BT-Reveal™ Early Pancreatic Cancer Test

Clinical Utility of the BT-Reveal Early Pancreatic Cancer Test

Overview

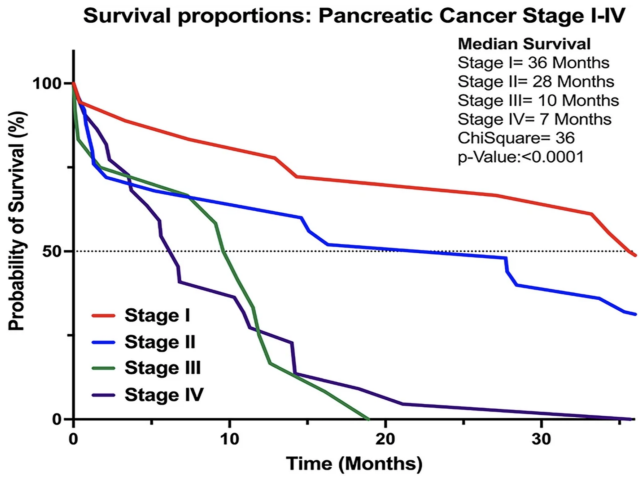
The BT-Reveal™, Early Pancreatic Cancer Detection test, is a non-invasive, qualitative, and Next Generation Sequencing (NGS)-based test for the detection of specific DNA methylation haplotype changes associated with pancreatic cancer which are present in cell-free tumor DNA fragments found in blood samples.

Test is clinically validated to detect early-stage as well as late-stage pancreatic ductal adenocarcinoma (PDAC) with high sensitivity and high specificity. In April 2023, the FDA granted this test a "Breakthrough Device" designation because it demonstrated that it effectively addressed an unmet medical need and, furthermore, that it had the potential to save lives.

Intended Use Population

The intended use patient population for the test are individuals determined to be at high risk of having or developing pancreatic ductal adenocarcinoma (PDAC). The American College of Gastroenterology (AGA), the National Comprehensive Cancer Network (NCCN) and other professional organizations consider patients to be at high risk if they have strong family history of the disease (at least one first degree relative), or have genetic predispositions such as familial atypical multiple mole melanoma syndrome, Peutz-Jeghers syndrome (PJS), Lynch syndrome, or hereditary pancreatitis (HP). Additionally, new onset diabetes (diabetes mellitus) in adults over 50 has also been recognized as associated with a higher risk to develop pancreatic cancer and should be included for high-risk surveillance and monitoring.

This test is not intended for the general population.



Background

Pancreatic ductal adenocarcinoma (PDAC) is widely considered as one of the most lethal diseases worldwide. Despite being a relatively rare form of cancer with a lifetime incidence of 1.6% [1,2], PDAC is the fourth leading cause of cancer-related death in the United States [3]. Since PDAC does not present with specific symptoms, 88% of disease is diagnosed at late stage due to the lack of an effective early detection method[4,5]. With increasing incidence of pancreatic cancer in the US, there are 66,440 estimated new cases and 51,750 estimated cancer deaths in 2024 [2].

The prognosis of PDAC is highly associated with the disease stage at diagnosis [6,7]. As reported, the actual 5-year survival was 31.7% for stage IA tumors (1.3% of the patient population) and decreased to 11.8% in stage IB patients (4.4% of patients), while stage IV tumors (56% of patients) showed an actual 5-year survival rate of 0.5% [6]. However, among the 84,275 PDAC patients in the SEER database, only 7.1% had a localized tumor, 31.2% had regional spread, 56% had distant disease, and 5.7% were unstaged [6]. A study of pancreatic cancer survival in seven high-income countries (ICBP SURVMARK-2) showed that about two-thirds (64%) of pancreatic cancer cases presented with the advanced (metastatic) disease, with survival ranging from 9-19% at 1 year and <5% at 3 years after diagnosis [7].

BREAKTHROUGH GENOMICS

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The Need for Early Detection

The reason most PDAC cases are detected in late stages without surveillance programs is the lack of effective early detection methods. Symptoms, which typically include abdominal and back pain, diarrhea, weight loss, and jaundice, are non-specific for PDAC and may be associated with other gastrointestinal diseases. This complication is particularly common in the diagnosis of chronic pancreatitis (CP), especially because CP patients have a significantly higher risk of developing PDAC in the long run. Thus, accurate PDAC diagnosis methods are needed to screen PDAC patients among these high-risk CP patients; however, current differential diagnoses between CP and PDAC have an accuracy of 65% or lower, leaving much to be desired.

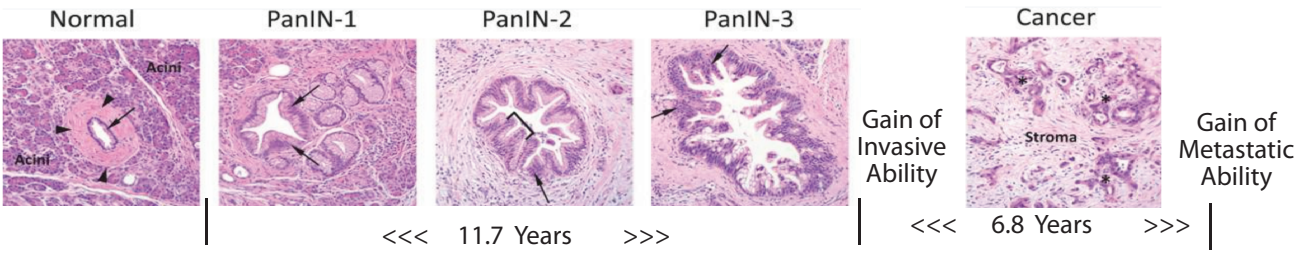
Individuals at increased risk of pancreatic cancer are recommended to undergo screening with endoscopic ultrasound (EUS) or magnetic resonance imaging (MRI) [8-10]. EUS is an invasive procedure which may be associated with increased risk of adverse effects from anesthesia or the procedure itself. MRI also has contraindications such as claustrophobia, contrast allergy, implanted metal, or renal failure. Even though both imaging tests are safe, they can be costly and may result in overdiagnosis [9].

The Earliest Stages of Pancreatic Cancer and Pre-malignant Tumors

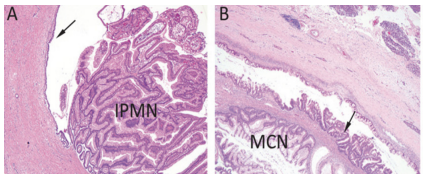
According to the International Cancer of the Pancreas Screening Consortium (CAPS), the primary goal of pancreatic cancer high risk surveillance and early detection program should be to identify and treat precuror lesions. These lesions occur in three categorates: pancreatic neuroendocrine tumours (PanNETs), mucinous cystic neoplasms (MCNs) and intraductal papillary mucinous neoplasms (IPMNs) with varying degrees of potential malignancy [10]. It is important to note that while up to 50% of patients in high risk programs are often found (through imaging) to have pancreatic cysts, only a minority of these cysts will develop into cancer. This creates a signficant challenge for continued surveillance since the majority of these cysts are small, asymtomatic, and present with non-specific characteristics in EUS imaging.

Another confounding factor in resolving the malignancy of pancreatic cysts is that there is currently no safe procedure to determine if a cyst is cancerous or not. Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) is the commonly used gold-standard method to obtain pathological diagnosis; however, this procedure is invasive and has been linked to bleeding and/or tumor dissemination. Highlighing the limitations of clinical imaging for diagnosing early stage pancreatic cancer, the AGA in the their most recent clinical practice update emphasizes the need for "alternative or complementary approaches, including biomarkers." [8]

Morphological Progression of Pancreatic Cancer Carcinogenesis



Above - In its eary stages PDAC develops slowly, demonstrating the overall feasibility of early detection. PanIN is the term used to describe microscopic precursor lesions tha arise in small calibre (<5mm) pancreatic ducts that give rise to conventional ductal adenocarcinomas (PDAC).



Left - low power view of 2 types of pancreatic cysts. IPMN and MCN cysts are considered to be precursor stages to PDAC development, but can be difficult for MRI or EUS procedures to resolve. Note In this example (B) the neoplasm also contains foci of borderline to high-grade dysplasia (indicated by arrow) suggesting this cyst is malignant.

Iacobuzio-Donahue, C Gut 2012;61:1085e1094

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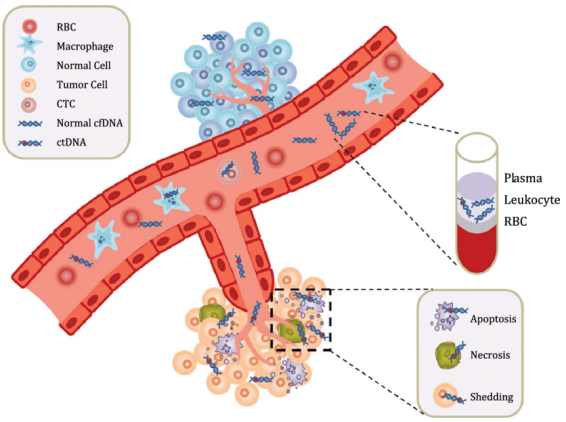
BT-Reveal - Biochemical Overview and Analytical Performance

Understanding How the Test Works



Non-invasive early detection in cell-free DNA. Majority of cf-DNA is from normal cells or food uptake. When a patient has pancreatic cancer, a small percentrage of their cf-DNA will have unique DNA methylation signatures that can be identified and are specific to pancreatic cancer.

The Breakthrough Genomics BT-Reveal™ test is a laboratory-developed next-generation sequencing (NGS) assay for the qualitative detection of aberrant methylation from bisulfite-treated cell-free DNA isolated from patient plasma, where whole blood is collected and transported in Streck tubes. The assay results are analyzed by bioinformatics pipeline which interrogates 59 specific methylation haplotype regions associated with the occurrence of pancreatic ductal adenocarcinoma. The DNA methylation haplotype technology is licensed from University of California San Diego (UCSD).

Starting with cell-free DNA (extracted from plasma out of whole bood), the test includes a patented technology that is able to retain up to 80% of input DNA (compared to 20% with other technologies) which significantly aids in the detection of rare tumor DNA moleculres. Then the 59 specific gene regions bearing the PDAC associated methylation signals are amplified by PCR. One region may have as few as a single CpG methylation site or up to dozens of CpG sites, and contiguous methylation patterns are recognized as a 'methylation haplotype' (i.e., a string of contiguous methylation states interpreted in cis)[18].

Published Clinical Study

The clinical validity of the test has been described in a published study which included 90 tissues and 546 plasma samples collected from 232 PDAC patients, 23 chronic pancreatitis (CP) patients, and 323 healthy controls19. Among 223 PDAC cases with known stage information, 43/119/38/23 cases were of Stage I/II/III/IV. This test showed a sensitivity of 80% (59-93%) in Stage I, 68% (52-81%) in Stage II, and 87% (66-97%) in Stage III/IV PDAC cases. The test specificity was determined to be 89% (75-97%)[19]. If combined with CA 19-9, the assay sensitivity further improved to as high as 92% (74-99%) in Stage I PDAC cases which surpasses all current PDAC screening tests.

FDA Submission and Further Improvement

Since the publication the paper (Nov 2022), the test was further improved by deploying deep neural network (DNN) machine learning based models within the bioinformatic analysis.

Clinical Validation Performance Overview (PDAC Detection)

Overall Sensitivity | Overall Specificity

Test Sensitivity and Specificity Scores submitted to the FDA include both early and late stage PDAC detection and are based on a training set of 334 unique samples and a pre-clinical validation set of 199 samples that were submitted to the FDA as part of the application for the FDA's Breakthrough Device Designation. As part of FDA application, the test also surpassed the FDA's requirements for evaluating the test's performance after taking into account compounding factors such as age, sex, race, obesity, and smoking/non-smoking.

BT-Reveal™ Early Pancreatic Cancer Test

Clinical Utility - Early Detection and Resolving Inconclusive Clinical Imaging and improved Surveillance of Pancreatic Cysts

Type of Risk Factor	Specific Factor	Germline Mutation / Gene	Increased Lifetime Risk	Qualify for Test?	Recommended Age of Initiation of screening regime	Frequency of Recommended Surveillance
Genetic Risk	Peutz–Jeghers syndrome	STK11	132x	YES	Age 35	Annual or less
Genetic Risk	Hereditary Pancreatitis	Predominantly PRSS1 but also PINK1, and CFTR	40% lifetime risk related to chronic pancreatitis	YES	Age 40	Annual or less
Genetic Risk	familial atypical multiple mole melanoma syndrome	CDKN2A	13x - 39x	YES	Age 40	Annual or less
Genetic Risk	Lynch Syndrome	MLH1, MSH2, MSH6	8.6x - 11x	YES	Age 50**	Annual
Genetic Risk	Li-Fraumeni syndrome	TP53	4x - 13x	YES	Age 50**	Annual
Genetic Risk	Hereditary Pancreatic Cancer	BRCA2* PALB2 BRCA1	3.5x - 6.2x	YES	Age 50**	Annual
Genetic Risk	Hereditary Pancreatic Cancer	CDKN2A, ATM,	4x - 13x	YES	Age 50**	Annual

Clinical Utility: Early Detection and Interval Screening of High-Risk Individuals

Both the AGA and the American Society of Gastrointestinal Endoscopy (ASGE) recommended pancreatic cancer screening in high-risk individuals after having systemically reviewed published studies showing survival benefits of the surveillance programs. A similar screening regimen has also been recommended by the CAPS Consortium[10] showing that most pancreatic cancers diagnosed within the CAPS high-risk cohort in the recent years (2014-2021) have had Stage I disease and long-term survival[11]. In a long-term follow-up study, most PDACs detected during surveillance (9/10) were resectable, and 85% of these patients survived for 3 years or more[12].

The AGA, NCCN, and others recommend annual screening of high risk individuals with shortened intervals for individuals with specific genetic risk factors. Survival benefits as well as cost-effectiveness of pancreatic cancer surveillance have also been demonstrated in Netherland (Leiden University) in individuals carrying CDKN2A-p16 gene mutations where 71% of PDAC tumors identified during surveillance could be resected[13,14]. These patients had considerable better survival and the annual surveillance was found to be cost-effective[14].

Clinical Utility: Resolving Inconclusive Clinical Imaging and Improved Surveillance of Pancreatic Cysts

As evidenced by statements by the AGA, there is a clear need within secondary Gastroenterology Centers and centers performing pancreatic endoscopies for a safe and non-invasive biochemical test to complement existing MRI and EUS technology. Most critically, a blood-based biochemical test can help practicioners determine whether more invasive diagnostic procedures are necessary for individuals patients who present with non-specific or asymptomatic pancreatic cysts. This not only will address the problem of overdiagnosis and the complications that can result from these procedures, but will also have significant cost savings to both institutions and individual patients.

Clinical Utility: Informing diagnosis of worrisome, non-specific GI symptoms

Primary care doctors and GI specialists also encounter patients with pancreatitis and other non-specific symptom that can be difficult to resolve with traditional modalities, including the need to rule out pancreatic cancer. Included in this category where the BT-Reveal can play a role in diagnosis is pancreatitis (which can present in a variety of ways), unspecified back and stomach pain, jaundice, and unintended weight loss .

Therefore, as a noninvasive, highly sensitive and specific test, BT-Reveal™ has great clinical utility in three distinct use cases. First, as an essential and safe tool for periodic pancreatic cancer surveillance in high-risk individuals, second as a complementary test in GI endoscopy centers who are confronted with indeterminate imaging results of pancreatic cysts and other precursor lesions that may or may not be malignant, and third when patients present with potential clinical indications for pancreatic cancer, such as pancreatitis, unspecifc back or stomarch pain, jaundice, or unintended weight loss.

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