA, Zill OA, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. PLoS One 2015;10(10):e0140712.