Invitae Personalized Cancer Monitoring

Invitae Personalized Cancer Monitoring (PCM™) is a pan-cancer, tumor-informed liquid biopsy assay that uses next-generation sequencing powered by Anchored Multiplex PCR (AMP™) to monitor molecular residual disease with high sensitivity at low variant allele fractions.

Introduction

Determining the best treatment plan for a cancer patient after surgical resection or completion of adjuvant chemotherapy depends on several factors, including the results of current disease monitoring. Standard methods for monitoring disease after surgery include radiological imaging and analysis of circulating tumor markers such as carcinoembryonic antigen (CEA) and serum cancer antigen 125 (CA-125). However, standard imaging detects only macroscopic disease,[1] and the sensitivity and specificity of methods for detecting circulating markers are not always reliable.[2] Therefore, more accurate methods of detection are warranted. One such method, which is evolving quickly with great promise, is to monitor circulating tumor DNA (ctDNA) as a biomarker for molecular residual disease (MRD).

Over time, tumor cells accumulate DNA variants that differ from DNA variants in healthy cells. Both healthy cells and tumor cells shed fragmented DNA into the bloodstream, and the fragmented DNA is referred to as cell-free DNA. The portion that is released from the tumor cells is known as ctDNA. When DNA sequencing is performed on a plasma sample, tumor-derived variants appear in only a fraction of all sequenced DNA. The proportion of a tumor-derived variant in relation to the background of cell-free DNA is known as a variant allele fraction. The collection of tumor-derived variants present in a plasma sample serves as a signature for that particular tumor, and detecting very low levels of ctDNA carrying this unique signature provides a basis for MRD monitoring.

Applications in medicine

Recurrence monitoring: Studies have demonstrated a strong correlation between the presence of ctDNA in plasma and cancer recurrence.[1,3] Growing evidence further suggests that MRD monitoring can detect recurrence sooner than standard imaging in patients who have undergone treatment with curative intent. In one study among 40 patients with stage I to III lung cancer who underwent first-line treatment, ctDNA was detected a median of 5.2 months before radiographic progression in 72% of the patients who relapsed.[1] In another study, researchers monitored MRD in 240 patients with stage II and III colorectal cancer who had been treated with surgery and adjuvant chemotherapy if needed. During a median follow-up of about 27 months, radiological imaging detected recurrence in 32 of the patients. Serial analysis of ctDNA detected recurrence a mean of 5.01 months ahead of imaging with an accuracy of 92%.[4]

Therapy response monitoring: Research has shown that MRD monitoring can reliably predict progression in patients on immunotherapy,[5] and studies are being implemented to see if it can determine how patients are responding to other types of therapy as well.[6] MRD monitoring may also have the potential to identify whether a therapy is effective against all or only some cell types in a tumor and to track the development of therapy-resistant DNA variants.[7]

Therapy guidance: Because MRD monitoring can detect post-surgical recurrence earlier than standard imaging, it may allow additional treatment to be started when tumor burden is still relatively low. For patients who are already being treated after surgery, the type and level of ctDNA burden detected could help clinicians and patients decide to continue with a given therapy, switch to an alternative one, or cease treatment altogether in the event of a cure.

Clinical research: Clinical trials in adjuvant populations usually require large heterogeneous populations and many years of follow-up to adequately power the studies. Because MRD monitoring could identify patients at high risk of relapse (i.e., those in immediate need of adjuvant therapy), it could allow smaller and shorter clinical trials in more homogeneous populations. MRD also has the potential to be a surrogate for clinical trial endpoints, such as progression-free survival,[8] which could further shorten clinical trials and accelerate the development of new drugs.

"MRD monitoring with methods such as PCM has the potential to determine a therapy's effectiveness much sooner than current monitoring methods, allowing clinicians to more efficiently refine and optimize treatment plans," says Invitae Chief Medical Officer Dr. Robert Nussbaum.

"Patients whose cancer has been cured by tumor resection may be spared from unnecessary and potentially harmful adjuvant therapy, while those at risk of relapse may be diagnosed earlier and treated with the necessary therapies."

Methods for detecting molecular residual disease

First used as a clinical metric in hematological cancers, MRD was historically measured with cell-based assays such as flow cytometry. Only recently, with the development of highly sensitive methods of DNA analysis, has ctDNA in plasma samples been shown to be a reliable biomarker for MRD.[9,10] Some of the earliest pilot studies proving ctDNA's utility as a biomarker for MRD were conducted among patients with breast cancer,[11-16] but a growing number of studies have also confirmed its applicability to colorectal cancer,[17,18] early-stage non-small cell lung cancer (NSCLC),[19,20] pancreatic cancer,[21] and bladder cancer.[22,23]

The presence of tumor-specific variants in ctDNA from patients with solid tumor malignancies increases as the disease progresses (**Figure 1**). Variant allele fractions are usually less than 0.1% for patients with early-stage cancers that have been surgically resected with intent to cure, less than 1% for patients with locally advanced disease, and less than 10% for patients with advanced metastatic disease.[10] The amount of ctDNA shed into the bloodstream can also differ by tumor type and stage.[24] Methods for measuring ctDNA as a biomarker for MRD must therefore be very sensitive to be able to accurately and reliably detect the earliest signs of disease progression in a variety of cancer types.[10]

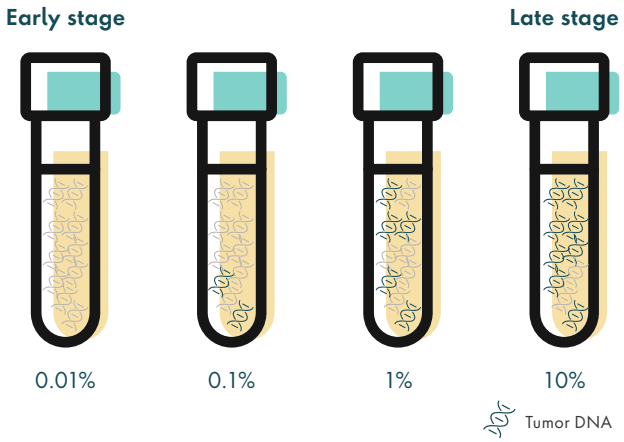


Figure 1. Examples of increasing variant allele fractions as cancer progresses

One of the first methods used to detect and quantify ctDNA in a patient's plasma was Sanger sequencing, although it was limited by its low sensitivity.[9,10] Alternative methods in use today include a variety of PCR assays and next-generation sequencing (NGS) approaches.[9,10] Allele-specific, digital, and BEAMing (beads, emulsions, amplification, and magnetics) PCR target known variants, typically a single locus or multiple common tumor alterations. NGS is a high-throughput platform that enables massively parallel sequencing of many genomic targets simultaneously. NGS can be used in an untargeted manner to sequence whole genomes or whole exomes or it can be used in a targeted manner (e.g., NGS based on multiplex PCR or hybrid capture) to sequence specific genes or intragenic segments.

NGS-based MRD assays can be either tumor-informed or tumor-agnostic (**Figure 2**). Tumor-informed assays typically begin with non-targeted NGS of tumor tissue (e.g., a surgical specimen) to determine which tumor-derived variants are present and amenable to tracking in cell-free DNA. That information can then be used to design a targeted NGS panel unique to the patient's tumor. Instead tumor-agnostic assays include fixed panels aimed at detecting a specific number of genomic and/or epigenomic alterations commonly associated with a particular tumor type. To detect MRD, tumor-informed and tumor-agnostic assays both rely on how well a tumor sheds DNA into the bloodstream, but tumor-informed approaches have the benefit of predicting which unique variants in a patient's tumor are the best alterations to detect and monitor.

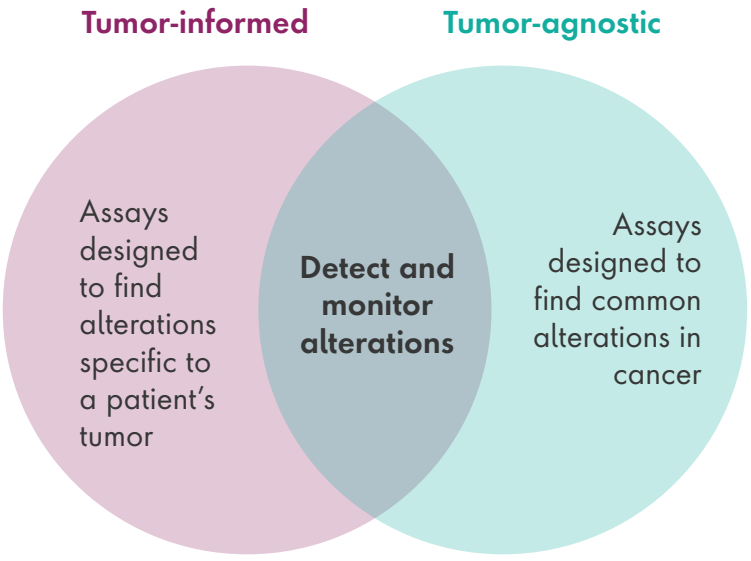


Figure 2. Assays based on next-generation sequencing

The Invitae platform

Our assay

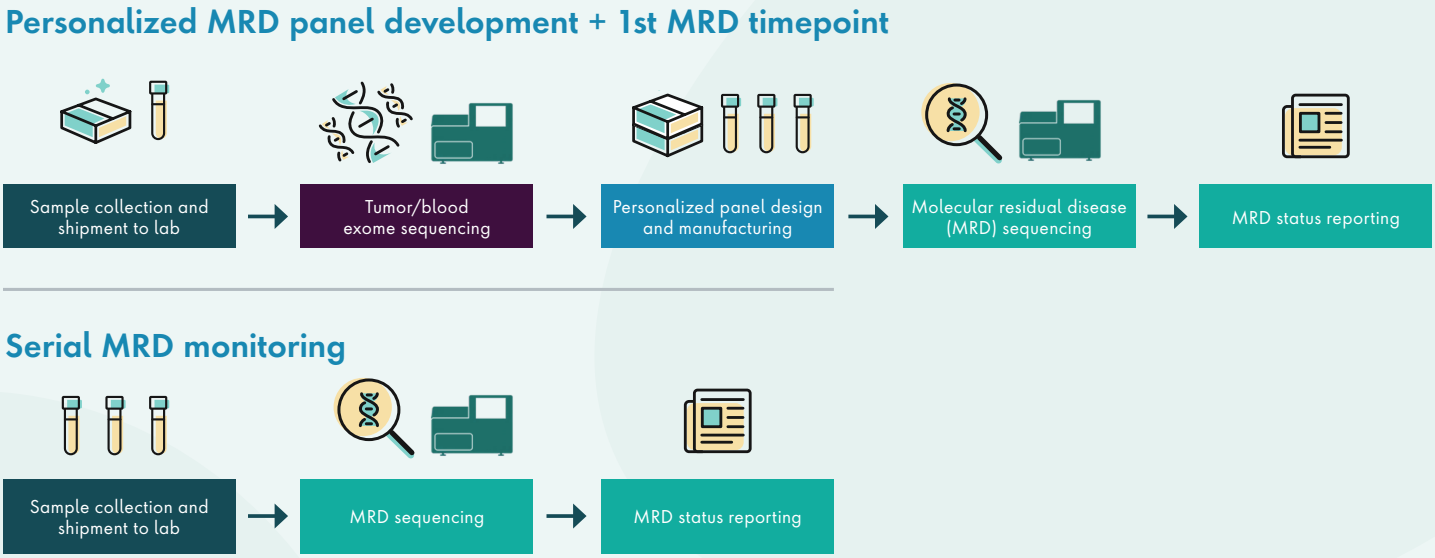
Invitae has developed a personalized cancer monitoring (PCM) assay to monitor MRD. PCM is a pan-cancer, tumor-informed liquid biopsy assay that uses NGS powered by Anchored Multiplex PCR (AMP) chemistry to analyze ctDNA in a patient's plasma. Each assay is custom designed to detect a patient's unique tumor signature, allowing for personalized results to guide personalized treatment decisions.

AMP is a target-enrichment strategy for NGS that enables open-ended capture of target sequences. Built for NGS biomarker detection, AMP has unique advantages in detecting both simple and complex DNA variants, setting it apart from traditional primer-based techniques for preparing NGS libraries.

The PCM assay includes an adapter that enables molecular barcoding to uniquely tag each DNA fragment analyzed, allowing deduplicate reads and advanced sequencing error correction for high-confidence MRD calls at low variant allele fractions. Furthermore, AMP is uniquely suited for low-input sample types, such as liquid biopsies, making the technology particularly useful in clinical settings.

A simple five-step PCM workflow (**Figure 3**) and a powerful bioinformatics suite was built to leverage the advantages of AMP chemistry.

Figure 3. PCM workflow



STEP 1: Tumor tissue and blood collection

After initial tumor resection, a tumor sample is sent to the laboratory where DNA will be extracted for input into whole exome sequencing. A blood sample is also sent to the laboratory to be processed in parallel with the tumor tissue sample.

STEP 2: Whole exome sequencing to identify variants

Whole exome sequencing is performed on the tumor sample to identify unique variants of the somatic genome sequence, accounting for genetic heterogeneity of subclones. Whole exome sequencing is also performed on the paired blood sample to account for the unique germline genetic makeup of each patient.

STEP 3: Variant selection and panel design

Based on the results of whole exome sequencing of both tumor and blood samples, our proprietary variant selection software chooses up to 50 tumor-specific variants for inclusion on a personalized ctDNA panel. The software is designed to minimize background error by prioritizing low-noise variants for optimal panel design.

STEP 4: Detection of molecular residual disease

After the personalized panel is manufactured, MRD reagents specific to the patient's tumor are gathered and transferred to the laboratory. The patient's ctDNA is extracted from plasma and processed through the customized assay, including amplification and sequencing of the tumor-specific variants to provide an initial MRD result. Our proprietary MRD calling algorithm uses built-in error correction and sequencing noise modeling to enable our test's high sensitivity.

STEP 5: Serial monitoring

Over time, additional MRD results can be obtained for comparison with earlier results. At each time point, ctDNA from a new plasma sample is sent to the laboratory and processed using the patient's same personalized panel. The number of time points for monitoring can be adjusted to fit each patient's needs based on tumor type and stage.

Analytical validation

A full analytical validation of Invitae's PCM assay was conducted at our Metro Park, NJ, laboratory, with the aim to ultimately seek U.S. Food and Drug Administration (FDA) approval. The laboratory is accredited by the College of American Pathologists (CAP), and the assay's performance was determined according to current Clinical Laboratory Improvement Amendments (CLIA) guidance.

Limit of detection

Because ctDNA levels in plasma decrease to variant allele fractions of less than 0.1% after treatment of most solid tumors with curative intent,[9,10] we sought to validate the ability of PCM to detect allele fractions well below this level. Limit of detection (LOD) was defined as the lowest targeted variant allele fraction at which the PCM assay, using Invitae's MRD calling algorithm, could detect control ctDNA in at least 95% of replicates. Using commercial reference material, LOD was estimated using 562 libraries and then confirmed at minimum and maximum DNA inputs. The study established an LOD of 10 ng of DNA at 0.03% variant allele fraction and 60 ng at 0.008% variant allele fraction.

Accuracy

The accuracy of the PCM assay to consistently make the correct MRD call for a given sample was evaluated on the basis of agreement between calls made by PCM and the sample's known MRD status. Various contrived samples containing known NSCLC variants were mixed with pooled plasma from healthy donors to generate 100 samples with known variant allele fractions. Additional samples of cell-free DNA from eight healthy donors were used as negative controls, and all 108 samples were evaluated at DNA inputs near the LOD.

Results found that the overall percent agreement between MRD calls made by PCM and the sample's known MRD status was 96.3% (**Table 1**).

Table 1. High percent agreement between PCM and an orthogonal method

Metric	% (n/N)	95% CI lower bound	95% CI upper bound
Precision	100% (8/8)	63.1%	100%
Reproducibility	96.3%(104/108)	90.8%	99.0%

CI = confidence interval

Sensitivity

The same 108 samples were used to determine the sensitivity of PCM, or the probability that the assay would detect ctDNA at low variant allele fractions. The study showed greater than 99% sensitivity with inputs of cell-free DNA ranging from 10 ng to 60 ng and in allele fractions as low as 0.005% (**Figure 4**), demonstrating the assay's potential to detect variant alleles across most solid tumor types.

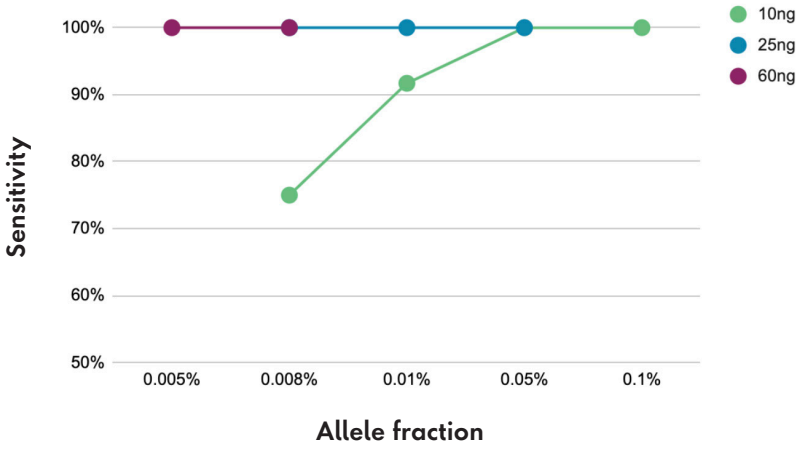


Figure 4. High sensitivity at low allele fractions

Clinical data

TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]) is a nine-year prospective study using an earlier iteration of the PCM assay to follow the clonal evolution of early-stage NSCLC from diagnosis through relapse. Funded by Cancer Research UK, the study is a collaboration among University College London, Invitae, and several other institutions in the United States and Europe.

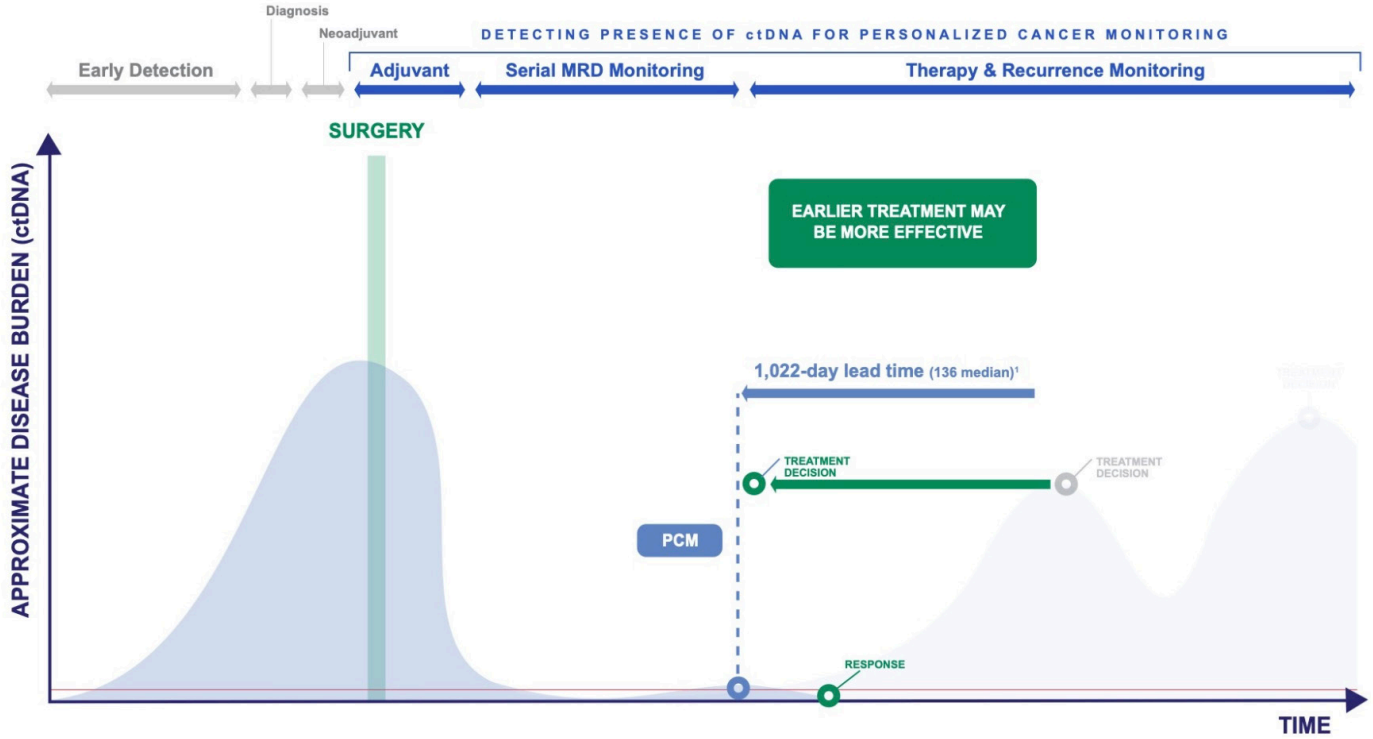
Results from the first 100 of approximately 850 study participants with stage I to III NSCLC showed that PCM predicted relapse after resection in 13 (93%) of 14 cases and detected recurrence a median of 70 days earlier than standard computed tomography imaging.[7] In an additional set of patients, PCM detected ctDNA at or before clinical relapse in 37 (82%) of 45 patients who relapsed after undergoing resection of their primary tumors. The data from this cohort also suggest that PCM may detect recurrence even earlier than initially predicted, as the assay had a median lead time of 151 days over standard imaging.[25] The study validated the sensitivity of PCM in a clinical setting, detecting ctDNA at a sensitivity of 89% at an allele fraction of 0.008%.[25]

Another iteration of the PCM assay is being incorporated into ongoing international clinical trials. Currently, two phase III randomized controlled trials are using PCM to detect MRD in patients with stage II and III NSCLC whose tumors have been resected.[26-28] Patients with MRD but no clinical evidence of recurrence are being randomized to receive adjuvant treatment with either standard-of-care chemotherapy alone or standard-of-care chemotherapy plus the immunotherapy durvalumab. Disease-free survival rates will be evaluated in both groups to help determine whether intensifying adjuvant therapy in patients at high risk of relapse (based on MRD monitoring) will improve patient outcomes.

Looking forward

In a clinical setting, PCM has the potential to detect ctDNA following surgical resection or completion of adjuvant chemotherapy, to assess therapy response, and to monitor detectable ctDNA in the months or even years after a patient finishes treatment. If an MRD-positive result were to be obtained at any point in a patient's cancer journey, the clinician and patient could discuss the implications of the result and the most appropriate treatment or clinical trial options. As research continues to address questions in support of meaningful clinical applications of MRD monitoring, PCM and other liquid biopsy approaches have the potential to become a mainstay in precision oncology.[29]

Figure 5. Potential of PCM in a clinical setting[25]



References

1. Chaudhury AA, Chabon J, Lovejoy AF, *et al.* Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. *Cancer Discov.* 2017;7(12):1394-1403.
2. Newton KF, Newman W, Hill J. Review of biomarkers in colorectal cancer. *Colorectal Dis.* 2012;14(1):3-17.
3. Tie J, Wang Y, Tomasetti C, *et al.* Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. *Sci Transl Med.* 2016;8(346):346ra92.
4. Chen G, Peng J, Xiao Q, *et al.* Postoperative circulating tumor DNA as markers of recurrence risk in stages II to III colorectal cancer. *J Hematol Oncol.* 2021;14(1):80.
5. Hellman MD, Nabet BY, Rizvi H, *et al.* Circulating tumor DNA analysis to assess risk of progression after long-term response to PD-(L)1 blockade in NSCLC. *Clin Cancer Res.* 2020;26(12):2849-58.
6. Naidoo M, Gibbs P, Tie J. ctDA and adjuvant therapy for colorectal cancer: time to re-invent our treatment paradigm. *Cancers (Basel).* 2021; 13(2):346.
7. Abbosh C, Birkbak NJ, Wilson GA, *et al.* Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature.* 2017;545 (7655):446-51.
8. Hrebien S, Citi V, Garcia-Murillas I, *et al.* Early ctDNA dynamics as a surrogate for progression-free survival in advanced breast cancer in the BEECH trial. *Ann Oncol.* 2019;30(6):945-52.
9. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease—latest advances and implications for cure. *Nat Rev Clin Oncol.* 2019;16:409-24.
10. Chin RI, Chen K, Usmani A, *et al.* Detection of solid tumor molecular residual disease (MRD) using circulating tumor DNA (ctDNA). *Mol Diagn Ther.* 2019;23(3):311-31.
11. Garcia-Murillas I, Schiavon G, Weigelt B, *et al.* Mutation tracking in circulating tumor DNA predicts relapses in early breast cancer. *Sci Transl Med.* 2015;7(302):302ra133.
12. Chen Y-H, Hancock BA, Solzak JP, *et al.* Next-generation sequencing of circulating tumor DNA to predict recurrence in triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy. *NPJ Breast Cancer.* 2017;3:24.
13. Olsson E, Winter C, George A, *et al.* Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. *EMBO Mol Med.* 2015;7(8):1034-47.
14. Riva F, Bidard F-C, Houy A, *et al.* Patient-specific circulating tumor DNA detection during neoadjuvant chemotherapy in triple-negative breast cancer. *Clin Chem.* 2017;63(3):691-99.
15. McDonald BR, Contente-Cuomo T, Sammut S-J, *et al.* Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. *Sci Transl Med.* 2019;11(504):eaax7392.
16. Magbanua MJM, Brown-Swigart L, Wu H-t, *et al.* Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann Oncol.* 2021;32(2):229-239.
17. Reece M, Saluja H, Hollington P, *et al.* The use of circulating tumor DNA to monitor and predict response to treatment in colorectal cancer. *Front Genet.* 2019;10:1118.
18. Dasari A., Morris VK., Allegra CJ. *et al.* ctDNA applications and integration in colorectal cancer: an NCI Colon and Rectal–Anal Task Forces whitepaper. *Nat Rev Clin Oncol.* 2020;17(12):757-70.
19. Chae YK, Oh MS. Detection of minimal residual disease using ctDNA in lung cancer: current evidence and future directions. *J Thorac Oncol.* 2019; 14(1):16-24.
20. Ho GYF, Wang T, Kwok HH, *et al.* Longitudinal multi-gene panel assessment of circulating tumor DNA revealed tumor burden and molecular characteristics along treatment course of non-small cell lung cancer. *Transl Lung Cancer Res.* 2020;9(5):1873-1884.
21. Sausen M, Phallen J, Adleff V, *et al.* Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. *Nat Commun.* 2015;6:1-6.
22. Dudley JC, Schroers-Martin J, Lazareschi DV, *et al.* Detection and surveillance of bladder cancer using urine tumor DNA. *Cancer Disc.* 2019;9(4):500-9.
23. Springer SU, Chen C-H, Rodriguez Pena MDC, *et al.* Non-invasive detection of urothelial cancer through the analysis of driver gene mutations and aneuploidy. *Elife.* 2018;7:e32143.
24. Bettegowda C, Sausen M, Leary RJ, *et al.* Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med.* 2014;6(224):224ra24.
25. 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. Abstract CT023. Presented at the 2020 American Association for Cancer Research Annual Meeting, April 27-28, 2020. Accessed August 2, 2021. https://www.abstractsonline.com/pp8/#!/9045/presentation/10734collaboration-at-2020-aacr-virtual-annual-meeting-301048632.html
26. AstraZeneca. AstraZeneca collaborates with ArcherDX to use personalised cancer assays to detect minimal residual disease in lung cancer trials. Press release. May 26, 2020. Accessed August 19, 2021. 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
27. U.S. National Library of Medicine (ClinicalTrials.gov). 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). Accessed August 19, 2021. https://clinicaltrials.gov/ct2/show/NCT04385368?term=mermaid-1&draw=2&rank=1
28. U.S. National Library of Medicine (ClinicalTrials.gov). Phase III study to determine efficacy of durvalumab in stage II-III non-small cell lung cancer (NSCLC) after curative intent therapy (MERMAID-2). Accessed August 2, 2021. https://clinicaltrials.gov/ct2/show/NCT04642469?term=mermaid-2&draw=2&rank=2
29. Babayan A, Pantel K. Advances in liquid biopsy approaches for early detection and monitoring of cancer. *Genome Med.* 2018;10(1):21.