Invitae Personalized Cancer Monitoring

Invitae Personalized Cancer Monitoring (PCM™) is a pan-cancer,* tumor-informed liquid biopsy test that uses next-generation sequencing to monitor minimal residual disease with high sensitivity at low tumor concentration levels or variant allele fractions.

*With exceptions – such as CNS malignancies and sarcomas – this test is not intended to be used in hematological malignancies such as leukemias or lymphomas.

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 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 (cfDNA). 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 cfDNA 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.

How MRD monitoring may fit into patient care

Risk stratification studies have shown the utility of ctDNA MRD in risk stratifying to determine which patients have a high risk of relapse following their therapy.

Therapy response assessment and tailoring treatments: Because MRD monitoring may 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.

Surveillance and longitudinal monitoring can refine and complement current imaging protocols and may allow for earlier detection of recurrence than standard imaging. Research has shown that MRD monitoring can reliably predict progression in patients on immunotherapy,[3] and studies are being implemented to see if it can determine how patients are responding to other types of therapy as well.[4]

"There is a clinical need for risk stratification across solid tumor types as well as the need for molecular tools to complement and improve upon standard of care methods for recurrence detection. MRD monitoring and methods such as PCM have the potential to identify relapse prior to current monitoring methods, allowing clinicians to to optimize available information for treatment planning."

Robert Nussbaum, MD, Chief Medical Officer, Invitae

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.[5,6] Some of the earliest pilot studies proving ctDNA's utility as a biomarker for MRD were conducted among patients with breast cancer,[7-12] but a growing number of studies has also confirmed its applicability to colorectal cancer,[13,14] early-stage non-small cell lung cancer (NSCLC),[15,16] pancreatic cancer,[17] and bladder cancer.[18,19]

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.[6] The amount of ctDNA shed into the bloodstream can also differ by tumor type and stage.[20] 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.[6]

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. Alternative methods in use today include a variety of PCR assays and next-generation sequencing (NGS) approaches, and have proven to be more effective.[5,6]

PCR assays: PCR methods target known variants, typically a single locus or multiple common tumor alterations. Although PCR assays are highly sensitive, NGS is a high-throughput platform that enables massively parallel sequencing of many genomic targets simultaneously.

NGS approaches: The Sanger method only sequences a single DNA fragment at a time. NGS sequences millions of fragments, simultaneously. In addition, NGS can be used in an untargeted manner to sequence whole genomes or whole exomes, or it can be used in a targeted manner to sequence specific genes or intragenic segments. Also, NGS-based MRD assays can be either tumor-informed or tumor-agnostic (**Figure 2**):

- *Tumor-informed* assays typically begin with 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.
- *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 potential benefit of predicting which unique variants in a patient's tumor are the best alterations to detect and monitor. **The Invitae Platform, PCM, leverages a NGS and tumor-informed approach for detecting MRD.**

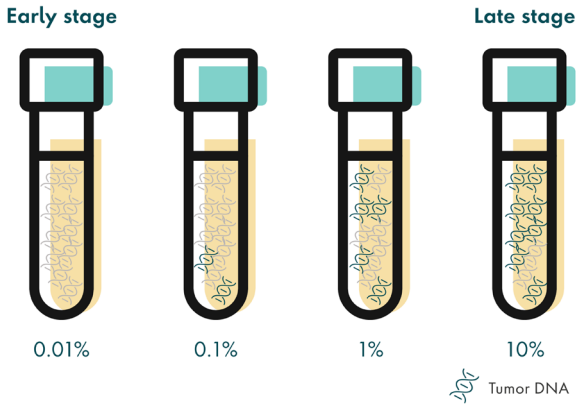

Figure 1. Examples of increasing variant allele fractions as cancer progresses

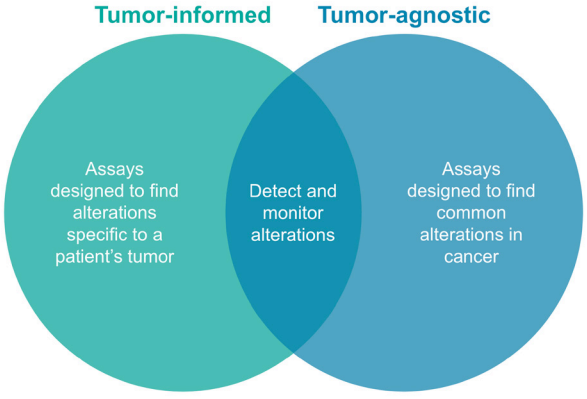

Figure 2. Assays based on next-generation sequencing

The Invitae Platform

Our test

PCM is a pan-cancer,* tumor-informed liquid biopsy test that uses NGS to analyze ctDNA in a patient's plasma. Each test is custom designed to detect a patient's unique tumor signature, allowing for personalized results. PCM requires both blood and tumor tissue samples from the patient to conduct tumor-normal whole exome sequencing (WES). Based on the results, Invitae's proprietary algorithm selects 18–50 tumor-specific variants to include on the patient's custom-designed ctDNA panel.

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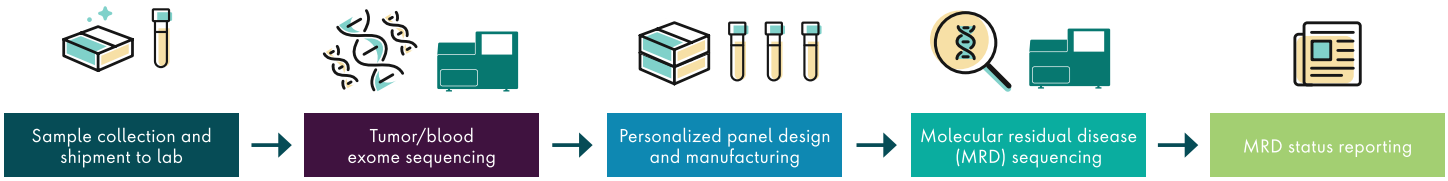
This algorithm's validated selection criteria uses the whole exome sequencing data and selection of the best 18–50 tumor variants based on several factors, including:

- Variant clonality
  - Our proprietary algorithm prioritizes **passenger alterations** with higher allele frequency because these variants tend to be less susceptible to the impacts of therapeutic pressure
- Variants in low noise genomic regions

Personalized MRD panel development + 1st MRD time point



Serial MRD monitoring

Figure 3. A simple 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.

The PCM test is for patients diagnosed with solid tumor cancer and available formalin-fixed, paraffin-embedded (FFPE) and plasma sample sets.

Analytical validation

A full analytical validation of the PCM test was conducted at our Metro Park, NJ, laboratory. 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.

Accuracy

The accuracy of the PCM test 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 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. 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**).

METRIC	% (N/N)	95% CI LOWER BOUND	95% CI UPPER BOUND
Precision	>99.9% (8/8)	63.1%	>99.9%
Reproducibility	96.3% (104/108)	90.8%	99.0%

CI = confidence interval

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

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 test's potential to detect variant alleles across most solid tumor types.

Specificity

PCM performs at >99.99% at 18 single nucleotide variants (SNPs).

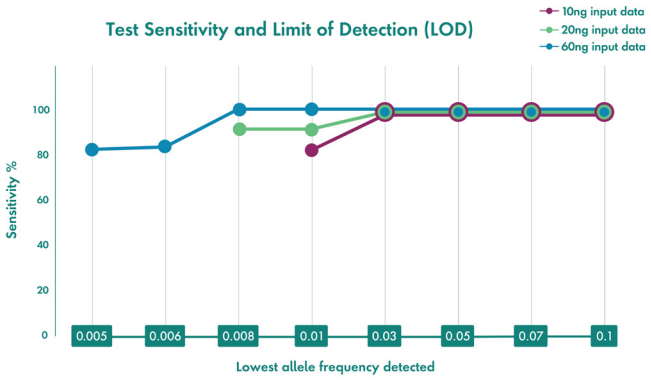


Figure 4. High sensitivity at low tumor concentration levels or variant allele fractions

Clinical data

TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]) is a nine-year prospective clinical 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.[21] 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 136 days over standard imaging. 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%.[22]

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.[23–25] 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.

Ongoing data

Invitae is taking a global approach to research for PCM. We have ongoing studies in Japan, Europe and the United States, and continue to strive to prioritize studies in community settings to ensure a diverse cohort of patients.

There are ongoing retrospective and clinical studies in NSCLC, breast cancer, pancreatic cancer, CRC and melanoma.

These studies are designed to assess how PCM could be used:

- to identify patients who are potentially at risk for relapse
- for escalation or de-escalation of therapies during treatment
- as an additional tool to inform adjuvant therapy decisions
- during the surveillance period to detect recurrence after a patient has finished treatment

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.

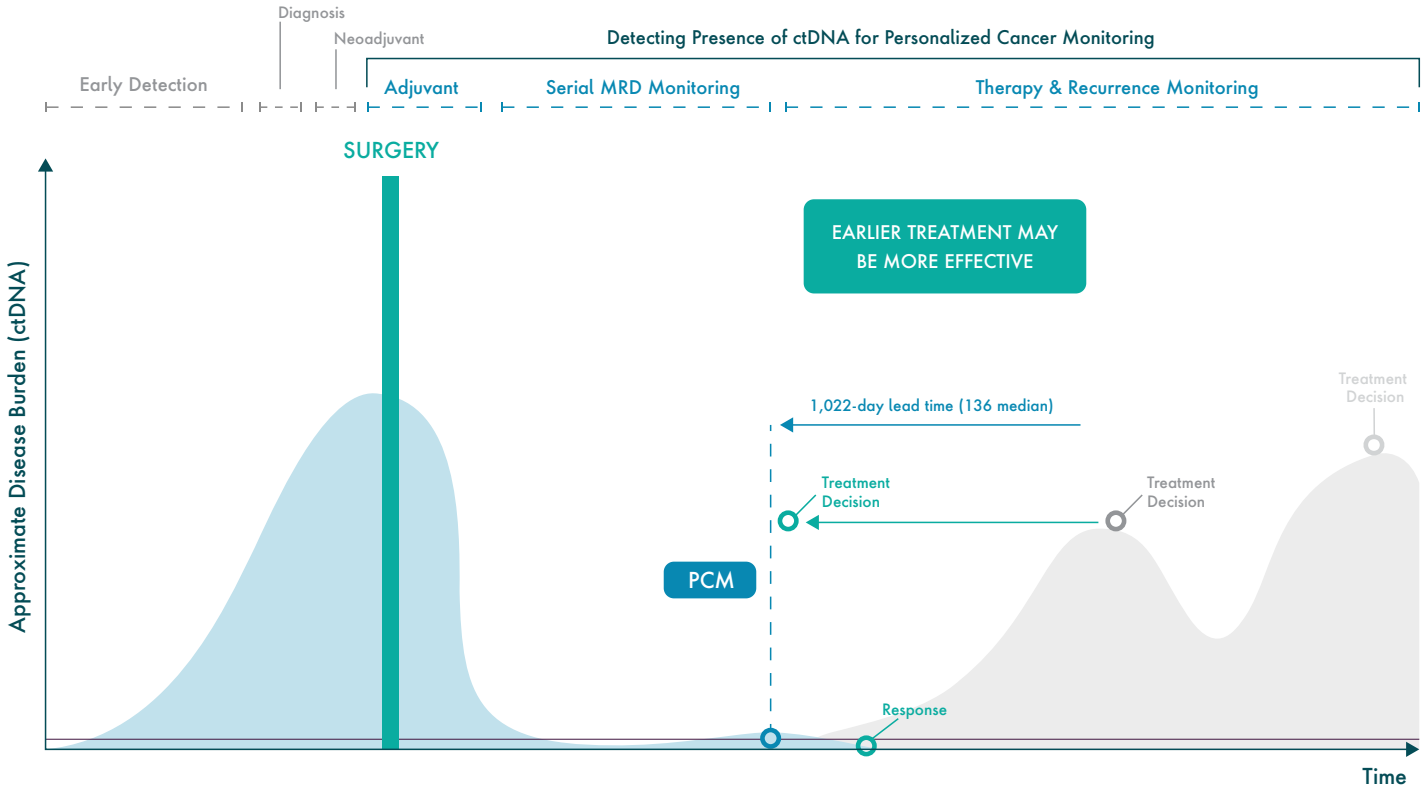


Figure 5. Potential of PCM in a clinical setting[24]

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.[26]

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