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Previstage™ GCC Colorectal Cancer Staging Test

A New Molecular Test to Identify Lymph Node Metastases and Provide More Accurate Information about the Stage of Patients with Colorectal Cancer

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Abstract

- Colorectal cancer (CRC) is the third most common cancer and a leading cause of death for both men and women in North America.
- The staging of the CRC at the time of diagnosis is the single most important prognostic factor in determining recurrence and survival.
- Until 2008, accurate evaluation of CRC stages I and II was based on examination of regional lymph nodes (LNs) under a microscope to identify cancer cells. This method can detect one cancer cell in 200 normal cells, but analyzes only a fraction of the available tissue from the LN (less than 0.1%).
- Up to 30% of patients assessed by traditional histopathology methods as having stage II disease (negative LNs) experience a recurrence of their cancer.
- Previstage™ GCC Colorectal Cancer Staging Test, a new molecular diagnostic test, is able to identify patients at high risk of recurrence by examining their LNs for guanylyl cyclase C (GCC).
- GCC is a marker found in cells lining the lumen of the gastrointestinal tract. The expression of GCC is conserved in CRC and metastatic disease.
- Using an ultrasensitive quantitative reverse transcription (RT)-PCR, the test interrogates a patient's LN tissues to identify GCC levels consistent with metastatic (stage III) disease.
- The technology employed in Previstage™ GCC is nearly 100 000 times more sensitive than microscopic staging methods. This molecular diagnostic test allows a more thorough examination of LNs and has an analytic sensitivity of 92% and a specificity of 98%. Such a test can be used to overcome the limitations of staging by traditional histopathology alone.

Previstage™ GCC Colorectal Cancer Staging Test – key features	
Diagnostic indication	
To investigate the lymph nodes (LNs) of patients with colorectal cancer to detect the marker, guanylyl cyclase C (GCC), for metastatic disease	
Assay characteristics	
Technology basis	Quantitative RT-PCR is used to detect GCC in LN tissues
Measured parameter	GCC mRNA
Sample type	Formalin-fixed paraffin-embedded (FFPE) whole or half LNs A minimum of 12 LNs per patient is requested
Performance measures	
Sensitivity	92%
Specificity	98%
Requirements	
Test location	DiagnoCure Oncology Laboratories, West Chester, PA, USA
Equipment	Homogenizer instrument PCR System
Training	DiagnoCure Oncology Laboratories is CLIA-certified and staffed by pathologists, oncologists and laboratory professionals
Time to result	7 business days from the date of receipt of the LNs
Cost	
$US3500 for 15 LNs	

1. Disease Background

Colorectal cancer (CRC) is the second most common cause of cancer death (men and women combined) in North America, where in 2008 it was estimated that more than 170 000 new cases of CRC would be diagnosed and more than 58 000 people would die of the disease.[1,2]

In Canada, one in 14 men is expected to develop colorectal cancer during their lifetime and one in 27 will die of it. One in 16 women is expected to develop colorectal cancer during their lifetime and one in 31 will die of it.[1] In the US, about 72% of cases arise in the colon and about 28% in the rectum.[2]

At the time of diagnosis, 36% of CRC patients have localized or lymph node-negative disease (stages I and II) and 37% have regional or lymph node-positive disease (stage III).[1,2] It is critically important to accurately differentiate between these groups of patients because of the significant effect of staging on prognosis and further treatment.

The most significant risk factor for disease recurrence in CRC is the stage of the disease at diagnosis.[3,4] Staging a patient with colorectal cancer is crucial because it establishes the patient's course of treatment and helps determine the need for additional treatment following surgery to remove the tumor. Appropriate treatment decisions depend on accurate staging.

Current staging methods are not sufficiently sensitive to detect occult metastases, which could indicate a patient's increased risk of recurrence and need for systemic chemotherapy. Examination of lymph nodes (LNs) for metastases by traditional histopathologic methods results in understaging in up to 30% of patients who later go on to develop metastatic disease. Consequently, some centers recommend chemotherapy for all patients with stage II (LN-negative) disease in order to prevent a recurrence in the approximately 25–30% who are at increased risk.

It is important to improve methods for the identification of patients who appear to be free of disease after surgical removal of their tumor, but later develop recurrent cancer, and to identify with more certainty those patients who can be spared unnecessary chemotherapy treatment because their risk of recurrence is low.

2. Current Diagnostic and Staging Methods

Most patients diagnosed with CRC will undergo some type of surgery. Adjuvant treatments, such as chemotherapy and radiation, may also be recommended. Treatment decisions are based on the stage and location of the cancer as well as the risks and benefits associated with the available treatment options.

The stages of CRC include stage 0 (a small tumor limited to the inside lining of the colon or rectum), stage I (the tumor has invaded the colon or rectum, but has not spread beyond the wall), stage II (the tumor has spread beyond the wall into local tissue, but has not spread to the LNs), stage III (the tumor has spread to LNs, but not to distant organs or tissues), and stage IV (the tumor has spread to distant organs or tissues).

Staging begins at the time of diagnosis when patients undergo a series of non-invasive tests, including computed tomography (CT) scans, magnetic resonance imaging (MRI) and positron emission tomography (PET) scans, to evaluate the extent of their cancer.[5]

Testing identifies most patients with stage IV disease prior to surgery. Once patients are identified as having stage IV CRC, the goal of surgery is to relieve or prevent blockage and prevent other complications.

For patients without evidence of distant spread, staging will rely on findings at surgery and examination of the surgical specimens for tumor spread.

For patients with rectal cancer, surgery to remove the tumor is the main treatment. Treatment with chemotherapy and/or radiation may be used before surgery (neoadjuvant) and after surgery (adjuvant) to reduce the risk of recurrence.

The standard surgical treatment for localized colon cancer is removal of a length of colon on either side of the tumor as well as removal of regional LNs for further examination for metastases. If cancer cells are detected in regional LNs by traditional histopathology, the patient is identified as having stage III disease, which signifies an increased risk of cancer recurrence following surgery to remove the tumor. Adjuvant therapy is currently recommended for all patients with stage III disease.

The current method for examining LNs for metastases relies on visual examination of a single small sample taken from each LN harvested at surgery. With traditional histopathology, LNs are fixed, sectioned, and stained with hematoxylin and eosin for examination under a microscope. The standard practice is to remove a 5 μm slice from each LN for visual inspection. This method can detect one cancer cell in 200 normal cells,[6] but analyzes only a fraction of the available tissue from the LN (less than 0.1%).

Based on this limited sampling of harvested LNs, patients are classified as having LN-negative (stage I or stage II) disease or LN-positive (stage III) disease. Up to 30% of patients with LN-negative CRC as classified by traditional histopathology (stage I or stage II disease) will experience a recurrence of their cancer.

For patients with melanoma and breast cancer, intraoperative LN mapping and sentinel LN biopsy have been demonstrated to accurately predict regional LN involvement. The value of these procedures is to spare patients unnecessarily extensive surgery and the postoperative sequelae of regional

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LN dissection. However, since most patients with localized CRC undergo bowel resection with regional lymphadenectomy, the value of this approach in CRC has not been established. Importantly, sentinel LN biopsy does not address the possibility of error due to limited sampling within the LN.

Carcinoembryonic antigen (CEA), cytokeratin-19 (CK-19), gastrointestinal tumor-associated antigen-733.2 (GA733.2), and mucin-1 (MUC-1) are among the markers that have been evaluated as messenger RNA (mRNA) markers to detect metastases in breast cancer using an ultrasensitive RT-PCR assay. However, they do not have diagnostic value as mRNA markers for the detection of micrometastases because they are also expressed in the blood and lymph nodes of patients without cancer.[7]

Immunohistochemistry (IHC) has been used to assess the location and frequency of micrometastases in patients with CRC, but was found to be of limited value due to its limited reproducibility.[8] Likewise, this procedure does not address the possibility of a sampling error within the LN.

Improvements in diagnostic technology are warranted to overcome the known limitations of current methodologies, which include sampling errors and a lack of sensitivity, in order to identify patients at increased risk of recurrence following surgery.

3. Previstage™ GCC Colorectal Cancer Staging Test

Guanylyl cyclase C (GCC) shows promise as a marker to augment the traditional staging of CRC and to identify those patients with stage I and II CRC who have metastases not detected by visual examination and for whom adjuvant treatment may be warranted. Unlike other methods of LN evaluation, Previstage™ GCC examines 100% of the available tissue from each LN tested.

GCC is a transmembrane receptor protein expressed specifically in the gastrointestinal tract from the duodenum to the rectum.[9] It remains expressed in colorectal cancer and in metastases and can be easily measured by quantitative RT-PCR with <100 copies of GCC mRNA/reaction.[10] In CRC, GCC has been shown to be able to identify cohorts of patients at high risk of recurrence as well as those patients whose cancer did not recur.[11,12]

The novel assay evaluates 12 or more LNs (whole or half, formalin-fixed paraffin embedded [FFPE]) from an individual patient for the presence of GCC mRNA. Using ultrasensitive quantitative RT-PCR, the assay interrogates LNs to identify levels of GCC mRNA consistent with those found in LNs with histologically-confirmed metastases from patients with stage III CRC.

This test is designed to detect occult metastases that would have been identified by histopathologic examination if the entire node (or half-node) were examined instead of a single 5 μm section. This unique methodology minimizes the sampling error inherent in traditional histopathologic examination of LNs and provides more accurate information for staging the patient with CRC. The assay shows great promise in identifying occult metastases in LNs from patients classified as having stage I or II disease by histopathology alone.

3.1 Measuring GCC mRNA in the Previstage™ Assay

FFPE LN halves are homogenized and the nucleic acids are extracted and amplified. GCC mRNA levels are measured by quantitative RT-PCR using an analytical standard curve for accurate GCC load determination. The lower limit of detection of this assay is 100 copies per reaction (312.5 ng of LN RNA). β-actin, a housekeeping gene, serves as an internal control to ensure proper reaction amplification. The analytical cut-off is the level above the limit of detection of the assay, and is a value comparable to the levels detected in histopathology-positive stage III patients, who are known to harbor LN metastases. A positive result is one in which the level of GCC mRNA in a patient's LN is consistent with that of a true histopathology-positive LN from a patient with stage III disease, and indicates the presence of metastases. A negative result indicates that the patient's LN had a GCC mRNA value below the limit of detection of the assay and below that characteristic of a LN harboring true CRC metastases.

3.2 Analytical Performance of the Previstage™ Assay

Amplification reaction efficiencies average 98 ± 4% and reaction linearity averages 0.994 ± 0.004%, with a limit of detection of 100 copies of GCC mRNA per reaction. The dynamic range is 4 logs. The analytical accuracy and precision (coefficient of variation [CV]) are 86 ± 4% and 5.1%, respectively, when LN extracts are spiked with 1000 GCC synthetic mRNA transcripts.

The Previstage™ GCC Colorectal Cancer Staging Test was validated as a laboratory-developed test on >1000 LN specimens from patients with stage I, II, and III colon cancer and non-cancerous gastrointestinal diseases.

4. Discussion

The Previstage™ GCC Colorectal Cancer Staging Test provides useful information for patients and their physicians to

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help guide treatment decisions. By providing more sensitive detection of nodal metastases, the test allows more accurate CRC staging, thereby informing clinical decisions regarding additional treatment after surgery. The Previstage™ GCC Colorectal Cancer Staging Test can be used as an adjunct to traditional histopathology to confirm that LNs are negative and no additional treatment is needed. This should result in fewer low-risk patients being exposed unnecessarily to chemotherapy, and more high-risk patients being accurately identified and treated. More accurate staging has the potential to lead to more effective treatment, fewer recurrences and lower healthcare costs.

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