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REVIEW ARTICLE

Commercial ctDNA Assays for Minimal Residual Disease Detection of Solid Tumors

Kevin Chen[1] · Misty D. Shields[2,3] · Pradeep S. Chauhan[1] · Ricardo J. Ramirez[4,5] · Peter K. Harris[1] · Melissa A. Reimers[5,6] · Jose P. Zevallos[4,5] · Andrew A. Davis[5,6] · Bruna Pellini[2,3] · Aadel A. Chaudhuri[1,5,7,8,9,10]

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Abstract

The detection of circulating tumor DNA via liquid biopsy has become an important diagnostic test for patients with cancer. While certain commercial liquid biopsy platforms designed to detect circulating tumor DNA have been approved to guide clinical decisions in advanced solid tumors, the clinical utility of these assays for detecting minimal residual disease after curative-intent treatment of nonmetastatic disease is currently limited. Predicting disease response and relapse has considerable potential for increasing the effective implementation of neoadjuvant and adjuvant therapies. As a result, many companies are rapidly investing in the development of liquid biopsy platforms to detect circulating tumor DNA in the minimal residual disease setting. In this review, we discuss the development and clinical implementation of commercial liquid biopsy platforms for circulating tumor DNA minimal residual disease detection of solid tumors. Here, we aim to highlight the technological features that enable highly sensitive detection of tumor-derived genomic alterations, the factors that differentiate these commercial platforms, and the ongoing trials that seek to increase clinical implementation of liquid biopsies using circulating tumor DNA-based minimal residual disease detection.

Kevin Chen and Misty D. Shields contributed equally to this work.

✉ Andrew A. Davis
aadavis@wustl.edu

✉ Bruna Pellini
bruna.pellini@moffitt.org

✉ Aadel A. Chaudhuri
aadel@wustl.edu

1 Division of Cancer Biology, Department of Radiation Oncology, Washington University School of Medicine, 4511 Forest Park Avenue, St. Louis, MO 63108, USA

2 Department of Oncologic Sciences, Morsani College of Medicine, University of South Florida, Tampa, FL, USA

3 Department of Thoracic Oncology, Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA

4 Department of Otolaryngology-Head and Neck Surgery, Washington University School of Medicine, St. Louis, MO, USA

5 Siteman Cancer Center, Barnes Jewish Hospital and Washington University School of Medicine, St. Louis, MO, USA

6 Division of Medical Oncology, Department of Medicine, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA

7 Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA

8 Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, MO, USA

9 Department of Biomedical Engineering, Washington University School of Medicine, St. Louis, MO, USA

10 Department of Computer Science and Engineering, Washington University in St. Louis, St. Louis, MO, USA

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Key Points

The landscape of commercial liquid biopsy technologies for circulating tumor DNA detection of minimal residual disease is rapidly evolving.

We broadly review the commercial assays available for clinical or research use, focusing on their technological features, input requirements, performance measures, clinical trial data demonstrating proof of concept, and ongoing clinical trials that could lead to more personalized circulating tumor DNA minimal residual disease-based management in the future.

1 Introduction

Liquid biopsies have emerged as noninvasive diagnostic tools for the interrogation of tumor-derived genomic alterations and dynamic tumor assessment, while also offering the potential to detect micrometastatic disease prior to radiographic evidence of recurrence [1, 2]. Technological advances in the detection of circulating tumor DNA (ctDNA), the tumor-derived component of cell-free DNA (cfDNA), have led to a recent surge in the research and clinical applications of ctDNA assays for a variety of solid tumor types. Research applications include early cancer detection, molecular profiling, minimal residual disease (MRD) detection, and surveillance of post-treatment tumor burden [3, 4]. Currently, clinical applications are primarily limited to molecular profiling of patients with advanced-stage disease to identify mutations for targeted therapies [5].

The first liquid biopsies approved by the US Food and Drug Administration (FDA) were single-locus polymerase chain reaction (PCR) assays that queried plasma cfDNA for specific actionable mutations in patients with advanced-stage cancer. The Roche cobas® Mutation Test v2, approved as a companion diagnostic in June 2016 [6], enabled clinicians to screen patients with metastatic non-small cell lung cancer (NSCLC) for actionable or resistance *EGFR* mutations to guide treatment decisions regarding the use of EGFR tyrosine kinase inhibitors [7]. Similarly, the Qiagen therascreen® PIK3CA RGQ PCR kit was approved in May 2019 as a companion diagnostic for patients with advanced-stage, HR-positive/HER2-negative breast cancer to identify patients with *PIK3CA* mutations for treatment with the PIK3CA alpha-isoform inhibitor alpelisib in combination with fulvestrant [8, 9]. These PCR-based single-locus assays have a rapid turnaround time, allowing clinicians to make therapeutic decisions in a timely manner. PCR-based single-locus assays, however, are restricted to the interrogation of a limited number of genomic alterations and typically have a limit of detection above 0.5% variant allele fraction (VAF), which significantly restricts the ability to detect ctDNA in the MRD setting [10]. As MRD is characterized by a small volume of tumor cells that remain after curative-intent treatment without an obvious macroscopic correlate on radiographic imaging, detecting ctDNA MRD requires a significantly higher level of sensitivity to detect low VAFs [11, 12]. Moreover, ctDNA MRD assays commonly track multiple genomic alterations in parallel to minimize the probability of sampling error and increase the likelihood of detecting ctDNA in plasma [10, 13].

Targeted next-generation sequencing (NGS) has paved the way for highly sensitive MRD detection by using gene panels to query multiple loci in parallel across a genomic scale [14–17]. Compared to early cancer detection, the sensitivity of MRD detection can be enhanced by prior knowledge of a tumor mutational profile (i.e., tumor-informed), thus enabling the sequencing of patient-specific mutations expected to be present as opposed to variant-calling de novo with a preselected panel of hotspot or actionable mutations (i.e., tumor-naïve). The enhanced sensitivity of tumor-informed sequencing is the product of reduced background noise from non-tumor-derived mutations, thus allowing for a lower limit of detection via higher sequencing depths [10]. Conversely, tumor-informed approaches for MRD detection require available tumor tissue to genotype, a prolonged turnaround time due to assay personalization, restricted sequencing to known variants originating from the primary tumor sample, and more resources for personalized assay development than tumor-naïve assays, which generally only require plasma [18].

Regardless of the approach, ctDNA enrichment strategies using either PCR amplicons (e.g., tagged amplicon deep sequencing or TAM-Seq) or hybrid capture with molecular barcoding (e.g., Cancer Personalized Profiling by deep Sequencing or CAPP-Seq) allow both tumor-informed and tumor-naïve assays to maintain high specificity while querying an array of genomic targets. PCR-based assays have limited capacity for multiplex sequencing in a single reaction pool, typically restricting genomic breadth to 2000 base pairs per reaction [10, 12, 15]. Therefore, assays utilizing multiplexed PCR generally require multiple separate reaction pools running in parallel to achieve adequate multi-mutation assessment, which is challenging when cfDNA input is limited. In contrast, hybrid capture-based assays enable sequencing across thousands of base pairs in a single reaction but may be associated with a higher rate of false-positive findings given the increased opportunity for technical noise across a larger genomic space and longer sequencing library preparation times compared with PCR-based methods [10, 16]. Both of these sequencing methods

may be complemented by fragment size selection, which has been shown to enrich for ctDNA when selecting for fragment sizes between 90 and 150 base pairs owing to the shorter length of ctDNA relative to normal cfDNA [19, 20]. Using molecular barcodes and bioinformatic approaches, artifactual mutations derived from PCR and sequencing errors are reduced, allowing for high sequencing depth and low limits of detection for detecting molecular alterations, such as single nucleotide variants (SNVs) and insertions/deletions (indels) [17, 21]. Moreover, sequencing of paired peripheral blood mononuclear cells to a similar depth as cfDNA enables the filtration of false-positive variants that arise from clonal hematopoiesis [22]. While hybrid capture-based cfDNA technologies, such as Guardant360® CDx and FoundationOne® Liquid CDx, were approved in 2020 by the FDA as companion diagnostics for querying the mutational status of multiple genes with targetable drugs for patients with advanced-stage cancer [23, 24], there have been no liquid biopsy assays to date receiving full approval by the FDA for MRD detection of solid tumors [5].

A different approach to ctDNA MRD detection entails whole genome sequencing (WGS) or whole exome sequencing (WES) [13], which strongly prioritizes the genomic breadth of sequencing over depth and is better equipped to detect genome-wide copy number alterations (CNAs) compared with targeted NGS [25, 26]. Targeted NGS and broad WGS can also be integrated via a combinatorial approach to interrogate a more heterogeneous spectrum of molecular alterations [27–30]. Genome-wide CNA-based approaches have demonstrated promise for early treatment response assessment in the advanced setting [31], and more recently were applied to distinguish malignancy from pre-malignancy in the setting of NF1 cancer predisposition syndrome [32].

With each strategy offering distinct advantages, many companies are now adopting these technologies to design cfDNA assays for ctDNA MRD detection. Several observational studies have correlated ctDNA MRD detection with worse prognosis in patients with different solid tumor malignancies, including cancers of the lung, breast, colon, pancreas, stomach, esophagus, head and neck, and bladder [33–40]. Furthermore, several prospective randomized trials are utilizing ctDNA MRD detection to guide intervention. In this review article, we provide a comprehensive overview of commercial platforms used to detect ctDNA MRD. We introduce the stages of development that are required for integrating a commercial assay into clinical practice. Then, we present several commercial platforms for ctDNA MRD detection that are available for either clinical use (Table 1) or research use (Table 2), focusing on technological differences and clinical trials that demonstrate proof of concept data based on information searchable through PubMed, Google Scholar, and conference proceedings. We conclude by discussing the importance of MRD detection from a clinical perspective and future directions in the field that will enhance the implementation of ctDNA liquid biopsies moving forward.

Table 1 ctDNA MRD platforms available for clinical practice

Platform	Technology	Variants queried	Samples required	Tissue dependence	Reported LOD	Turnaround time	Tumor types
Signatera™ (Natera)	Multiplex PCR-based NGS	SNVs, indels	Baseline: tumor tissue; whole blood (6 mL) MRD/monitoring: plasma (from 20 mL blood)	Tumor-informed	0.01% VAF	3 weeks for tumor sequencing and personalized PCR primer design 1–2 weeks after plasma sample is received	Multi-cancer
Guardant Reveal™ (Guardant Health)	Hybrid capture-based NGS	SNVs, indels, methylation	MRD/monitoring: plasma (from 20 mL blood)	Tumor-naïve	0.01% VAF	1 week from sample receipt	CRC
NavDx™ (Naveris)	Digital droplet PCR	Queries HPV16, 18, 31, 33, 35	MRD/monitoring: plasma (from 10 mL blood)	Tumor-naïve	10 viral copies/mL	1 week from sample receipt	HPV+ OPSCC

A comparison of technological features and performance specifications between Signatera™ (Natera), Guardant Reveal™ (Guardant Health), and NavDx™ (Naveris), which are commercially available platforms with clinical application for ctDNA MRD detection.

CRC colorectal cancer, *ctDNA* circulating tumor DNA, *HPV* human papilloma virus, *indel* insertion or deletion, *LOD* limit of detection, *MRD* minimal residual disease, *NGS* next-generation sequencing, *OPSCC* oropharyngeal squamous cell carcinoma, *PCR* polymerase chain reaction, *SNV* single nucleotide variant, *VAF* variant allele fraction

Table 2 ctDNA MRD platforms currently for research use only

Platform	Technology	Variants queried	Samples required	Tissue dependence	Reported LOD
AVENIO (Roche)	Hybrid capture-based NGS	SNVs, indels, CNAs, fusions	MRD/monitoring: plasma (4 mL)	Tumor-naïve	0.1% VAF
PCM™ (ArcherDX)	Multiplex PCR-based NGS	SNVs, indels, CNAs	Baseline: tumor tissue MRD/monitoring: plasma	Tumor-informed	0.003% VAF
RaDaR™ (Inivata)	Multiplex PCR-based NGS	SNVs, indels, CNAs	Baseline: tumor tissue MRD/monitoring: plasma (from 20 mL blood)	Tumor-informed	0.001% VAF
PredicineALERT™ (Predicine)	Hybrid capture-based NGS	SNVs, indels, CNAs, fusions	Baseline: tumor tissue or plasma (from 10 mL blood) MRD/monitoring: plasma	Tumor-informed or tumor-naïve	0.005% VAF
MRDetect (C2i Genomics)	WGS	SNVs, CNAs	Baseline: tumor tissue MRD/monitoring: plasma (1 mL)	Tumor-informed	0.001% TF
PhasED-Seq (Foresight Diagnostics)	Hybrid capture-based NGS	PVs, SNVs	Baseline: tumor or plasma MRD/monitoring: plasma	Tumor-informed	< 0.0001% TF

A comparison of technological features and performance specifications among ctDNA MRD platforms that are available for translational research and clinical trial use only: AVENIO (Roche), PCM™ (ArcherDX), RaDaR™ (Inivata), PredicineALERT™ (Predicine), MRDetect (C2i Genomics), and PhasED-Seq (Foresight Diagnostics).

CNA copy number alteration, *ctDNA* circulating tumor DNA, *indel* insertion or deletion, *LOD* limit of detection, *MRD* minimal residual disease, *NGS* next-generation sequencing, *PCR* polymerase chain reaction, *PV* phased variant, *SNV* single nucleotide variant, *TF* tumor fraction, *VAF* variant allele fraction, *WGS* whole genome sequencing

2 From Research and Development to Clinical Practice

Validation is critical to assay development [5, 41, 42]. The Evaluation of Genomic Applications in Practice and Prevention initiative establishes three evidence-based components required for validation: analytical validity, clinical validity, and clinical utility [43]. Analytical validity is an assay's ability to accurately and reliably measure the analyte or genotype of interest (e.g., ctDNA), as determined by sensitivity, specificity, reproducibility, and robustness [44]. This is generally assessed by in vitro experiments that apply the assay to known varying concentrations of fragmented tumor genomic DNA diluted into mixtures of normal cfDNA (i.e., synthetic DNA spike-in), which allow for the developers to determine the exact concentration at which an assay reaches its limit of detection [45]. Clinical validity reflects the strength of the correlation with clinically meaningful events (e.g., disease recurrence), as determined by sensitivity, specificity, positive predictive value, and negative predictive value [46]. Lastly, clinical utility is demonstrated by an assay's ability to inform treatment and impact long-term patient outcomes (e.g., adjuvant therapy guided by ctDNA MRD improves survival), as determined by prospective randomized trials [47]. For example, IMvigor011 is a prospective randomized trial utilizing major commercial technology that will compare outcomes between ctDNA MRD-positive patients randomly assigned to a control arm or treatment arm following radical cystectomy for high-risk bladder cancer [48]. This type of study design is representative of other trials that are aiming to demonstrate the clinical utility of ctDNA MRD detection using commercial assays (Fig. 1).

The subsequent stages of assay development involve the acquisition of regulatory clearance and implementation into routine clinical practice [5]. The success of these stages largely depends on how well analytical and clinical validity are demonstrated for a specific indication. An important topic of debate in the field, however, is the extent to which clinical utility needs to be demonstrated before regulatory approval. While an increase in overall survival (OS) is certainly the gold standard, these clinical trials can take considerable time to complete and are difficult to control given that patients often undergo multiple lines of therapy, thus confounding the results [49, 50]. Therefore, clinical trials that demonstrate an assay's ability to improve disease-free survival (DFS) or other surrogate endpoints may be considered a sufficient benchmark for clinical implementation and potential regulatory approval, although this area remains actively debated and may depend on the degree of therapeutic benefit

observed in the adjuvant setting. For example, the ADAURA trial met its primary endpoint of improved DFS in a study of patients with stage IB–IIIA resected NSCLC harboring an *EGFR* mutation (exon 19 deletion and/or L858R) who were randomized to either osimertinib or placebo, which led to the unblinding of patients in the trial even though this compromised the analysis of OS [51, 52].

As there is no universal standard for MRD detection, clinicians must also carefully consider several other variables that could affect which platform is most appropriate to utilize. One such variable is cost, which is based on the extent of insurance or government payer reimbursement [5]. Commercial assays that are granted breakthrough device designations for intended use, and are subsequently approved by the FDA, may receive an accelerated path to reimbursement by a policy recently proposed by the Centers for Medicare and Medicaid Services [53]. Another consideration is assay turnaround time, as results should be returned within a reasonable time frame to avoid potentially adverse delays in adjuvant treatment after MRD detection [18]. Given these nuances, it is important not only to review the data validating assay performance but also to consider these logistical factors that may impact implementation into clinical practice [5]. Nonetheless, the demonstration of clinical utility remains the most important factor that impacts the application of these technologies to clinical practice.

3 Commercial Platforms Available for Clinical Use

3.1 Signatera™ (Natera, Inc., San Carlos, CA, USA)

Signatera™ is a tumor-informed ctDNA MRD assay that utilizes patient-specific tumor mutations to perform multiplex PCR amplification and subsequent NGS of cfDNA from plasma [54]. The top 16 somatic variants (SNVs or indels) from WES of primary tumor tissue are first selected using paired whole blood sequencing to filter out germline variants and clonal hematopoiesis. Somatic variants are prioritized based on clonality, detectability, and frequency of mutation. Primers are designed against each of the top 16 tumor variants, followed by 16-plex PCR of plasma cfDNA isolated from subsequent blood samples collected after definitive treatment in the MRD setting. This approach enables ultra-deep sequencing of each target to an average depth of 100,000× and reduces background noise from non-tumor variants. Signatera™ has been shown to detect at least two tumor variants per sample among the 16 queried in the panel, with over 98% analytical sensitivity at ctDNA concentrations of 0.01%–0.02% [54, 55]. This limit of detection of 0.01% VAF translates to the detection of two mutant haploid genomes among a background of 20,000 normal haploid genomes.

Despite the increased specificity associated with targeted interrogation of patient-specific mutations, variant detection is limited to those that originate in the primary tumor sample. For this reason, tumor-informed assays can miss clonal variants that are unaccounted for in the primer pool and emerge in the MRD setting, which could include important mutations that confer treatment resistance [34, 38, 56]. As a potential solution, Natera is developing an expanded WES platform that queries approximately 20,000 genes in plasma cfDNA, which may enable better detection of *de novo* resistance mutations [57]. Another limitation of Signatera™ is that the dependence on tumor sequencing could theoretically be a limitation if it prolongs the overall turnaround time and delays adjuvant treatment decision making [58].

While not yet approved by the FDA, Signatera™ has been granted a total of three breakthrough device

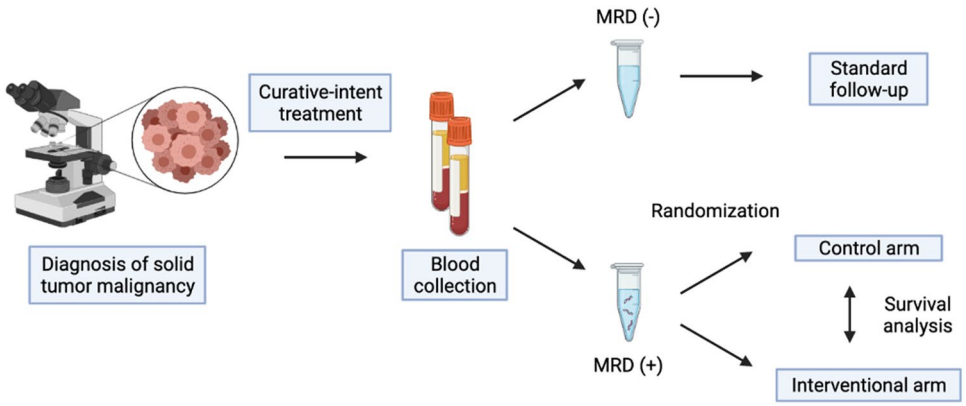

Fig. 1 Prospective randomized trial design to demonstrate clinical utility of circulating tumor DNA (ctDNA) minimal residual disease (MRD) detection. The IMvigor011 trial utilizes Signatera™ to detect ctDNA, and then randomizes patients with positive MRD to either a placebo arm or interventional arm with adjuvant atezolizumab. This study design is representative of clinical trials that aim to demonstrate the clinical utility of ctDNA MRD detection.

designations, one in May 2019 and two in March 2021, to accelerate phase III development and approval as a companion diagnostic for three different clinical indications [59, 60]. In September 2020, Medicare finalized a plan to approve full coverage for the use of Signatera™ for patients with stage II and III colorectal cancer (CRC) to inform adjuvant treatment after surgery and monitor recurrence [61]. The turnaround time after receiving the tumor tissue specimen is around two weeks for tumor sequencing results, followed by an additional week for designing patient-specific PCR probes. Once the patient-specific assay is designed, subsequent Signatera™ results based on post-treatment blood samples are available one to two weeks after a physician orders the test.

Signatera™ has shown evidence of clinical validity in prospective multicenter cohort studies for multiple solid tumor types, including CRC, NSCLC, breast cancer, and bladder cancer. In a study of 130 patients with stage I–III CRC treated with surgery and adjuvant chemotherapy, a positive ctDNA test during longitudinal analysis (i.e., at least one ctDNA positive among plasma samples collected every three months for up to three years) with Signatera™ after definitive treatment predicted 14 of 16 clinical relapses (87.5%) and corresponded to more than a 40-fold increase in the likelihood of disease recurrence compared to a negative ctDNA test (hazard ratio [HR] = 43.5; 95% confidence interval [CI]: 9.8–193.5) [62]. Serial ctDNA analyses also detected disease recurrence at a median lead time of 10.1 months (range: 0.8–16.5 months) before radiologic detection.

For patients with stage I–III NSCLC treated with surgery, the Signatera™ assay was applied to pre-surgical and post-surgical plasma samples from 24 patients enrolled in the TRACERx study (NCT01888601), which is a multicenter prospective trial with longitudinal sampling that aims to study the evolutionary phylogenetic landscape and dynamics of intratumor heterogeneity in > 800 patients at the time of diagnosis, therapeutic intervention, and disease relapse [63–65]. Among 14 patients who ultimately relapsed, 13 of them had positive ctDNA detection before or at the time of clinical relapse (93% sensitivity). Furthermore, relapse was detected by ctDNA prior to radiographic evidence by a median lead time of 2.3 months (range: 0.3–11.4 months). Among ten patients without clinical evidence of relapse, ctDNA detection was positive in just one patient (90% specificity). Interestingly, this patient with a supposed false-positive result received both adjuvant chemotherapy and radiation therapy, which likely ablated their ctDNA MRD. In support of this hypothesis, ctDNA findings were negative at each of three serial timepoints following adjuvant therapy completion. The results for using ctDNA to predict adjuvant treatment response were also promising. One patient with high ctDNA levels after surgery and before adjuvant chemoradiotherapy was found to have undetectable ctDNA after the completion of adjuvant therapy and ultimately never recurred. Conversely, ctDNA also potentially reflects chemotherapy resistance, as three patients who had positive ctDNA detection after surgery and then showed increased ctDNA levels after adjuvant chemotherapy ultimately recurred within a year of surgery. Altogether, these results demonstrate the potential of using Signatera™ to monitor treatment response versus resistance in patients with localized NSCLC.

Promising results were also observed using the Signatera™ assay in a study of 49 high-risk patients with stage I–III breast cancer recruited within three years after treatment with surgery and adjuvant chemotherapy. Serial plasma analysis with Signatera™ predicted 16 of 18 relapses (89%) with no false positives and demonstrated a median lead time of 8.9 months (range: 0.5–24.0 months) compared to clinically detected relapse [66]. A separate study of ctDNA measured using Signatera™ in 84 high-risk patients with stage II–III breast cancer in the multicenter I-SPY 2 trial (NCT01042379) detected ctDNA pretreatment in 73% of patients. After completing neoadjuvant chemotherapy, 17 of the 60 ctDNA pretreatment-positive patients who achieved a pathologic complete response (pCR) were concordantly ctDNA MRD-negative [67]. Among the 43 patients who did not achieve a pCR after neoadjuvant chemotherapy, 14% were concordantly ctDNA MRD-positive and experienced significantly worse risk of metastatic recurrence (HR = 10.4; 95% CI: 2.3–46.6). Interestingly, the remaining 86% of patients who did not achieve a pCR were found to be ctDNA MRD-negative after neoadjuvant chemotherapy and had excellent outcomes comparable to those who achieved a pCR (HR = 1.4; 95% CI: 0.2–13.5). These results suggest that the lack of ctDNA clearance from pre-treatment to post-chemotherapy is associated with poor treatment response and ultimately a higher rate of metastatic recurrence, while ctDNA clearance, even among those who fail to achieve a pCR, may predict improved survival. The wide CI reinforces the importance of validating whether ctDNA MRD may be a better marker than pCR for predicting disease recurrence.

Signatera™ has also been applied successfully in patients with muscle-invasive bladder cancer (MIBC). Among 68 patients with MIBC treated with neoadjuvant chemotherapy and surgery, post-operative surveillance with Signatera™ identified cases of metastatic relapse with 100% sensitivity, 98% specificity, and a median lead time of 3.2 months (range: 2.7–8.0 months) [56]. Plasma ctDNA detected during disease surveillance after cystectomy was highly prognostic, with ctDNA status being the strongest predictor of DFS after cystectomy in a multivariate analysis (HR = 129.6; $p < 0.001$) and associated with significantly worse OS ($p < 0.001$). Circulating tumor DNA detection after neoadjuvant chemotherapy, but before cystectomy and

at the time of diagnosis prior to neoadjuvant chemotherapy, was also significantly associated with worse DFS and inferior OS ($p < 0.001$).

Neoadjuvant chemotherapy followed by cystectomy has been a standard curative-intent treatment for MIBC for decades. However, despite this aggressive multidisciplinary therapeutic approach, many MIBC patients develop disease recurrence. To assess whether adjuvant atezolizumab would reduce the risk of recurrence in patients undergoing curative-intent treatment, the phase III, open-label IMvigor010 trial (NCT02450331) randomized 809 patients with high-risk muscle-invasive urothelial carcinoma who had undergone either radical cystectomy or nephroureterectomy 1:1 to receive either adjuvant atezolizumab or observation [68]. Plasma for ctDNA was prospectively collected on the IMvigor010 trial.

Among unselected patients, the addition of atezolizumab did not confer a significant improvement in DFS (HR = 0.89; 95% CI: 0.74–1.08) or OS (HR = 0.85; 95% CI: 0.66–1.09). To assess whether adjuvant atezolizumab would benefit selected patients who were positive for ctDNA MRD at cycle 1 day 1 (C1D1), and to test if ctDNA MRD in plasma at C1D1 and cycle 3 day 1 (C3D1) was associated with inferior DFS, plasma samples from 581 patients enrolled onto IMvigor010 were analyzed using the Signatera™ assay [39]. At C1D1 (median of 11 weeks after surgery), 37% (214/581) of patients had positive ctDNA MRD in blood plasma, which conferred an increased risk of disease recurrence compared to those who were ctDNA MRD-negative (HR = 6.3; 95% CI: 4.45–8.92; $p < 0.0001$ for patients in the observation arm). However, patients with positive ctDNA MRD who received adjuvant atezolizumab had improved DFS (HR = 0.58; 95% CI: 0.43–0.79; $p = 0.0024$; median DFS: 5.9 vs 4.4 months) and OS (HR = 0.59; 95% CI: 0.41–0.86; median OS: 25.8 vs 15.8 months) compared with patients with positive ctDNA MRD in the observation arm. In contrast, there was no clinical benefit observed with atezolizumab therapy over observation in patients who had negative ctDNA MRD at C1D1. Also at C3D1, 38% (186/485) of patients were positive for ctDNA MRD and were at higher risk for disease recurrence than patients who were negative for ctDNA MRD (HR = 8.65; 95% CI: 5.67–13.18; $p < 0.0001$ for patients in the observation arm). Circulating tumor DNA dynamics were additionally assessed in this study. The authors observed that patients in the atezolizumab arm whose ctDNA was detectable at C1D1 but became undetectable at C3D1 (18 of 99; 18%) had superior DFS when compared with their counterparts whose ctDNA remained detectable at C3D1 (HR = 0.26; 95% CI: 0.12–0.56; $p = 0.0014$; median DFS: not reached vs 5.7 months). These findings suggest that postoperative ctDNA MRD detection and clearance can inform the administration of adjuvant immune checkpoint inhibitors in patients with MIBC after surgery.

A broader application of Signatera™ will likely be driven by an increase in large prospective studies demonstrating its clinical utility, which would encourage regulatory approval and expanded payer support. One such trial in progress is the IMvigor011 study (NCT04660344), a phase III, randomized, double-blind, placebo-controlled trial initiated in May 2021 in which 495 patients with MIBC who are ctDNA positive within 20 weeks after undergoing radical cystectomy will be randomized to either adjuvant atezolizumab or placebo for one year. The primary outcome measure is DFS, with OS as a secondary outcome [48]. This prospective study was developed based on the findings from the IMvigor010 study, which showed the potential of ctDNA MRD-based personalization of adjuvant therapy via a randomized open-label trial. IMvigor011 aims to validate these findings, which could provide strong evidence for approving ctDNA MRD detection via Signatera™ to guide adjuvant treatment with atezolizumab for patients with high-risk bladder cancer after a radical cystectomy in the future [68].

The DARE study (NCT04567420) is a phase II, randomized, multicenter trial that is enrolling about 100 patients with stage II–III, HR-positive/HER2-negative breast cancer who are currently receiving adjuvant endocrine therapy and test positive with the Signatera™ assay [69]. These ctDNA-positive patients will then be randomized to either continue standard of care endocrine therapy or start treatment with the CDK4/6 inhibitor palbociclib in combination with fulvestrant for two years. Palbociclib, as well as other CDK4/6 inhibitors, are not yet approved in the adjuvant setting. The PALLAS trial (NCT02513394) showed that the addition of adjuvant palbociclib to endocrine therapy did not improve DFS compared to endocrine therapy alone [70, 71]. However, the MonarchE trial (NCT03155997), which randomized patients to either abemaciclib with endocrine therapy or endocrine therapy alone, has yielded early data showing significant differences in DFS between these two study arms [72, 73]. A hypothesis that warrants prospective validation is to assess whether adjuvant treatment with a CDK4/6 inhibitor may only benefit a subset of patients with HR-positive/HER2-negative breast cancer who are ctDNA MRD-positive [74]. For this reason, the DARE trial aims to determine if ctDNA-guided adjuvant treatment with a CDK4/6 inhibitor leads to significant improvement in DFS, using Signatera™ to identify those who are ctDNA MRD-positive. Similarly, the LEADER study (NCT03285412) is a phase II randomized trial of ribociclib for the treatment of patients with ER-positive early-stage breast cancer who are ctDNA MRD-positive based on the Signatera™ assay [75].

3.2 Guardant Reveal™ (Guardant Health, Inc., Redwood City, CA, USA)

Guardant Reveal™ is a ctDNA MRD assay that does not require personalized tumor sequencing and could help clinicians identify patients at high risk for recurrence after curative-intent resection of CRC, potentially informing the need for adjuvant chemotherapy [76, 77]. Using plasma from peripheral blood, Guardant Reveal™ applies a fixed 500-kilobase gene panel to perform hybrid capture-based NGS. The assay utilizes proprietary bioinformatics software to simultaneously query methylation and genomic alterations, while aiming to filter out biological noise from clonal hematopoiesis without requiring the sequencing of paired peripheral blood mononuclear cells [78, 79]. By integrating genomic and epigenomic signatures, Guardant Reveal™ was able to detect ctDNA in patients with early-stage CRC with 91% sensitivity down to a limit of detection of 0.01% VAF and a turnaround time of approximately seven days [76, 80]. Circulating tumor DNA MRD testing with Guardant Reveal™ can be performed serially at multiple intervals, including both four to six weeks and nine to eleven weeks after surgery, to improve sensitivity and inform clinicians of high-risk patients with detectable MRD for adjuvant chemotherapy. Circulating tumor DNA monitoring can be performed again at 16–18 weeks after surgery to identify patients who may have persistent disease despite adjuvant treatment [81].

With more than 150,000 patients diagnosed each year with CRC in the USA [82], the majority of CRC is diagnosed early (stages I–III), in part because of the implementation of preventative strategies, such as screening colonoscopies and stool DNA testing. After surgical resection with curative intent plus adjuvant chemotherapy for stage IIB CRC and higher, standard of care surveillance includes examination, carcinoembryonic antigen protein blood test monitoring, interval computed tomography scans, and surveillance colonoscopies. Despite these interventions, CRC recurrence rates remain high at 30–40%, with 80% of recurrences occurring early within the first two years after resection [83, 84]. Adjuvant chemotherapy provides a significant survival benefit in high-risk colon cancer [85], thus reinforcing the appeal of using ctDNA MRD detection to select patients who may benefit most from an escalation of care. The Guardant Reveal™ assay has not yet been cleared by the FDA but is commercially available for clinical use in CRC [86].

Guardant Reveal™ showed promising performance when used to detect ctDNA MRD in a prospective observational study in patients with stages I–IV CRC receiving curative-intent therapy [77]. One hundred and three patients were enrolled in this study, and 70 patients met criteria for the final analysis, which required available plasma after completion of definitive therapy (one month after surgery or adjuvant therapy) with sufficient cfDNA quantity and quality. Seventeen patients had detectable ctDNA post-definitive therapy, 15 of whom relapsed, while 12 of 53 ctDNA MRD-negative patients also developed recurrence (sensitivity = 55.6%, specificity = 95.4%). Restricting analysis to patients with over a year of follow-up (n = 64), 15 patients had detectable ctDNA MRD with all recurring, while 12 of 49 patients with undetectable ctDNA MRD also relapsed (sensitivity = 55.6%, specificity = 100%). Finally, when incorporating all available surveillance blood draws in these patients with over a year of follow-up, assay sensitivity for recurrence increased further to 69% with 20 patients harboring detectable post-treatment ctDNA among 29 relapses, while maintaining 100% specificity. These findings demonstrate that post-treatment ctDNA analysis using Guardant's tumor-naïve technology can detect residual disease in patients with CRC.

The GEMCAD 1402 study (NCT02340949), a phase II, multicenter trial from 20 Spanish hospitals, randomized 180 patients with high-risk locally advanced rectal cancer to a modified schedule of total neoadjuvant treatment (TNT) with 5-fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) ± aflibercept, followed by chemoradiation and surgery [87, 88]. Blood samples were collected to investigate ctDNA value to predict tumor response, recurrence, and survival in locally advanced rectal cancer treated with TNT. Utilizing the Guardant Reveal™ assay (formerly called LUNAR-1), baseline and pre-surgery samples were analyzed from 72 patients [89]. At baseline, 83% of patients had detectable ctDNA vs 15% following TNT (pre-surgery). No association was identified between ctDNA status (detectable vs undetectable) and pathological response. However, detectable ctDNA pre-surgery was significantly associated with systemic recurrence (HR = 4; 95% CI: 1.0–16.2) and inferior OS (HR = 23; 95% CI: 2.4–212). This study highlights the prognostic potential of ctDNA detected following TNT in patients with locally advanced rectal cancer.

The PEGASUS study (NCT04259944) is an ongoing phase II, multicenter, prospective trial that is assessing the feasibility of using ctDNA liquid biopsy to guide the post-surgical management of 140 patients with microsatellite-stable high-risk stage II (T4N0) and stage III CRC, utilizing the Guardant Reveal™ platform for ctDNA detection [90, 91]. In this study, patients are randomized by ctDNA MRD status two to four weeks post-surgery to guide adjuvant treatment with either capecitabine or capecitabine/oxaliplatin (CAPOX). After adjuvant treatment, MRD status is reassessed among all patients to guide subsequent

treatment. Patients who are ctDNA MRD-positive upon the second round of testing receive an escalated treatment for six months (or until radiographic progression or toxicity) with either 5-fluorouracil plus leucovorin plus irinotecan (FOLFIRI) or CAPOX. Efficacy analyses from PEGASUS will be compared with the TOSCA trial (NCT00646607) for all known prognostic phenotypes [92], and the study complements AlfaOmega (NCT04120935), a master observational protocol that collects biospecimens including plasma from patients with CRC, and follows them clinically for at least five years or until death [93].

3.3 NavDx™ (Naveris, Inc., Natick, MA, USA)

NavDx™ is a commercial assay for monitoring HPV+ oropharyngeal squamous cell carcinoma. It was developed using digital droplet PCR to quantify circulating human papillomavirus DNA (ctHPVDNA) in blood plasma [94]. Longitudinal plasma samples can be monitored during radiotherapy and post-treatment surveillance to profile ctHPVDNA clearance using PCR primers against amplicons within the *E6* and *E7* genes of HPV 16 and *E7* genes in HPV 18, 31, 33, and 35 [94, 95].

NavDx™ was validated in a longitudinal trial that enrolled 115 patients with nonmetastatic HPV+ oropharyngeal squamous cell carcinoma undergoing curative-intent chemoradiotherapy [92]. In this study, blood was collected at baseline, once a week during therapy, and at each post-treatment follow-up visit. With a median follow-up time of 23 months, 87 patients had undetectable ctHPVDNA at all post-treatment timepoints, with a negative predictive value of 100% for disease recurrence. Of the 28 patients who were ctHPVDNA positive at any post-treatment timepoint, 16 patients had two consecutively positive ctHPVDNA tests, among whom 15 were diagnosed with biopsy-proven disease recurrence, suggesting potential utility in the post-treatment surveillance setting.

In another NavDx™ study, 46 patients undergoing transoral surgery had samples collected after surgery but prior to initiating adjuvant treatment [96]. Circulating human papillomavirus DNA was detectable in 64% (7/11) of patients that recurred compared to 29% (10/34) without recurrence, although this difference was not significant ($p = 0.1$). However, detectable ctHPVDNA was significantly associated with inferior 2-year progression-free survival (45 vs 84%; $p = 0.04$) and OS (80 vs 100%; $p = 0.02$). Thus, NavDx™ applied post-treatment has the potential to inform prognosis in patients with HPV+ oropharyngeal squamous cell carcinoma.

4 Commercial Platforms for Research Use Only

4.1 AVENIO (Roche Diagnostics, Indianapolis, IN, USA)

The AVENIO ctDNA analysis kits represent a portfolio of three hybrid capture-based NGS assays (Targeted Kit, Expanded Kit, and Surveillance Kit) for use in solid tumor research. The Surveillance Kit is designed to longitudinally monitor tumor burden in patients with either NSCLC or CRC after surgical resection. Using a tumor-naïve approach applied to cfDNA from four mL of plasma, the Surveillance Kit profiles 197 genes that represent recurrently mutated regions in NSCLC and CRC, achieving > 99% specificity and > 99% PPV for all classes of mutations (e.g., SNVs, indels, fusions, and CNAs) with a limit of detection of 0.1% VAF. The platform is under active clinical investigation, including in a phase II trial (NCT04585477) assessing whether there is a change in ctDNA levels after two cycles of adjuvant durvalumab in patients with stage I–III NSCLC with positive ctDNA MRD following curative-intent treatment [97]. The primary outcome is to detect a decrease in ctDNA MRD levels, specifically a ≥ 3-fold drop after two cycles of durvalumab treatment. The AVENIO ctDNA Surveillance Kit will be used for ctDNA analysis to assess MRD in this study.

The AVENIO Surveillance Kit was also recently utilized to determine whether ctDNA in plasma or urine can detect MRD in patients with oligometastatic CRC [98]. Plasma and urine samples were prospectively collected from 24 patients with oligometastatic CRC immediately prior to curative-intent surgery and generally following neoadjuvant chemotherapy. Using tumor-naïve plasma ctDNA analysis based on the AVENIO assay, MRD was detected with 95% sensitivity and 100% specificity. Among the 71% of patients who received neoadjuvant chemotherapy, sensitivity of MRD detection was 94%. The specificity of MRD detection remained high at 100% with a urine-based ctDNA analysis, but the sensitivity decreased to 64% because of ctDNA levels in urine being ~ 11-fold lower than in plasma. A further ctDNA-informed oncogenomic analysis showed that 81% of ctDNA MRD-positive patients may have benefitted from adjuvant therapy comprising either targeted systemic therapy or immune checkpoint inhibitors. More study is warranted to validate these findings and determine whether they can guide oligometastatic CRC treatment decision making in the future.

Finally, the AVENIO assay will be used in a phase III clinical trial (NCT04585490) to personalize the escalation of consolidation therapy for patients with unresectable stage III NSCLC with detectable ctDNA MRD after

curative-intent chemoradiation treatment. The main purpose of this study is to test whether detectable ctDNA levels in blood can be decreased by combining the standard treatment (durvalumab) with additional chemotherapy [99]. This prospective study could help validate the compelling findings published by Moding et al. that ctDNA MRD for locally advanced NSCLC following chemoradiation is potentially predictive for guiding consolidation systemic therapy [100].

4.2 PCM™ (ArcherDX, Inc., Boulder, CO, USA)

The Personalized Cancer Monitoring (PCM™) technology, developed by ArcherDX, applies the company's proprietary and minimally invasive anchored-multiplex PCR technology to detect ctDNA MRD in patients with early-stage cancer via a tumor-informed approach. The development of ArcherDX's PCM™ platform is supported by the TRACERx study [63], and the assay received breakthrough device designation from the FDA in January 2020 [101]. In a collaboration with AstraZeneca, ArcherDX is utilizing PCM™ to perform WES of resected tumor samples from NSCLC and generate patient-specific ctDNA assays, which then serve as companion diagnostics for AstraZeneca's associated therapies in the MERMAID-1 study (NCT04385368). This study is a phase III, randomized, multicenter, double-blind, placebo-controlled study designed to investigate the efficacy of adjuvant immunotherapy with durvalumab compared to placebo following standard of care platinum-based chemotherapy in 332 patients with stage II–III NSCLC who are ctDNA MRD-positive after surgical management [102]. Similarly, the MERMAID-II study (NCT04642469) is a phase III, randomized, double-blind, placebo-controlled trial that aims to evaluate the efficacy and safety of adjuvant durvalumab compared to placebo in patients with stage II–III NSCLC following curative-intent therapy (i.e., complete surgical resection with or without chemotherapy) who have no evidence of radiographic disease recurrence but become ctDNA positive during a 96-week surveillance period [103]. These studies will hopefully provide clinicians with additional tools to identify MRD and selectively intensify treatment for ctDNA MRD-positive patients with locally advanced NSCLC [104]. They may also help guide clinicians to avoid prescribing additional treatments to patients who are ctDNA MRD-negative following surgery and adjuvant chemotherapy.

Within the TRACERx study described previously, Abbosh et al. have also assessed the role of using PCM™ to monitor tumor-informed personalized ctDNA as an adjuvant biomarker following surgical tumor resection to predict disease relapse by MRD detection [105]. In 78 patients with stage I–III NSCLC, patient-specific anchored-multiplex PCR enrichment panels were generated, and 608 plasma samples were analyzed. Circulating tumor DNA enrichment panels targeted a median of 196 (range: 72–482) clonal and subclonal variants detected in the primary tumor tissue by multi-region exome sequencing. Background sequencing error was estimated and accounted for in instances of low VAFs to maximize ctDNA detection. The 50-variant anchored-multiplex PCR-MRD assay demonstrated 89% sensitivity at 0.008% VAF (with 25 ng DNA input) with 100% experimental specificity and 99.9% specificity in silico (95% CI: 99.67–99.99). Circulating tumor DNA MRD was detected in 37 of 45 patients (82%) who developed disease relapse with a median time from ctDNA detection to clinical relapse of 151 days (range: 0–984 days) and time to relapse from surgery of 413 days (range: 41–1242 days). These investigations are ongoing, and final study results are not yet available.

4.3 RaDaR™ (Inivata, Research Triangle Park, NC, USA and Cambridge, UK; Liquid Biopsy Division of NeoGenomics, Inc.)

RaDaR™ (Residual Disease and Recurrence) is a tumor-informed, multiplex PCR-based NGS assay built on Inivata's InVision® platform technology. RaDaR™ is designed to track 48 patient-specific variants for detecting MRD in multiple tumor types, either following curative-intent treatment or for early detection of relapse. RaDaR™ received breakthrough device designation by the FDA in March 2021 [106]. Inivata reports that RaDaR™ is sensitive to 10 parts per million with a VAF detection limit of 0.001% and a turnaround time of approximately seven days from collection to reporting [107].

Inivata presented two studies at the 2021 American Association for Cancer Research Annual Meeting, including data from breast cancer and head and neck squamous cell carcinoma [108, 109]. In a retrospective, multicenter, proof of principle study, 22 patients with nonmetastatic breast cancer were followed after curative-intent surgical resection [108]. Cell-free DNA was extracted from 147 plasma samples after tumor resection and sequenced with RaDaR™ assays (with a median of 41 patient-specific variants per panel) to determine ctDNA and its association with relapse. RaDaR™ identified post-surgical ctDNA in all patients with relapsed disease ($n = 17$; VAF range: 0.0007%–1.3%). Additionally, five of the relapsed cases detected by the RaDaR™ assay were not detected by single-gene digital droplet PCR. The detection of ctDNA after surgery was associated with a significantly higher rate of relapse (HR = 6.9; 95% CI: 2.5–19.2) with a median lead time from ctDNA detection to clinical relapse of 8.8 months. The five patients who did not relapse had undetectable ctDNA. These findings suggest that detection

of post-surgical ctDNA by the RaDaR^{TM} assay is associated with a high risk of relapse following nonmetastatic breast cancer treatment.

RaDaR™ is also being used in the LIONESS study (Liquid BIOpsy for MiNimal RESidual DiSease Detection). This study is a prospective, single-center, evidence-generating cohort study that investigated ctDNA detection in 11 patients with p16-negative head and neck squamous cell carcinoma after curative-intent surgical resection [109]. Baseline ctDNA levels in plasma samples taken prior to surgery ranged from 0.014% to 0.97% estimated VAF. In post-surgical samples, ctDNA could be detected at levels as low as 0.0006% VAF. Longitudinal monitoring of serial plasma samples showed that of the four patients who relapsed, ctDNA was detected ahead of clinical progression, with a lead time of 108 to 248 days. While the results of this small study are preliminary, they suggest that ctDNA can be used as a biomarker for monitoring HPV-negative head and neck squamous cell carcinoma after surgery.

4.4 PredicineALERT™ (Predicine, Inc., Hayward, CA, USA)

PredicineALERT™ is a platform for ctDNA MRD detection that uses available tissue or a biofluid sample (i.e., plasma) to establish a baseline molecular profile [110, 111]. PredicineWES is a 20,000-gene panel assay that sequences coding regions to a depth of coverage of 2500× and has a limit of detection of 1% VAF. PredicineATLAS is a 600-gene hybrid capture-based NGS assay with a depth of coverage of 20,000× and a limit of detection of 0.25% VAF. With a seven to ten day turnaround time after baseline profiling with PredicineWES and PredicineATLAS, PredicineALERT™ enables personalized baseline-informed ctDNA monitoring to VAF levels as low as 0.005%.

The platform's ability to detect genomic alterations is comparable to that of PredicineCARE, which is an assay developed to detect prognostic CNAs in the *PTEN-PI3K-AKT* and *AR* pathways using plasma cfDNA from patients with metastatic castration-resistant prostate cancer [111, 112]. The company has presented data to track ctDNA in the plasma of patients with metastatic castration-resistant prostate cancer based on 32 mutations identified from baseline profiling [111]. The assay was then applied to detect sheared tumor DNA diluted in normal plasma cfDNA to concentrations of 0.1% down to 0.005%. The assay reached its limit of detection at 0.005% VAF with an average of 3.75 mutations detected with 100% sensitivity, thus supporting its analytical validity. The company also reports that the assay can be used without a baseline sample (i.e., baseline-agnostic) and achieves a limit of detection of 0.025% VAF. As the assay is currently still in development, further trials exploring its clinical validity and utility are underway.

4.5 MRDetect (C2i Genomics, New York, NY, USA)

MRDetect is a tumor-informed, WGS-based cfDNA assay for MRD detection developed by C2i Genomics. The company asserts that limited DNA input, particularly in the MRD setting, still remains a major barrier to deep targeted sequencing [113]. They demonstrate that even with a sequencing depth of ~ 40,000× and bioinformatic error suppression, mutational detection was mostly limited to 0.1% VAF. This limit of detection appeared to be dependent on the number of cfDNA molecules available, which generally decreases with cancer stage [114], and a positive correlation was observed between the number of mutations detected and the number of unique cfDNA molecules sequenced. Therefore, C2i Genomics claims that overcoming this fundamental constraint imposed by limited cfDNA input in the setting of low disease burden requires a strategy that prioritizes increased sequencing breadth as opposed to depth. To implement this strategy, MRDetect applies WGS to tumor tissue and germline DNA from matched peripheral blood mononuclear cells to inform each personalized ctDNA assay. Then, each identified tumor somatic SNV is queried in cfDNA to calculate a cumulative genome-wide tumor signal from a sample of plasma. Similarly, CNAs are informed from tumor sequencing and queried in plasma cfDNA, which is combined with the SNV data to generate a single statistical detection score. In doing so, MRDetect capitalizes on signals from thousands of somatic mutations to enable a limit of detection less than one per total haploid genome equivalents. This is conceptually similar to mathematical modeling results using the binomial probability distribution shown by the developers of CAPP-Seq to demonstrate the ability of multi-mutational tracking to achieve ultra-low ctDNA limits of detection [16, 17].

By integrating signal from genome-wide mutations and subsequently using a machine-learning based filtering strategy to reduce noise, MRDetect was shown to achieve highly sensitive ctDNA detection with tumor fractions as low as 0.001%, with a genome-wide sequencing depth of 35× and requiring only two to three mL of whole blood (~ 1 mL of plasma) [113]. This was validated using a simulation of ctDNA detection with 700 in silico admixtures of varying tumor fractions, with a range from 0.001% to 20%, by combining tumor and matched germline WGS data from eight patients with different types of cancers (e.g., lung cancer, breast cancer, melanoma, and osteosarcoma). The combination of SNV-based and CNA-based detection using MRDetect was also applied to post-operative plasma collected from separate cohorts of 19 patients with CRC and 22 patients with lung adenocarcinoma. In patients with CRC,

positive ctDNA MRD detection ($n = 7$) was associated with a significantly shorter DFS ($p = 0.03$) compared with negative ctDNA MRD detection ($n = 12$). Similarly, in patients with lung adenocarcinoma, positive ctDNA MRD detection ($n = 10$) was associated with a significantly shorter DFS ($p = 0.009$) compared to negative ctDNA MRD detection ($n = 12$). However, hazard ratios were not reported for these survival analyses, which makes the exact effect sizes unclear.

A limitation inherent to low sequencing depth is a reduced level of confidence in the sensitivity of interrogating any individual locus, such as an actionable gene mutation [113]. However, genome-wide coverage of tumor sequencing strengthens the assay's ability to detect clonal variants that may have been missed via a targeted panel because of subsampling. Furthermore, integrating SNVs and CNAs across a genome-wide scale may increase the sensitivity of detecting ctDNA MRD in tumor types regardless of whether they are driven primarily by either a high mutational load or by aneuploidy [115, 116]. MRDetect offers a unique approach to ctDNA MRD detection, and further studies utilizing this technology in large prospective cohorts are anticipated.

4.6 PhasED-Seq (Foresight Diagnostics Inc., Aurora, CO, USA)

PhasED-Seq (Phased Variant Enrichment and Detection Sequencing) is a tumor-informed, hybrid capture-based sequencing approach that utilizes the detection of phased variants (PVs), which are defined as two or more mutations that occur within 150 base pair regions on the same DNA strand, to achieve highly sensitive ctDNA detection. This PV-based technique is an alternative to SNV-based duplex sequencing via CAPP-Seq, which relies on the detection of complementary somatic variants on both parent DNA strands to reduce background noise. However, duplex sequencing is limited by the rate of recovery of DNA duplexes, which is often a small fraction of total ctDNA input and thus makes it suboptimal in the MRD setting. By focusing on the detection of mutations on the same DNA molecule (in *cis*) rather than on duplex strands (in *trans*), PhasED-Seq enables a higher efficiency of genome recovery, which is a key factor that impacts the sensitivity of detecting lower burdens of disease.

The viability of PhasED-Seq as an assay for detecting PVs was first demonstrated in a study that utilized WGS data from 2538 tumors across 24 cancer types to identify genomic regions that recurrently harbored PVs [117]. These were found to be more enriched in B-cell lymphomas (e.g., diffuse large B-cell lymphoma or DLBCL; follicular lymphoma) compared with other cancer types, and associated with known mutational signatures (e.g., AID hypermutation in B-cell lymphomas; APOBEC3B in multiple solid cancer types). Interestingly, PVs detected in lymphoid neoplasms occurred in stereotyped regions corresponding to known hypermutated regions, such as *BCL2* in FL and *MYC* in Burkitt lymphoma, while PVs were present throughout the genome in other solid tumors.

To validate this approach for ctDNA detection, the authors applied PhasED-Seq to both lymphomas and solid tumors. A targeted sequencing panel was designed for lymphoma based on recurrent PVs identified within aggregated WGS data from patients with DLBCL. This PhasED-Seq panel was applied to 16 pretreatment tumor or plasma cfDNA samples from patients with DLBCL. Compared to a previously established panel designed to detect SNVs in B-cell lymphoma using duplex sequencing, the PhasED-Seq panel detected a significantly greater number of SNVs (median: 304.5 vs 114) and PVs (median: 2461 vs 423) per patient [118]. The analytical sensitivity of PhasED-Seq was determined by a technical experiment using ctDNA from patients with lymphoma diluted into healthy control cfDNA to simulate expected tumor fractions with a range from 0.1% to 0.00005%. PhasED-Seq significantly outperformed SNV-based duplex sequencing, with a limit of detection below one part per million. Moreover, PhasED-Seq applied to 12 unrelated control cfDNA samples revealed a significantly lower rate of background signal compared with duplex sequencing, thus further improving the limit of ctDNA detection.

When PhasED-Seq was applied to the detection of ctDNA in 88 patients with DLBCL after two cycles of standard immunochemotherapy, it detected ctDNA in an additional 25% of samples that were determined to be negative by SNV-based sequencing (i.e., potential false negatives), with ctDNA negativity defined as a 2.5-log reduction in ctDNA (i.e., major molecular response or MMR) and shown to be prognostic after two cycles [119]. At the end of therapy, PhasED-Seq was again compared to SNV-based sequencing in 19 patients with DLBCL. Among five patients who ultimately recurred after therapy, only two had detectable ctDNA by SNV-based sequencing, while all five had detectable ctDNA by PhasED-Seq. Therefore, PhasED-Seq demonstrates a strong proof of concept for sensitive ctDNA MRD detection in DLBCL.

In extending the application of PhasED-Seq to solid tumors, which in general do not harbor PVs concentrated in stereotyped genomic regions, targeted sequencing requires a tumor-informed approach using WGS to identify a personalized set of PVs. The authors assessed the utility of this approach by designing personalized PV panels via WGS for six patients with solid tumors (five with lung cancer and one with breast cancer). PhasED-Seq detected ctDNA in 15 out of 24 total plasma samples collected from these six patients, compared with nine detected by SNV-based sequencing. These six additional samples detected by PhasED-Seq harbored tumor fractions as low as 0.000094% (i.e., less than

one part per million). Serial samples from a patient with stage III lung adenocarcinoma treated with chemoradiation and consolidation immunotherapy were also analyzed. Single nucleotide variant-based sequencing failed to detect ctDNA MRD in three samples collected during treatment before ctDNA re-emerged at the time of disease recurrence, but PhasED-Seq captured ctDNA MRD in all three of these samples with a tumor fraction as low as 0.00016%. These results suggest that PhasED-Seq can be applied to solid tumors via a personalized approach for sensitive detection of low disease burden in the MRD setting.

5 Future Directions

In general, the benefit of adjuvant systemic therapy in unselected solid tumor patient populations is modest; therefore, many patients may receive toxic treatments with little therapeutic benefit. However, current limitations in detecting micrometastatic disease on conventional imaging can result in delayed receipt of effective adjuvant treatment, potentially leading to ultimately incurable metastatic disease. Therefore, the rapid development of commercial liquid biopsy platforms for ctDNA MRD detection offers the potential for enhancing the precision of oncologic treatments in the adjuvant setting. Furthermore, it may increase the cost effectiveness of adjuvant therapy by allowing clinicians to avoid overtreatment and reserve additional therapy for those with the highest risk of recurrence [18].

Moving forward, there are efforts by commercial and academic partners to optimize the standardization of pre-analytical variables (e.g., sample collection, storage, and processing) to maintain a high level of consistency among assay results. Basic standardization among commercial assays has included the requirement of plasma as opposed to serum, as the process of separating cellular material from whole blood to isolate plasma reduces the amount of cellular DNA contamination that interferes with cfDNA analysis. In addition, Streck tubes are becoming standard for collecting plasma, as these have been shown to increase cfDNA stability compared with EDTA tubes for longer storage and shipping periods [120, 121]. Patient-related factors are also associated with non-malignant conditions that could impact assay performance and must be further explored in prospective studies to enhance the interpretation of ctDNA MRD results [5]. Initiatives to define pre-analytical standards (e.g., NCI Biospecimen Evidence-Based Practices or BEBP; Blood Profiling Atlas in Cancer or BloodPAC), NGS standards (e.g., the number of genome equivalents to analyze), and post-analytical ctDNA interpretation (e.g., Friends of Cancer Research ctMoniTR project) are important steps for understanding how to best optimize these assays for clinical use [122–124].

The trials reviewed here aim to advance ctDNA-guided adjuvant therapy selection in the MRD setting. Moving forward, robust evidence of improved long-term clinical outcomes with this approach is still needed. The IMvigor010 trial is an example of a study that has successfully demonstrated improved DFS and OS among ctDNA MRD-positive patients who were treated with adjuvant atezolizumab, while no difference in these outcomes was observed between treatment arms among ctDNA MRD-negative patients. Predictively improving OS is the most compelling evidence for demonstrating clinical utility of a ctDNA MRD assay. However, assessing OS in a powered manner may be impractical for several tumor types from a study size and follow-up time standpoint. Therefore, the incorporation of ctDNA MRD detection into clinical practice may depend on trials that demonstrate reliable surrogate outcomes, such as DFS and pCR to neoadjuvant therapy. Other measures, such as treatment toxicity and patient quality of life, will also be important to assess in these trials.

Commercial ctDNA MRD assays are on the cutting edge of precision oncology, and the incorporation of ctDNA MRD detection into treatment paradigms continues to evolve rapidly across the solid tumor spectrum. The liquid biopsy technologies discussed in this review highlight the current landscape of advancements and progress being made in the field of oncology, with the potential for dramatically improved treatment personalization and patient survival in the future via ctDNA MRD detection.

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Declarations

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Conflict of interest KC, PSC, and RJR have patent filings related to cancer biomarkers. JPZ has patent filings related to cancer biomarkers, research support from Naveris, served as medical director to Summit Biolabs, and has ownership interests in Droplet Biosciences. BP has research support from BMS; speaker honoraria from BioAscend, OncLive, and MJH Life Sciences; and has served as an advisor/consultant to AstraZeneca, Guardant Health, and Guidepoint. AAC has patent filings related to cancer biomarkers, and has served as a consultant/advisor to Roche, Tempus, Geneoscopy, Daiichi Sankyo, AstraZeneca, NuProbe, Fenix Group International, and Guidepoint; AAC has stock options in Geneoscopy, research support from Roche, and ownership interests in Droplet Biosciences. MDS, PKH, MAR, and AAD declare

that they have no conflicts of interest that might be relevant to the contents of this review.

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Author contributions KC, MDS, AAD, BP, and AAC conceived of the study. KC, MDS, PSC, RJR, PKH, AAD, BP, and AAC performed the literature search, data collection, data curation, data interpretation, and writing of the manuscript. All authors edited the manuscript and commented on the manuscript at all stages.

References

1. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet. 2019;20(2):71–88.
2. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008;14(9):985–90.
3. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17(4):223–38.
4. Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. N Engl J Med. 2018;379(18):1754–65.
5. Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic: implementation issues and future challenges. Nat Rev Clin Oncol. 2021;18(5):297–312.
6. US Food and Drug Administration. cobas EGFR Mutation Test v2. 2016. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/cobas-egfr-mutation-test-v2. Accessed 1 May 2021.
7. Kwapisz D. The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? Ann Transl Med. 2017;5(3):46.
8. US Food and Drug Administration. The therascreen PIK3CA RGQ PCR Kit: P190001 and P190004. 2019. Available from: https://www.fda.gov/medical-devices/recently-approved-devices/therascreen-pik3ca-rgq-pcr-kit-p190001-and-p190004. Accessed 1 May 2021.
9. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019;380(20):1929–40.
10. Dudley JC, Diehn M. Detection and diagnostic utilization of cellular and cell-free tumor DNA. Ann Rev Pathol. 2021;16(1):199–222.
11. Abbosh C, Birkbak NJ, Swanton C. Early stage NSCLC: challenges to implementing ctDNA-based screening and MRD detection. Nat Rev Clin Oncol. 2018;15(9):577–86.
12. Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci. 2011;108(23):9530–5.
13. Chin R-I, Chen K, Usmani A, Chua C, Harris PK, Binkley MS, et al. Detection of solid tumor molecular residual disease (MRD) using circulating tumor DNA (ctDNA). Mol Diagn Ther. 2019;23(3):311–31.
14. Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DWY, Kaper F, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med. 2012;4(136):136ra68.
15. Gale D, Lawson ARJ, Howarth K, Madi M, Durham B, Smalley S, et al. Development of a highly sensitive liquid biopsy platform to detect clinically-relevant cancer mutations at low allele fractions in cell-free DNA. PLoS ONE. 2018;13(3):e0194630.
16. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014;20(5):548–54.
17. Newman AM, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, Scherer F, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. Nat Biotechnol. 2016;34(5):547–55.
18. Ijzerman MJ, De Boer J, Azad A, Degeling K, Geoghegan J, Hewitt C, et al. Towards routine implementation of liquid biopsies in cancer management: it is always too early, until suddenly it is too late. Diagnostics. 2021;11(1):103.
19. Chabon JJ, Hamilton EG, Kurtz DM, Esfahani MS, Moding EJ, Stehr H, et al. Integrating genomic features for non-invasive early lung cancer detection. Nature. 2020;580(7802):245–51.
20. Mouliere F, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Transl Med. 2018;10(466):eaat4921.
21. Schmitt MW, Fox EJ, Prindle MJ, Reid-Bayliss KS, True LD, Radich JP, et al. Sequencing small genomic targets with high efficiency and extreme accuracy. Nat Methods. 2015;12(5):423–5.
22. Abbosh C, Swanton C, Birkbak NJ. Clonal haematopoiesis: a source of biological noise in cell-free DNA analyses. Ann Oncol. 2019;30(3):358–9.
23. US Food and Drug Administration. Guardant360 CDx: P200010. 2020. Available from: https://www.fda.gov/medical-devices/recently-approved-devices/guardant360-cdx-p200010. Accessed 1 May 2021.
24. US Food and Drug Administration. FDA approves liquid biopsy NGS companion diagnostic test for multiple cancers and biomarkers. 2020. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-liquid-biopsy-ngs-companion-diagnostic-test-multiple-cancers-and-biomarkers. Accessed 1 May 2021.
25. Heitzer E, Ulz P, Belic J, Gutschi S, Quehenberger F, Fischereder K, et al. Tumor-associated copy number changes in the circulation of patients with prostate cancer identified through whole-genome sequencing. Genome Med. 2013;5(4):30.
26. Adalsteinsson VA, Ha G, Freeman SS, Choudhury AD, Stover DG, Parsons HA, et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. Nat Commun. 2017;8(1):1324.
27. Przybyl J, Chabon JJ, Spans L, Ganjoo KN, Vennam S, Newman AM, et al. Combination approach for detecting different types of alterations in circulating tumor DNA in leiomyosarcoma. Clin Cancer Res. 2018;24(11):2688–99.
28. Springer SU, Chen C-H, Rodriguez Pena MDC, Li L, Douville C, Wang Y, et al. Non-invasive detection of urothelial cancer through the analysis of driver gene mutations and aneuploidy. Elife. 2018;7:e32143.
29. O'Leary B, Cutts RJ, Liu Y, Hrebien S, Huang X, Fenwick K, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov. 2018;8(11):1390–403.

30. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018;359(6378):926–30.
31. Davis AA, Iams WT, Chan D, Oh MS, Lentz RW, Peterman N, et al. Early assessment of molecular progression and response by whole-genome circulating tumor DNA in advanced solid tumors. Mol Cancer Ther. 2020;19(7):1486–96.
32. Szymanski JJ, Sundby RT, Jones PA, Srihari D, Earland N, Harris PK, et al. Cell-free DNA ultra-low-pass whole genome sequencing to distinguish malignant peripheral nerve sheath tumor (MPNST) from its benign precursor lesion: a cross-sectional study. PLoS Med. 2021;18(8):e1003734.
33. Chaudhuri AA, Chabon JJ, Lovejoy AF, Newman AM, Stehr H, Azad TD, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov. 2017;7(12):1394–403.
34. Garcia-Murillas I, Schiavon G, Weigelt B, Ng C, Hrebien S, Cutts RJ, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med. 2015;7(302):302ra133.
35. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, 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.
36. Sausen M, Phallen J, Adleff V, Jones S, Leary RJ, Barrett MT, et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. Nat Commun. 2015;6(1):7686.
37. Leal A, Van Grieken NCT, Palsgrove DN, Phallen J, Medina JE, Hruban C, et al. White blood cell and cell-free DNA analyses for detection of residual disease in gastric cancer. Nat Commun. 2020;11(1):525.
38. Azad TD, Chaudhuri AA, Fang P, Qiao Y, Esfahani MS, Chabon JJ, et al. Circulating tumor DNA analysis for detection of minimal residual disease after chemoradiotherapy for localized esophageal cancer. Gastroenterology. 2020;158(3):494-505.e6.
39. Powles T, Assaf ZJ, Davarpanah N, Banchereau R, Szabados BE, Yuen KC, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. Nature. 2021;595(7867):432–7.
40. Chauhan PS, Chen K, Babbra RK, Feng W, Pejovic N, Nallicheri A, et al. Urine tumor DNA detection of minimal residual disease in muscle-invasive bladder cancer treated with curative-intent radical cystectomy: a cohort study. PLoS Med. 2021;18(8):e1003732.
41. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. J Clin Oncol. 2018;36(16):1631–41.
42. Coakley M, Garcia-Murillas I, Turner NC. Molecular residual disease and adjuvant trial design in solid tumors. Clin Cancer Res. 2019;25(20):6026–34.
43. Teutsch SM, Bradley LA, Palomaki GE, Haddow JE, Piper M, Calonge N, et al. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative: methods of the EGAPP Working Group. Genet Med. 2009;11(1):3–14.
44. Clinical and Laboratory Standards Institute. A framework for using CLSI documents to evaluate clinical laboratory measurement procedures, 2nd edn. Report EP19. Wayne: CLSI; 2015.
45. Deveson IW, Gong B, Lai K, LoCoco JS, Richmond TA, Schageman J, et al. Evaluating the analytical validity of circulating tumor DNA sequencing assays for precision oncology. Nat Biotechnol. 2021;39(9):1115–28.
46. US Food and Drug Administration. Design considerations for pivotal clinical investigations for medical devices. 2013. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/design-considerations-pivotal-clinical-investigations-medical-devices. Accessed 1 May 2021.
47. Grosse SD, Khoury MJ. What is the clinical utility of genetic testing? Genet Med. 2006;8(7):448–50.
48. National Institutes of Health. Hoffman-La Roche. A study of atezolizumab versus placebo as adjuvant therapy in patients with high-risk muscle-invasive bladder cancer who are ctDNA positive following cystectomy (IMvigor011) [ClinicalTrials.gov Identifier: NCT04660344]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
49. Driscoll JJ, Rixe O. Overall survival: still the gold standard: why overall survival remains the definitive end point in cancer clinical trials. Cancer J. 2009;15(5):401–5.
50. Saad ED, Buyse M. Statistical controversies in clinical research: end points other than overall survival are vital for regulatory approval of anticancer agents. Ann Oncol. 2016;27(3):373–8.
51. Wu Y-L, Tsuboi M, He J, John T, Grohe C, Majem M, et al. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. N Engl J Med. 2020;383(18):1711–23.
52. Gyawali B, West H. Lessons from ADAURA on adjuvant cancer drug trials: evidence, ethics, and economics. J Clin Oncol. 2021;39(3):175–7.
53. Centers for Medicare & Medicaid Services. Medicare program; Medicare Coverage of Innovative Technology (MCIT) and definition of "reasonable and necessary". 2020. Available from: https://www.federalregister.gov/documents/2021/03/17/2021-05490/medicare-program-medicare-coverage-of-innovative-technology-mcit-and-definition-of-reasonable-and. Accessed 1 May 2021.
54. Natera. Seeing beyond the limit: detect residual disease and assess treatment response. Natera. 2019. Available from: https://www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-Seeing-beyond-the-limit-Detect-residual-disease-and-assess-treatment-response-SGN_AV_WP.pdf. Accessed 1 May 2021.
55. Sethi H, Salari R, Navarro S, et al. Analytical validation of the Signatera™ RUO assay, a highly sensitive patient-specific multiplex PCR NGS-based noninvasive cancer recurrence detection and therapy monitoring assay. In: Proceedings from the American Association for Cancer Research Annual Meeting; 17 April 2019; Chicago (IL); abstract 4542.
56. Christensen E, Birkenkamp-Demtröder K, Sethi H, Shchegrova S, Salari R, Nordentoft I, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. J Clin Oncol. 2019;37(18):1547–57.
57. Natera. Natera announces plans to commercialize tumor whole exome sequencing from plasma. 2019. Available from: https://www.natera.com/company/news/natera-announces-plans-to-commercialize-tumor-whole-exome-sequencing-from-plasma/. Accessed 1 May 2021.
58. Chakrabarti S, Xie H, Urrutia R, Mahipal A. The promise of circulating tumor DNA (ctDNA) in the management of early-stage colon cancer: a critical review. Cancers. 2020;12(10):2808.
59. Natera. FDA grants breakthrough device designation to Natera's Signatera Test. 2019. Available from: https://www.natera.com/company/news/fda-grants-breakthrough-device-designation-to-nateras-signatera-test/. Accessed 1 May 2021.
60. Natera. FDA grants two new breakthrough device designations for Natera's Signatera™ MRD Test. Natera. 2021. Available from: https://www.natera.com/company/news/fda-grants-two-new-breakthrough-device-designations-for-nateras-signatera-mrd-test/. Accessed 1 May 2021.

61. Natera. Natera receives final Medicare voverage for its Signatera™ MRD Test in stage II-III colorectal cancer. 2020. Available from: https://www.natera.com/company/news/natera-receives-final-medicare-coverage-for-its-signatera-mrd-test-in-stage-ii-iii-colorectal-cancer/. Accessed 1 May 2021.
62. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. JAMA Oncol. 2019;5(8):1124.
63. Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, et al. Tracking the evolution of non-small-cell lung cancer. N Engl J Med. 2017;376(22):2109–21.
64. National Institutes of Health. University College, London. TRAcking Non-small Cell Lung Cancer Evolution Through Therapy (Rx) (TRACERx) [ClinicalTrials.gov Identifier: NCT01888601]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
65. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature. 2017;545(7655):446–51.
66. Coombes RC, Page K, Salari R, Hastings RK, Armstrong A, Ahmed S, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. Clin Cancer Res. 2019;25(14):4255–63.
67. Magbanua MJM, Swigart LB, Wu HT, Hirst GL, Yau C, Wolf DM, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol. 2021;32(2):229–39.
68. National Institutes of Health. Hoffman-La Roche. A study of atezolizumab versus observation as adjuvant therapy in participants with high-risk muscle-invasive urothelial carcinoma (UC) after surgical resection (IMvigor010) [ClinicalTrials.gov Identifier: NCT02450331]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
69. National Institutes of Health. Criterium, Inc. DNA-guided second line adjuvant therapy for high residual risk, stage II-III, hormone receptor positive, HER2 negative breast cancer (DARE) [ClinicalTrials.gov Identifier: NCT04567420]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
70. National Institutes of Health. Alliance Foundation Trials, LLC. PALbociclib CoLlaborative Adjuvant Study (PALLAS) [ClinicalTrials.gov Identifier: NCT02513394]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
71. Mayer EL, Dueck AC, Martin M, Rubovszky G, Burstein HJ, Bellet-Ezquerra M, et al. Palbociclib with adjuvant endocrine therapy in early breast cancer (PALLAS): interim analysis of a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2021;22(2):212–22.
72. National Institutes of Health. Eli Lilly and Company. Endocrine therapy with or without abemaciclib (LY2835219) following surgery in participants with breast cancer (monarchE) [ClinicalTrials.gov Identifier: NCT03155997]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
73. Johnston SRD, Harbeck N, Hegg R, Toi M, Martin M, Shao ZM, et al. Abemaciclib combined with endocrine therapy for the adjuvant treatment of HR+, HER2−, node-positive, high-risk, early breast cancer (monarchE). J Clin Oncol. 2020;38(34):3987–98.
74. Di Cosimo S, Porcu L, Cardoso F. CDK 4/6 inhibitors mired in uncertainty in HR positive and HER2 negative early breast cancer. Breast. 2021;55:75–8.
75. National Institutes of Health. Massachusetts General Hospital. CDK 4/6 inhibitor, ribociclib, with adjuvant endocrine therapy for ER-positive breast cancer (LEADER) [ClinicalTrials.gov Identifier: NCT03285412]. Available from: https://www.clinicaltrials.gov. Accessed 1 May 2021.
76. Guardant. Guardant Health launches Guardant Reveal™ liquid biopsy test for residual disease and recurrence monitoring in patients with early-stage colorectal cancer. 2021. Available from: https://investors.guardanthealth.com/press-releases/press-releases/2021/Guardant-Health-Launches-Guardant-Reveal-Liquid-Biopsy-Test-for-Residual-Disease-and-Recurrence-Monitoring-in-Patients-with-Early-Stage-Colorectal-Cancer/default.aspx. Accessed 1 May 2021.
77. Parikh AR, Van Seventer EE, Siravegna G, Hartwig AV, Jaimovich A, He Y, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. Clin Cancer Res. 2021. https://doi.org/10.1158/1078-0432.CCR-21-0410.
78. Parikh A, Van Seventer E, Boland G, Siravegna G, Hartwig A, Jaimovich A, et al. 419P Minimal residual disease (MRD) detection in colorectal cancer (CRC) using a plasma-only integrated genomic and epigenomic circulating tumor DNA (ctDNA) assay. Ann Oncol. 2020;31:S419.
79. Artieri C, Axelrod H, Baca A, Burke J, Chudova D, Dahdouli M, et al. Analytical validation of a tissue agnostic ctDNA MRD assay using tumor specific methylation and somatic variant profiles in early-stage CRC. J Clin Oncol. 2020;38(15):e15549.
80. Guardant. The first blood-only liquid biopsy test for residual disease and recurrence monitoring, starting with early-stage colorectal cancer. 2021. Available from: https://guardanthealth.com/wp-content/uploads/Backgrounder-Guardant-Reveal-2.10.pdf. Accessed 1 May 2021.
81. Peng J, Li Y, Mo S, Ma X, Hu X, Zhang L, et al. Prognostic value of circulating tumor DNA (ctDNA) detection during adjuvant chemotherapy in patients with stage III colorectal cancer: the interim report of a prospective, observational study. J Clin Oncol. 2020;38(4):29.
82. American Cancer Society. Key statistics for colorectal cancer. 2021. Available from: https://www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html. Accessed 1 May 2021.
83. El-Shami K, Oeffinger KC, Erb NL, Willis A, Bretsch JK, Pratt-Chapman ML, et al. American Cancer Society colorectal cancer survivorship care guidelines. CA Cancer J Clin. 2015;65(6):427–55.
84. Duineveld LAM, Van Asselt KM, Bemelman WA, Smits AB, Tanis PJ, Van Weert HCPM, et al. Symptomatic and asymptomatic colon cancer recurrence: a multicenter cohort study. Ann Fam Med. 2016;14(3):215–20.
85. Sobrero A, Andre T, Meyerhardt J, et al. OS and long-term DFS of 3 versus 6 months of adjuvant oxaliplatin and fluoropyrimidine-based therapy for patients with stage III colon cancer: final results from the IDEA collaboration. J Clin Oncol. 2020;38(15):4004.
86. Clinical OMICS. Philippidis A. Guardant throws hat in early cancer detection ring with CRC test. 2021. Available from: https://www.clinicalomics.com/topics/molecular-dx-topic/liquid-biopsies/guardant-throws-hat-in-early-cancer-detection-ring-with-crc-test. Accessed 1 May 2021.
87. National Institutes of Health. Grupo Espanol Multidisciplinario del Cancer Digestivo. Induction FOLFOX with or without aflibercept followed by chemoradiation in high risk locally advanced rectal cancer (RIA) [ClinicalTrials.gov Identifier: NCT02340949]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
88. Monjazeb AM, Giobbie-Hurder A, Lako A, Thrash EM, Brennick RC, Kao KZ, et al. A randomized trial of combined PD-L1 and CTLA-4 inhibition with targeted low-dose or hypofractionated radiation for patients with metastatic colorectal cancer. Clin Cancer Res. 2021;27(9):2470-80.
89. Vidal J, Casadevall D, Bellosillo B, Pericay C, Garcia-Carbonero R, Losa F, et al. Clinical impact of presurgery circulating tumor

DNA after total neoadjuvant treatment in locally advanced rectal cancer: a biomarker study from the GEMCAD 1402 trial. Clin Cancer Res. 2021;27(10):2890–8.
90. National Institutes of Health. IFOM, The FIRC Institute of Molecular Oncology. Post-surgical liquid biopsy-guided treatment of stage III and high-risk stage II colon cancer patients: the PEGASUS Trial [ClinicalTrials.gov Identifier: NCT04259944]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
91. Lonardi S ea. The PEGASUS trial: Post-surgical liquid biopsy-guided treatment of stage III and high-risk stage II colon cancer patients. J Clin Oncol. 2020;38(15)_suppl.
92. National Institutes of Health. Gruppo Italiano per lo studio dei Carcinomi dell'Apparato Digerente. FOLFOX-4 3 months versus 6 months and bevacizumab as adjuvant therapy for patients with stage II/III colon cancer (TOSCA) [ClinicalTrials.gov Identifier: NCT00646607]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
93. National Institutes of Health. IFOM, The FIRC Institute of Molecular Oncology. ALFAOMEGA Master Observational Trial (ALFAOMEGA) [ClinicalTrials.gov Identifier: NCT04120935]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
94. Chera BS, Kumar S, Beaty BT, Marron D, Jefferys S, Green R, et al. Rapid clearance profile of plasma circulating tumor HPV type 16 DNA during chemoradiotherapy correlates with disease control in HPV-associated oropharyngeal cancer. Clin Cancer Res. 2019;25(15):4682–90.
95. Chera BS, Kumar S, Shen C, Amdur R, Dagan R, Green R, et al. Plasma circulating tumor HPV DNA for the surveillance of cancer recurrence in HPV-associated oropharyngeal cancer. J Clin Oncol. 2020;38(10):1050–8.
96. Routman DM, Chera BS, Jethwa KR, Van Abel K, Kumar S, DeWees TA, et al. Detectable HPV ctDNA in post-operative oropharyngeal squamous cell carcinoma patients is associated with progression. Int J Radiat Oncol Biol Phys. 2019;105(3):682–3.
97. Stanford University. Adjuvant Durvalumab for Early Stage NSCLC Patients With ctDNA Minimal Residual Disease [ClinicalTrials.gov Identifier: NCT04585477]. National Institutes of Health. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
98. Pellini B, Pejovic N, Feng W, Earland N, Harris PK, Usmani A, et al. ctDNA MRD detection and personalized oncogenomic analysis in oligometastatic colorectal cancer from plasma and urine. JCO Precis Oncol. 2021;5:378–88.
99. National Institutes of Health. Stanford University. Personalized escalation of consolidation treatment following chemoradiotherapy and immunotherapy in stage III NSCLC [ClinicalTrials.gov Identifier: NCT04585490]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
100. Moding EJ, Liu Y, Nabet BY, Chabon JJ, Chaudhuri AA, Hui AB, et al. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small-cell lung cancer. Nat Cancer. 2020;1(2):176–83.
101. Clinical OMICS. ArcherDX's personalized cancer monitoring (PCM) tech gets FDA breakthrough device designation. 2020. Available from: https://www.clinicalomics.com/news-and-features/archerdxs-personalized-cancer-monitoring-pcm-tech-gets-fda-breakthrough-device-designation. Accessed 1 May 2021.
102. National Institutes of Health. AstraZeneca. Phase III study to determine the efficacy of durvalumab in combination with chemotherapy in completely resected stage II-III non-small cell lung cancer (NSCLC) (MERMAID-1) [ClinicalTrials.gov Identifier: NCT04385368]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
103. National Institutes of Health. AstraZeneca. Phase III study to determine efficacy of durvalumab in stage II-III non-small cell lung cancer (NSCLC) after curative intent therapy (MERMAID-2) [ClinicalTrials.gov Identifier: NCT04642469]. Available from: https://clinicaltrials.gov. Accessed 1 May 2021.
104. AstraZeneca. AstraZeneca collaborates with ArcherDX to use personalised cancer assays to detect minimal residual disease in lung cancer trials. 2020. Available from: https://www.astrazeneca.com/media-centre/press-releases/2020/astrazeneca-collaborates-with-archerdx-to-use-personalised-cancer-assays-to-detect-minimal-residual-disease-in-lung-cancer-trials.html. Accessed 1 May 2021.
105. Abbosh C, Frankell A, Garnett A, et al. Phylogenetic tracking and minimal residual disease detection using ctDNA in early-stage NSCLC: a lung TRACERx study. Proceedings from the American Association for Cancer Research Annual Meeting; 27-28 April 2020; Philadelphia (PA): abstract CT023.
106. Inivata. FDA grants breakthrough device designation for Inivata's RaDaR™ assay. 2021. Available from: https://www.inivata.com/fda-grants-breakthrough-device-designation-for-inivatas-radar-assay. Accessed 1 May 2021.
107. Marsico G, Sharma G, Perry M, Hackinger S, Forshew T, Howarth K, et al. Analytical development of the RaDaR™ assay, a highly sensitive and specific assay for the monitoring of minimal residual disease. In: Proceedings from the American Association for Cancer Research Virtual Annual Meeting II; June 2020: poster 3097.
108. Cutts RJ, Coakley M, Garcia-Murillas I, et al. Molecular residual disease detection in early stage breast cancer with a personalized sequencing approach. In: Proceedings from the American Association for Cancer Research Annual Meeting; 10 April 2021: poster 536.
109. Flach S, Howarth K, Hackinger S, Pipinikas C, McLay K, Marsico G, et al. Personalized circulating tumor DNA analysis in head and neck squamous cell carcinoma: preliminary results of the Liquid BIOpsy for MiNimal RESidual DiSease Detection in Head and NeckSquamous Cell Carcinoma (LIONESS) study. In: Proceedings from the American Association for Cancer Research Annual Meeting; 10 April 2021: poster 553.
110. PredicineALERT™. Predicine. 2021. Available from: https://www.predicine.com/solutions-overview/predicinealert. Accessed 1 May 2021.
111. Wang AX, Zhao Z, Dai C, Zhou K, Jia S, Du P, Luo S. Comprehensive analysis of cell-free DNA with whole-exome sequencing and its application to minimal residual disease (MRD) monitoring. In: Proceedings from the American Association for Cancer Research Annual Meeting; 10 April 2021: abstract 578.
112. Kwan EM, Dai C, Fettke H, Hauser C, Docanto MM, Bukczynska P, et al. Plasma cell-free DNA profiling of PTEN-PI3K-AKT pathway aberrations in metastatic castration-resistant prostate cancer. JCO Precis Oncol. 2021;5:622–37.
113. Zviran A, Schulman RC, Shah M, Hill STK, Deochand S, Khamnei CC, et al. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. Nat Med. 2020;26(7):1114–24.
114. Phallen J, Sausen M, Adleff V, Leal A, Hruban C, White J, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med. 2017;9(403):eaan2415.
115. Campbell BB, Light N, Fabrizio D, Zatzman M, Fuligni F, De Borja R, et al. Comprehensive analysis of hypermutation in human cancer. Cell. 2017;171(5):1042.e10-1056.e10.
116. Taylor AM, Shih J, Ha G, Gao GF, Zhang X, Berger AC, et al. Genomic and functional approaches to understanding cancer aneuploidy. Cancer Cell. 2018;33(4):676.e3-689.e3.
117. Kurtz DM, Soo J, Co Ting Keh L, Alig S, Chabon JJ, Sworder BJ, et al. Enhanced detection of minimal residual disease by targeted sequencing of phased variants in circulating tumor DNA. Nat Biotechnol. 2021. https://doi.org/10.1038/s41587-021-00981-w.
118. Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, et al. Distinct biological subtypes and patterns of genome

evolution in lymphoma revealed by circulating tumor DNA. Sci Transl Med. 2016;8(364):364ra155.
119. Kurtz DM, Scherer F, Jin MC, Soo J, Craig AFM, Esfahani MS, et al. Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma. J Clin Oncol. 2018;36(28):2845–53.
120. Medina Diaz I, Nocon A, Mehnert DH, Fredebohm J, Diehl F, Holtrup F. Performance of Streck cfDNA blood collection tubes for liquid biopsy testing. PLoS ONE. 2016;11(11):e0166354.
121. Parpart-Li S, Bartlett B, Popoli M, Adleff V, Tucker L, Steinberg R, et al. The effect of preservative and temperature on the analysis of circulating tumor DNA. Clin Cancer Res. 2017;23(10):2471–7.
122. Greytak SR, Engel KB, Parpart-Li S, Murtaza M, Bronkhorst AJ, Pertile MD, et al. Harmonizing cell-free DNA collection and processing practices through evidence-based guidance. Clin Cancer Res. 2020;26(13):3104–9.
123. Godsey JH, Silvestro A, Barrett JC, Bramlett K, Chudova D, Deras I, et al. Generic protocols for the analytical validation of next-generation sequencing-based ctDNA asays: a joint consensus recommendation of the BloodPAC's Analytical Variables Working Group. Clin Chem. 2020;66(9):1156–66.
124. Friends of Cancer Research. Friends of Cancer Research Launches ctMoniTR. 2019. Available from: https://friendsofcancerresearch.org/news/friends-cancer-research-launches-ctmonitr/. Accessed 1 May 2021.