CANCER SCREENING

Multi-cancer early detection liquid biopsy testing: A predictive genetic test not quite ready for prime time

Current cancer screening methods have helped save millions of lives. Now, applying developing technology that can detect circulating tumor DNA has the potential to identify up to 50 cancers with a simple blood test.

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CASE Patient inquires about new technology to detect cancer

A 51-year-old woman (para 2) presents to your clinic for a routine gynecology exam. She is up to date on her screening mammogram and Pap testing. She has her first colonoscopy scheduled for next month. She has a 10-year remote smoking history, but she stopped smoking in her late twenties. Her cousin was recently diagnosed with skin cancer, her father had prostate cancer and is now in remission, and her paternal grandmother died of ovarian cancer. She knows ovarian cancer does not have an effective screening test, and she recently heard on the news about a new blood test that can detect cancer before symptoms start. She would like to know more about this test. Could it replace her next Pap, mammogram, and future colonoscopies? She also wants to know—How can a simple blood test detect cancer?

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The power of genomics in cancer care

Since the first human genome was sequenced in 2000, the power of genomics has been evident across many aspects of medicine, including cancer care.[1] Whereas the first human genome to be sequenced took more than 10 years to sequence and cost over $1 billion, sequencing of your entire genome can now be obtained for less than $400—with results in a week.[2]

Genomics is now an integral part of cancer care, with results having implications for both cancer risk and prevention as well as more individualized treatment. For example, a healthy 42-year-old patient with a strong family history of breast cancer may undergo genetic

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testing and discover she has a mutation in the tumor suppression gene *BRCA1,* which carries a 39% to 58% lifetime risk of ovarian cancer.[3] By undergoing a risk-reducing bilateral salpingo-oophorectomy she will lower her ovarian cancer risk by up to 96%.[4,5] A 67-year-old with a new diagnosis of stage III ovarian cancer and a *BRCA2* mutation may be in remission for 5+ years due to her *BRCA2* mutation, which makes her eligible for the use of the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib.[6] Genetic testing as illustrated above has led to decreased cancer-related mortality and prolonged survival.[7] However, many women with such germline mutations are faced with difficult choices about surgical risk reduction, with the potential harms of early menopause and quality of life concerns. Having a test that does not just predict cancer risk but in fact quantifies that risk for the individual would greatly help in these decisions. Furthermore, more than 75% of ovarian cancers occur without a germline mutation.

FAST TRACK

Many women with BRCA1 or BRCA2 are faced with difficult decisions about surgical risk reduction, and liquid biopsies may be able provide quantifiable risk for certain cancers

Advances in genetic testing technology also have led to the ability to obtain genetic information from a simple blood test. For example, cell-free DNA (cfDNA), which is DNA fragments that are normally found to be circulating in the bloodstream, is routinely used as a screening tool for prenatal genetic testing to detect chromosomal abnormalities in the fetus.[8] This technology relies on analyzing fetal free (non-cellular) DNA that is naturally found circulating in maternal blood. More recently, similar technology using cfDNA has been applied for the screening and characterization of certain cancers.[9] This powerful technology can detect cancer before symptoms begin—all from a simple blood test, often referred to as a "liquid biopsy." However, understanding the utility, supporting data, and target population for these tests is important before employing them as part of routine clinical practice.

Current methods of cancer screening are limited

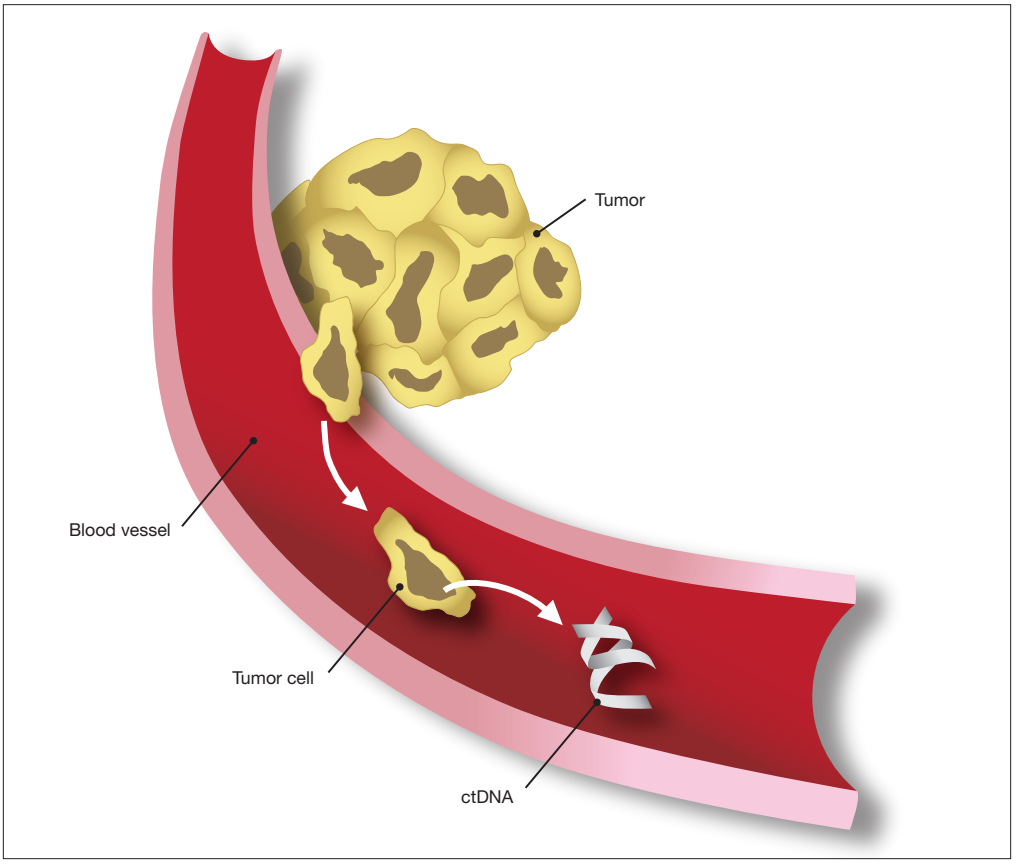
Cancer is a leading cause of death worldwide, with nearly 10 million cancer-related deaths annually, and it may surpass cardiovascular disease as the leading cause over the course of the century.[10,11] Many cancer deaths are in part due to late-stage diagnosis, when the cancer has already metastasized.[12] Early detection of cancer improves outcomes and survival rates, but it is often difficult to detect early due to the lack of early symptoms with many cancers, which can limit cancer screening and issues with access to care.[13]

Currently, there are only 5 cancers: cervical, prostate, breast, colon, and lung (for high-risk adults) that are screened for in the general population (see "Cancer screening has helped save countless lives on page 46").[14] The Pap test to screen for cervical cancer, developed in the 1940s, has saved millions of women's lives and reduced the mortality of cervical cancer by 70%.[15] Coupled with the availability and implementation of the human papillomavirus (HPV) vaccine, cervical cancer rates are decreasing at substantial rates.[16] However, there are no validated screening tests for uterine cancer, the most common gynecologic malignancy in the United States, or ovarian cancer, the most lethal.

Screening tests for cervical, prostate, breast, colon, and lung cancer have helped save millions of lives; however, these tests also come with high false-positive rates and the potential for overdiagnosis and overtreatment. For example, half of women undergoing mammograms will receive a false-positive result over a 10-year time period,[17] and up to 50% of men undergoing prostate cancer screening have a positive prostate-specific antigen (PSA) test result when they do not actually have prostate cancer.[18] Additionally, the positive predictive value of the current standard-of-care screening tests can be as low as <5%. Most diagnoses of cancer are made from a surgical biopsy, but these types of procedures can be difficult depending on the location or size of the tumor.[19]

The liquid biopsy. Given the limitations of current cancer screening and diagnostic tests, there is a great need for a more sensitive test that also can detect cancer from multiple organ sites. Liquid biopsy-based biomarkers

FIGURE Through GRAIL technology, cancer can be detected before symptoms begin



DNA from tumor cells is also known as circulating tumor DNA (ctDNA). ctDNA is found in much lower quantities in the blood stream compared with cell-free DNA, which are DNA fragments normally found to be circulating in the bloodstream, making it difficult to distinguish a cancerous versus a noncancerous cell, and to determine the tumor site of origin. Through innovation, GRAIL was able to optimize their methods of detecting both the presence of cancer cells and the tumor site of origin by focusing next-generation genomic sequencing and methylation. Their development of a methylation-based assay combined with machine-learning allows the test to determine first if there is cancer present or not, and second, the tissue of origin prediction.

can include circulating tumor cells, exosomes, microRNAs, and circulating tumor DNA (ctDNA). With advances in next-generation sequencing, ctDNA techniques remain the most promising.[20]

Methylation-based MCED testing: A new way of cancer screening

Multi-cancer early detection (MCED) technology was developed to address the need for better cancer screening and has the potential to detect up to 50 cancers with a simple blood test. This new technology opens the possibility for early detection of multiple cancers before symptoms even begin. MCED testing is sometimes referred to as "GRAIL" testing, after the American biotechnology company that developed the first commercially available MCED test, called the Galleri test (Galleri, Menlo Park, California). Although other biotechnology companies are developing similar technology (Exact Sciences, Madison, Wisconsin, and Freenome, South San Francisco, California, for example), this is the

Cancer screening has helped save countless lives

- Mammography has helped reduce breast cancer mortality in the United States by nearly 40% since 1990[1]
- Increases in screening for lung cancer with computed tomography in the United States are estimated to have saved more than 10,000 lives between 2014 and 2018[2]
- Routine prostate specific antigen screening is no longer recommended for men at average risk for prostate cancer, and patients are advised to discuss risks and benefits of screening with their clinicians[3]
- Where screening programs have long been established, cervical cancer rates have decreased by as much as 65% over the past 40 years[4]
- 68% of colorectal cancer deaths could be prevented with increased screening, and one of the most effective ways to get screened is colonoscopy[5]

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FAST TRACK

The process to develop and validate GRAIL's blood-based cancer screening test includes 4 large clinical trials of more than 180,000 participants, including those with cancer and those without

first test of its kind available to the public.[21]

The MCED test works by detecting the cfDNA fragments that are released into the blood passively by necrotic or apoptotic cells or secreted actively from tumor cells. The DNA from tumor cells is also known as circulating tumor DNA (ctDNA). CtDNA is found in much lower quantities in the blood stream compared with cfDNA from cells, making it difficult to distinguish a cancer versus a noncancer cell and to determine the tumor site of origin.[22]

Through innovation, the first example of detecting cancer through this method in fact came as a surprise result from an abnormal cfDNA test. A pregnant 37-year-old woman had a cfDNA result suggestive of aneuploidy for chromosomes 18 and 13; however, she gave birth to a normal male fetus. Shortly thereafter, a vaginal biopsy confirmed small-cell carcinoma with alterations in chromosomes 18 and 13.[23] GRAIL testing for this patient was subsequently able to optimize their methods of detecting both the presence of cancer cells and the tumor site of origin by utilizing next-generation genomic sequencing and methylation. Their development of a methylation-based assay combined with machine-learning allowed the test to determine, first, if there is cancer present or not, and second, the tissue of origin prediction. It is important to note that these tests are meant to be used in addition to standard-of-care screening tests, not as an alternative, and this is emphasized throughout the company's website and the medical literature.[24]

The process to develop and validate GRAIL's blood-based cancer screening test includes 4 large clinical trials of more than 180,000 participants, including those with cancer and those without. The Circulating Cell-Free Genome Atlas (CCGA) Study, was a prospective, case-controlled, observational study enrolling approximately 15,000 participants with 3 prespecified sub-studies. The first sub-study developed the machine-learning classifier for both early detection and tumor of origin detection.[25,26]

The highest performing assay from the first sub-study then went on to be further validated in the 2nd and 3rd sub-studies. The 3rd sub-study, published in the *Annals of Oncology* in 2021 looked at a cohort of 4,077 participants with and without cancer, and found the specificity of cancer signal detection to be

99.5% and the overall sensitivity to be 51.5%, with increasing sensitivity by cancer stage (stage I - 17%, stage II - 40%, stage III - 77%, and stage IV - 90.1%).[24] The false-positive rate was low, at 0.7%, and the true positive rate was 88.7%. Notably, the test was able to correctly identify the tumor of origin for 93% of samples.[24] The study overall demonstrated high specificity and accuracy of tumor site of origin and supported the use of this blood-based MCED assay.

The PATHFINDER study was another prospective, multicenter clinical trial that enrolled more than 6,000 participants in the United States. The participants were aged ≥50 years with or without additional cancer risk factors. The goal of this study was to determine the extent of testing required to achieve diagnosis after a "cancer signal detected" result. The study results found that, when MCED testing was added to the standard-of-care screening, the number of cancers detected doubled when compared with standard cancer screening alone.[27,28] Of the 92 participants with positive cancer signals, 35 were diagnosed with cancer, and 71% of these cancer types did not have standard-of-care screening. The tumor site of origin was correctly detected in 97% of cases, and there were less than 1% of false positives. Overall, the test led to diagnostic evaluation of 1.4% of patients and a cancer diagnosis in 0.5%.

Currently, there are 2 ongoing clinical trials to further evaluate the Galleri MCED test. The STRIVE trial that aims to prospectively validate the MCED test in a population of nearly 100,000 women undergoing mammography,[29] and the SUMMIT trial,[30] which is similarly aiming to validate the test in a group of individuals, half of whom have a significantly elevated risk of lung cancer.

With the promising results described above, the Galleri test became the first MCED test available for commercial use starting in 2022. It is only available for use in people who are aged 50 and older, have a family history of cancer, or are at an increased risk for cancer (although GRAIL does not elaborate on what constitutes increased risk). However, the Galleri test is only available through prescription—therefore, if interested, patients must ask their health care provider to register with GRAIL and order the test (https://www.galleri.com/hcp/the-galleri-test/ordering). Additionally, the test will cost the patient $949 and is not yet covered by insurances. Currently, several large health care groups such as the United States Department of Veterans Affairs, Cleveland Clinic, and Mercy hospitals have partnered with GRAIL to offer their test to certain patients for use as part of clinical trials. Currently, no MCED test, including the Galleri, is approved by the US Food and Drug Administration.

Incorporating MCED testing into clinical practice

The Galleri MCED test has promising potential to make multi-cancer screening feasible and obtainable, which could ultimately reduce late-stage cancer diagnosis and decrease mortality from all cancers. The compelling data from large cohorts and numerous clinical trials demonstrate its accuracy, reliability, reproducibility, and specificity. It can detect up to 50 different types of cancers, including cancers that affect our gynecologic patients, including breast, cervical, ovarian, and uterine. Additionally, its novel methylation-based assay accurately identifies the tumor site of origin in 97% of cases.[28] Ongoing and future clinical trials will continue to validate and refine these methods and improve the sensitivity and positive-predictive value of this assay. As mentioned, although it has been incorporated into various large health care systems, it is not FDA approved and has not been validated in the general population. Additionally, it should not be used as a replacement for recommended screening.

FAST TRACK

Although it is not FDA approved at this time, MCED testing has been shown to accurately identify the tumor site of origin in 97% of cases

CASE Resolved

The patient is eligible for the Galleri MCED test if ordered by her physician. However, she will need to pay for the test out-of-pocket. Due to her family history, she should consider germline genetic testing (either for herself, or if possible, for her father, who should meet criteria based on his prostate cancer).[3] Panel testing for

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germline mutations has become much more accessible, and until MCED testing is ready for prime time, it remains one of the best ways to predict and prevent cancers. Additionally, she should continue to undergo routine screening for cervical, breast, and colon cancer as indicated. ●

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