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Next-Generation Novel Noninvasive Cancer Molecular Diagnostics Platforms Beyond Tissues

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OVERVIEW

In recent years, there has been a revolutionary expansion in technologic advances and therapeutic innovations in cancer medicine. Cancer diagnostics has begun to move away from a sole dependence on direct tumor tissue biopsy for cancer detection, diagnosis, and treatment monitoring. The need for improvement in molecular cancer diagnostics has never been more important, with not only the advent of cancer genomics and genomics-guided precision medicine but also the recent arrival of cancer immunotherapies. Owing to the practical limitations and risks associated with tissue-based biopsy diagnostics, novel noninvasive cancer diagnostics platforms have continued to evolve and expand in recent years. Examples of these platforms include the liquid biopsy, which is used to interrogate ctDNA or circulating tumor cells, proteomics, metabolomics, and exosomes; the urine biopsy, which is used to assay ctDNAs; saliva and stool biopsies, which are used for molecular genomics assays; and the breath biopsy, which measures volatile organic compounds. These next-generation noninvasive molecular diagnostics assays beyond tissues fundamentally transform the potential utilities of cancer diagnostics to enable repeat, prospective, and serial longitudinal “biopsies” to monitor disease response resistance and progression on therapies. Moreover, they allow continual interrogation and molecular in-depth analysis of the evolving tumor’s pan-canceromics under therapeutic stress. These technological and diagnostic advances have already brought about paradigm-changing next-generation cancer therapeutic strategies to enhance overall treatment efficacies. This article reviews the key noninvasive next-generation molecular diagnostics platforms beyond tissues, with emphasis on clinical utilities and applications.

In recent years, there has been a revolutionary expansion in technologic advances in cancer medicine. For decades, cancer diagnostics has been predominantly dependent on direct

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tumor tissue biopsy for histologic and pathologic analysis. Modern state-of-the-art next-generation DNA sequencing and genomics bioinformatics analysis have brought forth a new paradigm shift in recent years from microscopic levels of histologic diagnostics to molecular genomics levels of cancer molecular diagnostics. Coupled with the revolution in remarkable drug development innovation and efficiency in the new era of small molecule chemistry and therapeutics, these exciting new trends engendered the birth of genomics-guided personalized cancer therapy, with examples of inhibitors adopted in the treatment of *EGFR*-mutant,¹ and *ALK*-fusion lung cancer,² and *BRAF*-mutant melanoma,³ among others. Nonetheless, with the current widespread clinical applications of genomics-matching personalized cancer therapy, it has also become increasingly evident that the need to obtain a large amount of tumor tissue through invasive tumor-needle or surgical biopsy procedures is self-defeating. The risks and inconvenience of such invasive procedures limit the scope of molecular-genomic profiling achievable in the tissues. This problem is substantially compounded by the arrival of cancer immunotherapies in the last few years, for which the need for diagnostics and predictive biomarkers is paramount. As a result, many research studies have been emphasized in achieving next-generation novel cancer diagnostics beyond the use of tumor tissues obtained from primary or metastatic tumor sites. These include the innovative utility of various body fluids or substrates from different body compartments that allow more noninvasive and low-risk access. In this article, we provide a broad overview of the emerging or well-developed technologic platforms (Fig. 1) for these novel cancer diagnostic applications.

LIQUID BIOPSY

Cellular Compartment

Circulating tumor cells.—Circulating tumor cells (CTCs) are cells that have disseminated away from the primary tumor or secondarily from metastatic sites and further deposited into the circulatory bloodstream.^{4–6} Previous studies suggested that epithelial-to-mesenchymal transition (EMT) may be involved in this active mobilization and dissemination of tumor cells, increasing their plasticity and *trans*-migratory potential.^{7,8} EMT is thought to assist tumor cells in their active intravasation mechanisms into the vasculature. During this process of dissemination, epithelial tumor cells lose their cell-cell contact and apical-basal polarity to gain a more elongated cell phenotype associated with enhanced cell motility and invasiveness.^{9,10} Interestingly, recent evidence also suggests that platelets adhering to CTCs in the circulation could induce EMT itself.¹¹ The EMT activation process leading to a more motile and invasive phenotype is also induced by the milieu of the tumoral microenvironmental make-up.¹² EMT-triggering signals include transforming growth factor β secreted by the activating platelets,¹¹ integrin-macrophage Interactions through epidermal growth factor supply,¹³ and proinflammatory cytokines secreted by fibroblasts.^{4,14}

Circulating tumor cell enrichment and detection.—Because CTCs clearly represent the tumor cell populations responsible for and involved in the process of metastasis, which is the leading cause of cancer morbidity and mortality, there has been intense interest in enhancing the ability to enrich, capture, detect, isolate, and characterize CTCs. Nonetheless,

there are numerous intrinsic challenges in this approach. The size dimension of CTCs varies depending on the different cancer types of origin; for example, small cell lung cancer (SCLC) cells are only approximately 10 μm in diameter, but prostate CTCs can be larger than 100 μm .¹⁵ Moreover, CTCs are extremely rare, and the sensitivity of CTC detection in patient blood is highly dependent on detection methods. No validated formula is available to estimate the true number of CTCs for each patient with cancer, or even for any cancer type. Importantly, how to define what constitutes a CTC and whether even the detected CTCs are functionally important is also quite controversial and challenging. It was recently suggested that even EMT in the CTCs belongs to a continuum rather than a binary phenotype.

CellSearch (Menarini Silicon Biosystems Inc., San Diego, CA) is currently the only U.S. Food and Drug Administration-approved detection system for enrichment, detection, and enumeration of CTCs, based on the epithelium-specific cell adhesion molecule (EpCAM) expressed on the surfaces of epithelium-derived CTCs. Allard et al¹⁶ reported average recovery sensitivity of 85% or greater. Beyond enumeration, which obviously has limitations in the underlying biologic information and applications to induce wide clinical adoption, newer approaches to achieve CTC capture have been developed, including microfluidic platforms such as the CTC-Chip, in which CTCs interact with EpCAM-coated micro-posts under laminar flow conditions.¹⁷ However, despite a more streamlined workflow, this positive-selection capture platform and other similar available platforms still depend on EpCAM detection, which may not best define a bona fide CTC.^{18–21}

To overcome the challenge of CTCs possessing the EMT phenotype and potentially having low to negative EpCAM expression, a number of studies have pioneered the use of EpCAM-Independent enrichment strategies. Examples include using CD45-conjugated magnetic beads to enrich CTCs through leukocyte depletion by the EasySepR bi-antibody leukocyte depletion kit (STEMCELL Technologies, Vancouver, BC, Canada) in an negative electromagnetic separation.²² Another novel platform that has emerged recently is the VTX-1 liquid biopsy system (Vortex Biosciences, Menlo Park, CA), which is capable of capturing and isolating CTCs that may remain viable and requires no preprocessing or labeling of blood samples. Sollier et al²³ reported using this system to extract and enumerate CTCs from the blood of patients with lung cancer (23–317 CTCs/7.5 mL) and breast cancer (25–51 CTCs/7.5 mL) and lung cancer.

By using these new technologies to fully interrogate CTCs molecularly or genomically, one could further unleash the power of the molecular diagnostics platform with multitudes of possible clinical applications. Fan et al²⁴ demonstrated high sensitivity and specificity in hepatocellular cancer diagnosis using CTCs and could apply them as a real-time parameter for risk prediction and treatment monitoring, enabling early decision making to tailor effective antitumor strategies. Miyamoto et al²⁵ used microfluidic cell enrichment to establish a sensitive and high-throughput strategy for analyzing prostate CTCs and reported its potential utility in guiding treatment selection in both metastatic and localized prostate cancer. Wang et al²⁶ combined a postsurgical quantity of small triploid CTCs (five or more cells per 6 mL blood) and multiploid (pentasomy of eight or greater) circulating tumor stem cells (CTSCs) or circulating tumor microembolus (CTMs; either one or more than one), which correlated with poor prognosis for patients with hepatocellular carcinoma and helped

predict recurrence. Ilié et al²⁷ collected peripheral blood samples with CTCs and circulating white blood cells from 106 patients with advanced non-small cell lung cancer (NSCLC). The authors demonstrated that PD-L1 status in CTCs and circulating white blood cells correlates with PD-L1 status in tumor tissue, revealing the potential of CTC assessment as a noninvasive real-time biopsy to evaluate PD-L1 expression for patients with advanced-stage NSCLC.²⁷

A recent prospective study to evaluate CTC profiles in NSCLC molecular subgroups enrolled 125 patients with stage NIB to IV NSCLC without treatment.²⁸ Of these patients, 51 (40.8%) were found to be total CTC-positive and 26 (20.8%) were vimentin CTC-positive at baseline. Multivariate analysis showed that patients with five or more total CTCs had reduced overall survival compared with patients with fewer than five total CTCs. The baseline presence of five or more total CTCs was validated to be correlated with a poor prognosis. CTCs from patients with EGFR-mutant NSCLC expressed EMT characteristics not seen in CTCs from patients with KRAS-mutant adenocarcinoma.

Ilié et al²⁹ adopted the CellSearch and ISET (Rarecells, Paris, France) technologies to enrich patients with advanced stage III/IV NSCLC and to interrogate MET biomarker expression. With CellSearch, CTCs were found for 83 of 256 patients (32%). With ISET, CTCs were found for 80 of 106 patients (75%). MET expression was found on ISET CTCs in 72% of cases, whereas MET expression on matched-patient tissue was positive for 65% of patients (93% concordance). Quantitative MET expression analysis using H-scores revealed a strong correlation of MET expression between tissue and CTCs (Spearman correlation, 0.93). The study results identified CTCs as a potential method for noninvasive, real-time biopsy to determine the MET status of patients entering clinical trials.²⁹

Real-time prospective CTC measurement is an attractive platform to monitor disease treatment response and progression. In a recent study, Pailler et al³⁰ collected blood samples at baseline pretreatment and 2 months after crizotinib treatment from 39 patients with *ALK*-fusion NSCLC undergoing crizotinib therapy. The results suggested that the dynamic change in CTC numbers with *ALK*-copy number gain may be a predictive biomarker for crizotinib efficacy, lending further support for serial molecular analysis of CTCs as a promising method for real-time therapy monitoring and clinical outcome prediction for these patient populations.³⁰

Soluble Compartment

ctDNA.—Both cell-free DNA (cfDNA) and ctDNA originating from normal and cancer cells can be identified and isolated in the peripheral blood.^{4–31} The major fragment size of cfDNA is approximately 170 base pairs, which corresponds well to the nucleosomal fragments resulting from the process of cellular apoptosis.³² ctDNA is a fraction of cfDNA that originates from primary, metastatic tumors in the body or from CTCs themselves. As a result, ctDNA fragments are found to contain tumor-specific somatic alterations and epigenetic aberrations in the blood. The estimated half-life of free DNA in the circulation is reported to be between 16 minutes and 2.5 hours.^{31,33} Nonmutant cfDNA molecules are longer than ctDNA in plasma.^{34,35}

The detection of ctDNA for molecular diagnostics is fundamentally challenged by the large excess of background wildtype DNA present in the circulation. Tumor-specific markers that allow the distinction of tumor-derived cfDNA as ctDNA from the wild-type DNA fraction can include mutations, indels, gene copy number changes, chromosomal rearrangements, or aberrant methylation.³⁶ Levels of wild-type DNA can be released during serum preparation; hence, plasma is preferred as the biologic material for molecular extraction and assays. Chromosomal rearrangements can be detected with high sensitivity using polymerase chain reaction (PCR) but require genome-wide sequencing of the primary tumor to find potential target aberrations.³⁷ Copy number changes detected by low-coverage genome sequencing have been applied to detect ctDNA without knowledge about the primary tumor genome, but this approach requires relatively high ctDNA concentrations for a successful detection.³⁸ Point mutations and indels (short insertions and deletions) are the most frequently used diagnostic ctDNA markers.³¹ Studies have shown reasonably good concordance of tumoral genomic alterations in ctDNA compared with that detected from tumor tissue genomic assays.³⁹ However, there are also studies that highlight the high specificity of ctDNA mutation detection but low sensitivity compared with tumor tissue mutation assays, suggesting that negative ctDNA assays might need to be confirmed with tumor tissue DNA mutation detection, as in the case of the *EGFR*-T790M assay.^{40–43}

Liquid biopsy techniques for assaying ctDNAs for genetic/genomic marker alterations have been increasingly applied in recent years to longitudinally monitor patients with cancer,^{44–48} especially those with initial actionable genomic mutations/alterations who are receiving specific cancer therapies. To detect *ALK* fusions and *ALK* mutations and track the evolution of drug resistance during treatment, Dagogo-Jack et al⁴⁵ procured blood plasma from 22 patients who had *ALK*-positive disease with acquired resistance to *ALK* tyrosine kinase inhibitors (TKIs). The correlation between plasma *ALK* mutations and response to distinct *ALK* precision inhibitors brought attention to the potential for liquid ctDNA biopsy analysis to guide *ALK* precision therapy decisions.⁴⁵ In another recent study, Guibert et al⁴⁶ collected 168 specimens from a total of 46 patients with NSCLC and adopted enhanced tagged amplicon sequencing of hotspots and coding regions from 36 genes, as well as intronic coverage for detection of *ALK/ROS1* fusions in plasma cfDNA assays to detect driver and resistance mutations, compared with plasma droplet digital PCR and tumor genotype for diagnostic accuracy. Blinded analysis in this study demonstrated the capacity of amplicon-based plasma next-generation sequencing (NGS) to detect a full range of targetable genotypes in NSCLC with high accuracy.⁴⁶ Almdovar et al⁴⁸ recently reported a longitudinal plasma cfDNA genotyping analysis of patients with SCLC, in which they aimed to gain dynamic insights into treatment efficacy and disease relapse with 140 plasma samples from 27 patients over a 26-month blood sample collection period. The assay detects single nucleotide variants, copy number variation, and indels in 14 genes that are frequently mutated in SCLC, including *TP53*, *RBI*, *BRAF*, *KIT*, *NOTCH1/2/3/4*, *PIK3CA*, *PTEN*, *FGFR1*, *MYC*, *MYCL1*, and *MYCN*. Results showed that disease-associated mutations were detected in 85% of cases, with *TP53* (70%) and *RBI* (52%) being the most common, which is consistent with previous data from tissue analysis. Hence, the study demonstrated that cfDNA genotyping analysis using targeted sequencing in SCLC is feasible, with the results matching relatively well with those obtained from tissue analysis. It also appears that

genotypic alterations detected by longitudinal cfDNA analysis might be predictive of clinical or radiographic progression. cfDNA genotyping analysis may also be useful in detecting minimal residual disease after treatments. That said, in this particular aggressive disease category of SCLC, the utility of longitudinal cfDNA analysis in clinical practice can be considered debatable. It is doubtful, at least for now, whether chemoresistance detection can meaningfully impact clinical outcomes, given the very limited salvage treatment options available, especially in extensive disease.

Exciting reports highlighting the promising potential of ctDNA profiling in liquid biopsy as a tool for early cancer detection have appeared in the last few years. Abbosh et al⁴⁹ used a tumor-specific phylogenetic approach to profile the ctDNA of the first 100 participants enrolled in the TRACERx (Tracking Non-Small-Cell Lung Cancer Evolution Through Therapy [Rx]) study. They showed that a phylogenetic ctDNA profiling method tracked the subclonal nature of lung cancer relapse and metastasis, providing a new approach for ctDNA-driven therapeutic studies.⁴⁹ Most recently, a new liquid biopsy assay platform, CancerSEEK, sought to detect eight common types of cancer (i.e., esophagus, breast, lung, stomach, liver, pancreas, colorectum, and ovary) through assessment of the levels of circulating proteins and mutations in cfDNA.^{50,51} The authors reported a positive result of about 70% (median) across the eight common cancer types among more than 1,000 patients. The specificity was greater than 99%, and only 7 of 812 healthy controls had a positive score on the CancerSEEK test. Currently, there are numerous active ongoing clinical studies investigating the utilities of CTCs and cfDNA/ctDNA in human cancers (Table 1).

Blood proteomics.—Beyond DNA in the blood compartment, soluble protein and peptides represent other biomolecular analytes that can be measured for clinical diagnostics purposes. Technologically, it is more difficult to identify and measure individual protein peptides beyond proteomics profiling for peptide signatures, compared with the genomics platforms. As a result, advances in liquid proteomics biopsies trail behind liquid genomics, especially the ctDNA biopsy for clinical/translational applications. Nonetheless, steady progress has been made in this area over the past 1 to 2 decades.

Blood-based proteomic biomarker assays have recently met with notable milestones in bedside adoption. A blood-based proteomics biomarker signature test, VeriStrat (Biodesix, Boulder, CO), was developed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MS)^{52–54} for patients with wild-type *EGFR* lung cancer to stratify them into a “good signature” group and a “poor signature” group. Patients with a good signature are predicted to potentially derive benefits from platinum-based chemotherapy, single-agent chemotherapy, or *EGFR*-TKI therapy. Conversely, patients with a poor signature are predicted to be less likely to respond to platinum-based therapy and single-agent chemotherapy and not likely to respond to *EGFR*-TKI. A phase III study to test the predictive power of the VeriStrat test in the second-line therapy of patients with advanced NSCLC was published in 2014.⁵⁴ The investigators analyzed 129 patients (91%) randomly assigned to the chemotherapy treatment group and 134 patients (94%) randomly assigned to the erlotinib treatment group. A significant interaction between treatment and proteomic classification was found. Patients with a poor VeriStrat proteomic test classification had worse survival with erlotinib than chemotherapy (HR 1.72; 95% CI, 1.08–2.74; $p = .022$).

There was no overall survival difference between treatments for patients with a good proteomic test classification. The authors concluded that VeriStrat serum protein test status is predictive of differential benefit in overall survival for erlotinib versus chemotherapy in the second-line setting. Patients classified as likely to have a poor signature had better outcomes with chemotherapy than erlotinib.

More recently, the VeriStrat serum protein test was evaluated among patients with advanced squamous cell NSCLC treated with second-line afatinib or erlotinib in the phase III LUX-Lung 8 study of 795 patients.⁵⁵ Pretreatment VeriStrat status was correlated with overall survival, progression-free survival, and other endpoints. The investigators found that a good VeriStrat classification was strongly associated with favorable outcomes with either afatinib or erlotinib compared with a poor VeriStrat classification. Multivariate analysis showed that VeriStrat was an independent predictor of overall survival for afatinib-treated patients, regardless of Eastern Cooperative Oncology Group performance status or best response to first-line chemotherapy. For patients with a good VeriStrat classification, survival outcomes were found to be better with afatinib treatment (HR 0.79; 95% CI, 0.63–0.98) than with erlotinib treatment. A recent questionnaire study reported that the serum-based proteomic test impacted treatment recommendations among physicians ordering the VeriStrat test.⁵⁶

Blood metabolomics.—Although the development of molecular cancer diagnostics has been emphasized in the fields of genomics, transcriptomics, and proteomics in the past decade, there is renewed recognition and dedicated research effort in the area of cancer metabolism and metabolomics. With advances in and wider adoption of MS-based profiling technology, blood serum or plasma-based metabolite measurements can now be readily achieved, both in targeted individual metabolite measurement and in global metabolomics profiling. A number of studies focusing on blood-based metabolite assays for lung cancer diagnostics have been published over the past decade.^{57–63} Nonetheless, the platforms studied primarily remain investigative in nature and are not yet ready for clinical applications. Prospective validation studies are mostly still lacking. Various studies have also produced differing and sometimes conflicting metabolite identities and/or quantities, probably at least partly as a result of methodologic and instrumentation differences, making it difficult to draw more sound conclusions on the platform's utility. A deeper understanding of and insight into the interrelationship between normal and cancer metabolic pathways and the correlations with metabolites produced would certainly help the interpretation of future cancer metabolites/metabolomics biomarker research.

With the advances in MS instrumentation and computational bioinformatics methods, there has been a steep increase in literature reports in recent years on the use of blood-based global metabolomics profiling in various human cancers, ranging from cancer risk evaluation,⁶⁴ diagnostics for genotype-phenotype (e.g., SRCAL-mutated breast cancer),⁶⁵ early cancer detection,^{66,67} and surveillance of cancer recurrence/progression.⁶⁸

SALIVA BIOPSY

The utility of DNA extracted from saliva samples for genome-wide molecular research platforms has been studied and found to be feasible,⁶⁹ especially compared with blood

DNA.⁷⁰ A recent pilot study showed that a salivary DNA tumor-suppressor methylation gene panel could have the potential to detect early-stage tumors for patients with HPV-negative head and neck squamous cell carcinoma. Methylation levels of patients with HPV-positive head and neck squamous cell carcinoma were deregulated by HPV infection.⁷¹

In 2014, a novel platform of electric field-induced release and measurement was developed to detect *EGFR* mutations directly in body fluids with a multiplexable electrochemical sensor. The investigators demonstrated that *EGFR* mutations could be detected in the saliva of patients with NSCLC, with an area under the curve of 0.94 for Del19 and 0.90 for L858R.⁷² In 2016, Pu et al⁷³ used electric field-induced release and measurement and detected the classic oncogenic *EGFR* mutations in the saliva plasma ctDNA samples of 17 patients with lung adenocarcinoma. They reported complete concordance of the assay results, with an area under the curve of 1.0.

CEREBROSPINAL FLUID BIOPSY

The brain and the central nervous system (CNS) represent a sanctuary where tumor cells residing there could be protected from systemic therapy due to the blood-brain barrier as a therapeutic obstacle. Similarly, ctDNA from primary CNS tumors is rarely detected in the systemic blood circulation, unlike in other visceral primary solid tumors. Hence, the cerebrospinal fluid (CSF) compartment represents a unique and potentially highly informative space for molecular diagnostics development.

In 2006, Choong et al⁷⁴ identified CTCs in the CSF of a patient with lung cancer with advanced FGFR-mutant disease and symptomatic leptomeningeal metastases that had progressed during therapy. They further demonstrated the feasibility of detecting a unique *EGFR* gene mutation (E884K) in the laser-capture microdissected CSF CTCs responsible for drug resistance against erlotinib but hypersensitization to gefitinib.⁷⁵ In addition, CSF cfDNA from patients with cancer was adopted for quantitative detection of cancer gene mutations using digital PCR and targeted amplicon sequencing methods, compared with collected plasma, for seven patients with solid brain tumors. The authors identified seven somatic mutations from the CSF of a patient with leptomeningeal disease through the use of cancer panel sequencing, which was also in concordance with genotype testing on the corresponding primary tumor biopsy.⁷⁶

More recently, Pentsova et al⁷⁷ used NGS of 341 cancer-associated genes in the CSF of 53 patients with known or suspected CNS metastatic disease from solid malignancies. They reported detection of somatic genomic alterations for 63% (20 of 32) of patients with solid tumor CNS metastases, 50% (6 of 12) of patients with primary brain tumors, and 0% (0 of 9) of patients without CNS involvement by cancer. CSF tumor-associated cfDNA was also detected from primary CNS tumors (medulloblastomas, ependymomas, and high-grade gliomas) abutting a CSF space or cortical surface.⁷⁸

A recent study showed that the sensitivity of comprehensive NGS for somatic mutation detection of the CNS ctDNA was higher than that using plasma ctDNA for patients with CNS tumors (58% vs. 0%). For patients with abundant visceral disease, the sensitivity of

CNS DNA and plasma DNA was comparable (60.5% vs. 55.5%). Moreover, the results also showed that the CSF ctDNA derived from CNS tumors was more abundant than that in the plasma. Importantly, the authors further demonstrated that CSF ctDNA levels fluctuated longitudinally in a time-dependent and in parallel fashion mirroring the changes in brain tumor burden.⁷⁹ In another recent study, LI et al⁸⁰ detected CSF-associated ctDNA using droplet digital PCR for a patient with *BRAF*-V600E-positive melanoma to monitor the response of metastatic leptomeningeal disease treated with whole brain radiotherapy and *BRAF*-specific inhibitors. Using nine CSF samples over 6 months, the mutant CSF ctDNA fractions were found to match well with the disease burden and clinical course under treatment, and they corresponded well with the treatment response. Whole-exome sequencing (WES) revealed a canonical cancer gene mutation, *PTEN-R130**, in CSF ctDNA of a patient with leptomeningeal disease both before treatment response and after disease relapse.⁸⁰ In addition, similar mutational profiles were found in the cellular and ctDNA (cfDNA), supporting the notion that the ctDNA is a derivative and representative of leptomeningeal disease cells. Hence, CSF ctDNA represents a novel molecular diagnostics platform for use as (1) a method for genomic biomarkers and actionable mutation detection, (2) a complementary tool to diagnose CNS tumors and leptomeningeal carcinomatosis, and (3) a monitoring tool for disease burden and treatment progress in CNS malignancies.

URINE BIOPSY

Urine is an attractive body fluid compartment for cfDNA molecular assays for cancer diagnostics. The urine biopsy is truly noninvasive, without even the need for a needle phlebotomy as in the case of a blood biopsy. Patients can collect their own urine samples at home or in the hospital or clinic. Furthermore, serial sample collection is not difficult or risky for patients and is usually not restricted by patient performance status or disease conditions. Attempts to achieve molecular diagnostics in urine samples for genitourinary cancers date back decades. In 1991, *p53* gene mutations were first identified in the urine sediment of patients with bladder cancer.⁸¹ Subsequently, a number of reports followed that demonstrated various gene mutations (e.g., *FGFR* mutations) identified in the urinary ctDNA extracted from patients with genitourinary cancer.^{82,83} Besides gene mutations, urine can be used as a source for epigenetic alteration assays. In 1989, a three-marker DNA hyper- and hypomethylation panel (*IRAK3*, *L1-MET*, and *SOX1*) in urine sediment was reported to accurately predict bladder cancer recurrence among 80% of patients, which compared favorably to urine cytology (35%) and cystoscopy (15%).⁸⁴ Today, it is understood that there are two different fractions of ctDNA in the urine. First, ctDNA can be derived from the urinary tract and associated endothelial cells as high molecular weight DNA. Second, ctDNA can be originated as low molecular weight DNA from ctDNA fragments that were excreted into urine during the process of renal glomerular filtration. Studies recently reported that urinary ctDNA can be detected in nonurothelial cancers as well, such as in liver cancer with *TP53* mutations⁸⁵ and in NSCLC with *EGFR* mutations.^{86,87}

One of the challenges in urinary ctDNA molecular detection and diagnostic assays is related to the fact that not only is urinary ctDNA highly fragmented but it is also present with a very low abundance (< 0.01%) among the background of wild-type cfDNA. This is similar to the technical challenges experienced in the field of plasma-based blood biopsy molecular

assays. Despite these technical difficulties, a sensitive and quantitative detection of *EGFR*, *BRAF*, and *KRAS* mutations in urine was reported to be achievable using short-footprint mutation enrichment PCR coupled with an NGS approach.^{87–89} Using this platform, reported limits of detection for *EGFR* mutations L858R, Del19, and T790M in urine or plasma were 0.006%, 0.006%, and 0.01%, respectively, which compare favorably with the U.S. Food and Drug Administration–approved Cobas *EGFR* Mutation Test (limit of detection 0.2%; Roche Molecular Diagnostics, Pleasanton, CA).^{87,90,91}

Chen et al⁸⁶ reported dynamic tracking of *EGFR* mutations among patients with NSCLC using urinary ctDNA detection in a serial monitoring trial of patients receiving precision TKIs. Their results showed a concordance in the quantity of urinary cfDNA and plasma cfDNA at baseline as well as in ctDNA decline during treatment.⁸⁶ Using the short-footprint mutation enrichment NGS assay, Reckamp et al⁸⁷ reported detection of oncogenic *EGFR* mutations and T790M resistance mutation in the urine and plasma of patients with NSCLC with comparable sensitivity. The group retrospectively analyzed samples from 63 patients with advanced NSCLC enrolled in TIGER-X, a phase I/II study of rociletinib (a third-generation EGFR-TKI) focusing on the most common, recurrent *EGFR* hotspot mutations, using the mutation enrichment PCR/NGS platform. The sensitivity of *EGFR* mutation detection in urine was up to 80% to 93% in samples that met recommended urine volumes (90–100 mL). A high specificity of 94% to 100% was attained with the *EGFR* urine testing assay, as determined using samples from healthy volunteers. Further evaluation of the concordance between urine and tissue *EGFR* T790M results in an expanded data set yielded a somewhat improved positive percentage agreement of 81% and equivalence in the rate of detection compared with plasma. The negative percentage agreement was 31%, raising the issue of discrepancies between tissue and liquid biopsy results likely as a result of tumor sampling errors and intratumoral heterogeneity. Recently, some investigators proposed a patient treatment paradigm in *EGFR*-mutant NSCLC, in which plasma genotyping serves as the reflex test upon progression on first-generation FGFR-TKIs to ameliorate the challenges associated with repeat tumor tissue biopsies. A negative ctDNA assay result would prompt tumor tissue rebiopsy and genotyping. Most recent data on the clinical relevance and validity of urine ctDNA molecular testing suggest that it could be included in the molecular diagnostics model to combine urine- and plasma-based genotyping assays to precede tumor tissue biopsies.

STOOL BIOPSY

Potential use of stool samples for molecular detection of colorectal cancers has long been sought as a means to achieve early detection of malignancies. Reports of detecting oncogenes in patient stool samples date back to 1992, when Sidransky et al⁹² identified *RAS* oncogene mutations in the stool of patients with curable-stage colorectal cancer. *KRAS* represents one of the most well-known and well-characterized oncogenes in human cancers, which can be somatically mutated within several hotspots (codons 12, 13, and 61) in a variety of cancer types. As such, much effort has centered on achieving early cancer detection by assaying for the presence of *KRAS* mutations in human tumors such as those in the colon, rectum, lung, and pancreas.⁹³ Besides stool samples, molecular diagnostics tests using mutated *KRAS* detection have been reported in pancreatic and duodenal lavage fluid,

sputum, and lavage samples from patients with colorectal and lung cancers. In addition to *KRAS*, early studies have also detected adenomatous polyposis coli mutations in stool DNA from patients with colorectal tumors, raising the promise for noninvasive early detection of the disease.⁹⁴ Both proximal and distal colorectal cancers can be well detected similarly using stool DNA molecular assays.

Although colonoscopy screening is proven to be effective in the early detection of colorectal cancer and to impact survival rates, target population compliance remains low, partly because of the invasive and unpleasant nature of the procedure. Stool DNA detection has been found to notably improve sensitivity compared with fecal occult blood tests. Compared with colonoscopy screening, stool DNA detection is quite attractive because of its noninvasive and convenient nature. In 2014, the U.S. Food and Drug Administration approved Cologuard (Exact Sciences Corp., Madison, WI), a multitarget stool DNA test for colorectal cancer screening for average-risk adults age 50 and older.⁹⁵ The interval testing frequency was determined to be every 3 years with adequate Medicare coverage. Effective July 1, 2017, United Healthcare began covering Cologuard as a colon cancer screening test. Cologuard incorporates molecular assays for aberrantly methylated *BMP3* and *NDRG4* gene promoter regions, mutant *KRAS*, and β -actin as well as an immunochemical assay for human hemoglobin. Cologuard is based on a study by Imperiale et al,⁹⁶ which demonstrated notably improved sensitivity for colorectal cancer detection compared with the fecal immunochemical test. Although Cologuard is a one-time screening stool DNA test that detects 92% of cases of colorectal cancer among asymptomatic average-risk persons compared with 74% with the fecal immunochemical test, Cologuard was able to detect less than one-half of advanced precancerous lesions (42% sensitivity) and produced a substantial number of false-positive results.

Further molecular testing methods are in development to improve the detection of epigenetic changes (e.g., DNA methylation in plasma and stool DNA samples).⁹⁷ Li et al⁹⁷ developed a methyl-BEAMing (Beads, Emulsion, Amplification, Magnetics-ing) technology to enable absolute quantification of the number of methylated molecules in a biologic sample, achieving highly sensitive enumeration of as few as 1 methylated molecule in approximately 5,000 unmethylated molecules in DNA from plasma or stool samples. Digital quantification of rare methylation events in clinical samples could have translational applications in cancer diagnostic and prognostic uses as well as in preclinical evaluation of novel epigenetic biomarkers.

EMERGING NEW PLATFORMS

Liquid Biopsy

Exosome profiling.—Peripheral blood exosome (microvesicle) profiling is an area of exciting and potentially impactful liquid biopsy research that has emerged in recent years. Exosomes were first described in 1983 by Pan and Johnstone⁹⁸ as a subclass of extracellular nonvesicles with a 40- to 150-nm diameter membrane with an endocytic origin that contain biomolecules of nucleic acids (including DNA, mRNAs, and microRNAs), proteins, and lipids and nucleic acids that are released by all cell types, including cancer cells. Exosomes are believed to be involved in intercellular communication in physiologic and malignant

conditions. Cancerous exosomes carry a cargo load of malignant bioinformation in the form of nucleic acids, proteins, and lipids that are capable of reprogramming the recipient cells. Recently, exosomes have been highlighted as putative transforming agents in malignancies, having a potentially causative role in major steps or processes of tumor progression, including immune response modulation, tumor microenvironment reprogramming, invasion, and metastasis.^{99–103}

Of interest, recent studies suggested that exosomal microRNAs may be involved in disease progression, potentially via stimulating angiogenesis and promoting metastasis.¹⁰⁴ Most recently, another new study demonstrated for the first time that before tumor cells acquire metastatic capacity, the tumors alert the host immune system continuously by generating exosomes, which carry triggers of innate immune responses such as the reported pigment epithelium-derived factor.¹⁰⁵ Another recent study¹⁰⁶ demonstrated that certain microRNAs (e.g., miR-196a and miR-1246) are specifically enriched in pancreatic cancer cell-derived exosomes and are present in the plasma exosome at elevated levels among patients with localized pancreatic cancer. Fang et al¹⁰⁷ recently reported that tumor-derived exosomal miR-1247–3p showed positive correlation with lung metastasis for patients with hepatocellular carcinoma and could convert fibroblasts to cancer-associated fibroblasts by decreasing B4GALT3, which would then activate the β 1-integrin/nuclear factor- κ B signaling pathway in the lung premetastatic niche from hepatocellular cancer. Furthermore, cancer-associated fibroblasts displayed increased secretion of interleukin-6 and interleukin-8, resulting in the promotion of cancer stemness, EMT, chemoresistance, and tumorigenicity of tumor cells. Most recently, a novel liquid biopsy platform that can achieve immense coverage of system-wide, native biomolecules was developed for plasma exome profiling for patients with cancer using a next-generation systems biology approach.¹⁰⁸ Domenyuk et al¹⁰⁸ used this novel approach (named adaptive dynamic artificial poly-ligand targeting, which embodies an enriched library of single-stranded oli-godeoxynucleotides to profile complex biologic samples) as a highly specific profiling tool to distinguish women with or without breast cancer based on circulating blood exosomes. The study achieved an area under the curve of 0.73 when healthy donors were compared with patients with biopsy-positive breast cancer.

Cancer immunotherapy.—Clinically relevant and actionable predictive biomarkers for cancer immunotherapy remain an area of huge unmet need. Since the arrival of anti-CTLA-4 and anti-PD-1/PD-L1 cancer immunotherapy and its clinical fruition with various U.S. Food and Drug Administration approvals granted for its use as standard-of-care cancer treatment in multiple cancer types, a few genomic and tissue biomarkers have been validated and approved for cancer immunotherapeutics. These include tumoral PD-L1 expression levels (as companion diagnostics for pembrolizumab and as complementary diagnostics for nivolumab and atezolizumab)^{109–110} and, more recently, microsatellite instability status (with high microsatellite instability as a tumor type agnostic biomarker for pembrolizumab).¹¹¹ Furthermore, strong evidence of tumor mutational load (or mutational burden) has been found to support its use as a predictive immuno-oncology biomarker.^{112–114} Nonetheless, alternative means of achieving immunotherapy biomarkers have been keenly sought in recent years, at least in part because of the practical issues surrounding invasive tumor tissue

biopsies, potential sampling errors in needle biopsies especially in the setting of tumor heterogeneity, and limitations in attempting repeat tissue biopsies during therapies. Again, liquid biopsy represents one of the top priorities in the quest for optimal noninvasive immuno-oncology biomarkers.¹¹⁵

Again, ctDNA in liquid biopsy serves as a source for a more comprehensive representation of the tumor genomic status in immuno-oncology therapy and thus contributes as a potential substrate for immuno-oncology treatment biomarkers. Koeppel et al¹¹⁶ reported a study in which tumor mutation load was assayed from cfDNA (i.e., ctDNA) using WES, rather than in tumor tissue DNA. They performed WES on cfDNA from 32 patients with different types of metastatic cancer in the MOSCATO 01 and/or MATCHR molecular triage trials (NCT01566019 and NCT02517892, respectively). WES mutation detection sensitivity was 92% in cfDNAs compared with targeted sequencing, whereas mutation detection sensitivity was 53% when cfDNA-WES was compared with tissue DNA-WES. For samples in which the presence of tumor DNA was confirmed in cfDNA, the tumor mutation load from the liquid biopsy was correlated with the tumor biopsy. In another study, Goldberg et al¹¹⁷ enrolled 28 patients with metastatic NSCLC undergoing Immunotherapy treatment and compared ctDNA level changes with radiographic tumor size and survival outcomes. They found that the median time to Initial response was 24.5 days by ctDNA versus 72.5 days by imaging evaluation. ctDNA response was associated with superior progressionfree survival (HR 0.29; 95% CI, 0.09–0.89; $p = .03$) and superior overall survival (HR 0.17; 95% CI, 0.05–0.62; $p = .007$). A decline in ctDNA levels is therefore considered to be an early marker of therapeutic efficacy, predicting greater prolonged survival for patients with NSCLC treated with immune checkpoint Inhibitors.

Khagl et al¹¹⁸ interrogated for hypermutated ctDNA as mutational burden to correlate with the response to checkpoint Immunotherapy In a diverse group of cancers. Significant Improvement in PFS and overall survival was found to be associated with high versus low alteration number in variants of unknown significance (VUS > 3 alterations vs. VUS ≤ 3 alterations). These results provide support for further investigations into the ctDNA genomic landscape as immunotherapy predictive biomarkers. Besides ctDNA, isolated CTCs from patients undergoing immuno-oncology therapy were also used for biomarker interrogation. Boffa et al¹¹⁹ studied the cellular expression of PD-L1 In the peripheral blood circulating cells and associated with poorer survival. In a Cox model analysis adjusting for stage, high PD-L1(+) cell burden was a significant predictor of mortality (HR 3.85; 95% CI, 1.64–9.09; $p = .002$).

Finally, novel emerging technology platforms such as serum proteomics and immune repertoire NGS also enable promising research to be conducted among patients undergoing immuno-oncology therapy for biomarker analysis.^{120,121} Although these novel assay platforms are promising, more validation studies and larger prospective analyses are needed before they could be translated to clinical use.

Breath Biopsy/Volatile Organic Compound Profiling

Exhaled human breath is known to contain at least a few thousand volatile organic compounds (VOCs) and can potentially be exploited as a VOC biomarker in breath biopsies

for human disease diagnostics, including cancers.^{122–124} In general, VOCs are organic chemicals that possess a high vapor pressure at ordinary room temperature, resulting in a low boiling point. Thus, large numbers of the molecules would evaporate from the liquid or solid phases of the compound as “volatile” substances entering into the surrounding air space. In fact, VOCs are ubiquitous, numerous, and varied, including both naturally occurring as well as human-made chemical compounds. In the human body, VOCs are thought to be mainly blood borne and thus could enable the monitoring or diagnosis of disease processes in the body beyond just the oropharyngeal cavities and lungs.¹²³ Recent advances in MS-based assay platforms have brought about more modern studies in cancer diagnostics using nonvolatile metabolomics profiling, evaluating the spectral landscapes of metabolites in patient tissues or blood as biomarkers. Beyond these nonvolatile metabolites, VOCs can now also be identified and measured in various headspace gas phases of human tissues, blood, and urine and even in exhaled breath. An emerging body of literature over the past decades has suggested that the feasibility and promise of VOC profiling or fingerprinting could be an attractive noninvasive cancer diagnostic in the future.¹²⁵

Pilot and proof-of-concept studies in the lung cancer field have increasingly been published in recent years using VOC profiling with gas chromatography/MS and various chemical sensor matrices including microfluidic chip sensors. Hence, the spectrum and concentrations of VOCs in breath samples can be captured with the sensors as correlates of the broad chemical constitution of the biospecimen without the absolute need for the identities of the specific VOC components. Cancer is now more regularly classified through the prism of the genomic lens with genomic alterations guiding precision therapeutics. This is particularly true as seen in genomic discoveries in recent years in lung adenocarcinoma. Peled et al¹²⁶ previously reported a proof-of-concept in vitro pilot study using tumor cell VOC detection with nanomaterial-based sensors to profile genetic mutations of lung cancer cells (namely, *EGFR* mutations, *KRAS* mutations, and *EML4-ALK* fusion). Breath analysis has also been adopted for the study of pulmonary nodules and for identification and characterization of lung cancer. Mazzone et al¹²⁷ reported identification of exhaled breath VOC biosignatures of lung cancer using a colorimetric VOC sensor array in a study cohort with 229 participants (92 patients with lung cancer and 137 controls). The authors suggested optimizing the breath biosignature accuracy by incorporating clinical parameters (e.g., risk factors and histology). Peled et al¹²⁸ reported the use of VOC profiling breath analysis to discriminate benign from malignant pulmonary nodules in a high-risk cohort of 19 patients with benign disease and 53 with malignant disease with similar smoking histories and comorbidities. More recently, Nardi-Agmon et al¹²⁹ further reported the use of noninvasive breath biosignatures, as determined by gas chromatography/MS and a nanomaterial-based sensor array, to correlate with and monitor treatment response in advanced lung cancer in a small study of 39 patients and 143 collected breath samples. These applications could potentially have a notable impact on clinical decision making and therapeutics determination.

Various devices and technologies designed to detect VOCs for cancer diagnostics using not only exhaled breath but also other noninvasive body substrates such as urine are currently available. In addition to the more routine application of gas chromatography/MS used in electronic nose technology (eNose, Zutphen, Netherlands) with arrays of nonselective chemical sensors, other competing technologies are emerging such as the field asymmetric

ion mobility spectrometry microchip.¹³⁰ This microchip platform is a variant of Ion mobility spectrometry,¹³⁰ a method of distinguishing charged gaseous molecules according to differences in the speed at which they move through a buffer gas under the Influence of an oscillating electric field. It is claimed to have improved advantages in overcoming sample stability and sensitivity issues and in data analysis. VOC detection at low parts per billion (and in some cases, parts per trillion) levels is feasible in the field asymmetric ion mobility spectrometry microchip platform, enabling it to be well suited not only for clinical sample assaying but also for use as a potential future point-of-care measurement in a portable and convenient fashion. Another new exciting technology in development is the Nanobeak Sensor (Nanobeak Inc., New York, NY), which converges nanoelectronics, bioinformatics, and wire-less technology to achieve a mobile point-of-care screening application based on chemical VOC sensing within a small Bluetooth device. The Nanobeak technology uses carbon nanotubes to detect VOCs with the invention originated from the National Aeronautics and Space Administration when It launched the nanosensor into orbit in 2007 to test and determine that It works well in outer space. Currently, a number of pilot clinical studies using these new breath biopsy technologies for cancer diagnostics are ongoing or are being planned (NCT02888366, NCT02781857, NCT02123030, NCT03275688, NCT01292369, NCT01386203, and NCT02612532).

CONCLUSION

In recent years, we have witnessed a revolutionary advancement in cancer medicine among the whole spectrum of cancer genomics, molecular genomics diagnostics, technological platform innovations, and cancer therapeutics. As a result, we have seen an unprecedented pace of progress in the expansion of cancer diagnostics on tumor tissues as well as beyond tissues in novel noninvasive molecular assays, in parallel with the ever-growing list of cancer therapeutics such as precision therapies and immunotherapeutic agents. Noninvasive molecular diagnostics beyond tissues have already found clinical utility and application particularly in the area of cfDNA/ctDNA genotyping/genomic profiling as a blood biopsy (liquid biopsy) technique. They have also been increasingly applied in longitudinal monitoring of therapy for early disease detection and in therapeutic response-resistance monitoring. Various other promising noninvasive cancer diagnostics assay platforms also include saliva biopsy, urine biopsy, stool biopsy, CSF biopsy, other forms of blood biopsy beyond cfDNA/ctDNA (e.g., proteomics and metabolomics), and even breath biopsy for VOC measurement. It is Important to emphasize the crucial significance of committing appropriate resources and applying rigorous study designs for validating these promising assay platforms in large-scale prospective clinical studies to bring them to fruition. Moreover, rigorous scientific scrutiny of the various assay platforms remains indispensable to discern between hype and reality when assessing each novel molecular diagnostics platform that emerges.

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PRACTICAL APPLICATIONS

- Nontissue-based next-generation novel noninvasive molecular diagnostics platforms have been emerging and expanding in clinical applications to match the revolution in cancer precision medicine and immunotherapies.
- Novel noninvasive molecular diagnostics platforms have increasingly been applied to serial longitudinal biopsies to better monitor and interrogate tumor progression and evolution under therapies.
- Next-generation novel noninvasive molecular diagnostics platforms include liquid biopsies, salivary biopsies, stool and urine biopsies, and breath biopsies.
- The emergence of the cancer immunotherapy revolution poses unprecedented promise in cancer treatments but also extraordinary challenges in the need for novel and validated cancer diagnostics and biomarkers to optimize the therapeutic impact.
- A number of novel noninvasive molecular diagnostics need large-scale prospective validation studies to ascertain their role in bedside molecular diagnostics applications.

Abbreviations: CTC, circulating tumor cell; cfDNA, cell-free DNA; VOC, volatile organic compound; CSF, cerebral spinal fluid.

FIGURE 1.
Novel Noninvasive Cancer Molecular Diagnostics Platforms Beyond Tissues

TABLE 1.

Ongoing ctDNA and CTC Clinical Trials From 2017

ClinicalTrials.gov Identifier	Cont Content	Sponsor
ctDNA		
Lung cancer		
NCT03337711	Explores intratumor heterogeneity and prognostic value of postoperative blood ctDNA of stage IIA to IMA nonsquamous NSCLC based on the capture-based sequencing and UMI technology of burning rock	Sun Yat-sen University, China
NCT03172156	ctDNA dynamic monitoring and its role of prognosis in stage I NSCLC byNGS	Sun Yat-sen University, China
NCT03090815	ctDNA as a prognostic tool for patients with advanced lung adenocarcinoma	University of Hong Kong, Hong Kong
NCT03317080	Dynamic monitoring of ctDNA of surgical patients with lung cancer	West China Hospital, China
NCT03017183	Detection of heterogeneity in central lung cancer; EBUS-TBNA and ctDNA analysis vs. endobronchial forceps biopsy	Wissenschaftliches Institut Bethanien e.V., Germany
NCT03081741	A prospective study of ctDNA detection in surveillance for patients with lung cancer	Shenzhen Gene Health Bio Tech Co. Ltd., China
NCT03235765	Compare concordance rate between tissue-based and blood-based cancer panel analysis for patients with lung cancer	Seoul National University Hospital, Korea
NCT03373955	Using NGS to construct an immune repertoire to detect variation in patients' immune state and to monitor therapeutic effects in NSCLC	Sichuan University, China
Breast cancer		
NCT03079011	Palbociclib and ctDNA for ESR1 mutation detection in metastatic breast cancer	UNICANCER, France
NCT03145961	Using blood ctDNA to detect cancer cells after standard treatment to trigger additional treatment for patients with early-stage triple-negative breast cancer	Institute of Cancer Research, United Kingdom
NCT03260192	ctDNA to monitor response to neoadjuvant chemotherapy in breast cancer	Sun Yat-Sen University, China
NCT03318263	Monitoring of ESR1, PIK3CA, and AKT ctDNA mutations during real-life follow-up of patients with metastatic breast cancer treated with aromatase inhibitors	Institut de Cancérologie de Lorraine, France
NCT03182634	Plasma ctDNA screening for advanced breast cancer with targetable mutations	Institute of Cancer Research, United Kingdom
NCT03357120	Assessing the prognostic value of ctDNA mutations for invasive breast cancer treated with neoadjuvant chemotherapy	Institut Bergonié, France
Colorectal cancer		
NCT03416478	The implication of ctDNA in the recurrence surveillance of stage II and III colorectal cancer	Sun Yat-sen University, China
NCT03312374	ctDNA as a prognostic marker for postoperative relapse in early- and intermediate-stage colorectal cancer	Sun Yat-sen University, China
Other tumors		
NCT03302325	ctDNA as a liquid biopsy for patients with stage IV solid tumors	Medical University of South Carolina, United States
NCT03115138	Evaluation of ctDNA as a theranostic marker in the management of glioblastomas	Centre Hospitalier Universitaire, Amiens, France

ClinicalTrials.gov Identifier	ContContent	Sponsor
NCT03214991	Usefulness of ctDNA as a prognostic factor in pancreatic cancer	Ji Kon Ryu, Korea
CTC		
Prostate cancer		
NCT03050866	Phase II multicenter study determining the response to cabazitaxel for patients with metastatic prostate cancer with AR-V7+ CTCs	Erasmus Medical Center, Netherlands
NCT03089099	Development of a prognostic model for patients with metastatic castration-resistant prostate cancer by sequentially analyzing the expression of molecular markers in CTCs	Fudan University, China
NCT03327662	Utilizing CTC counts to optimize systemic therapy of metastatic prostate cancer	Institute of Cancer Research, United Kingdom
Breast cancer		
NCT03070002	Phase II study to evaluate denosumab in ER- and/or PR-positive, HER2-negative metastatic breast cancer with bone metastases and detectable CTCs	Northwestern University, United States
NCT03213041	Phase II study to evaluate the impact on PFS with combination carboplatin/pembrolizumab for patients with CTC-positive, HER2-negative metastatic breast cancer previously treated with anthracyclines and taxanes	Northwestern University, United States
Pancreatic cancer		
NCT03032913	Diagnostic accuracy of CTCs and onco-exosome quantification in the diagnosis of pancreatic cancer	University Hospital, Bordeaux, France
NCT03033927	CTC and tumor tissue models for predicting effective pancreatic cancer response	Memorial Sloan Kettering Cancer Center, United States
Other tumors		
NCT03295591	CTC enumeration and clinical factors in metastatic colorectal cancer for liver resection	University of Hong Kong, Hong Kong
NCT03162198	Frequency of CTCs and amount of cfDNA for patients with cirrhosis with hepatocellular carcinoma	Institute of Liver and Biliary Sciences, India

Abbreviations: CTC, circulating tumor cell; NSCLC, non-small cell lung cancer; UM I, unique molecular identifier; NGS, next-generation sequencing; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; ER, estrogen receptor; PR, progesterone receptor; PFS, progres si on-free survival.