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Previstage™ GCC test for staging patients with colorectal cancer

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Abstract

The presence of tumor cells in regional lymph nodes is the most important prognostic and predictive marker in staging patients with colorectal cancer. Cancer cells in lymph nodes are associated with a poorer prognosis and an increased risk of recurrent disease. Additionally, nodal metastases identify patients who derive maximum benefit from adjuvant therapy. However, traditional paradigms for staging patients with colorectal cancer underestimate the extent of metastases and patients whose lymph nodes are ostensibly free of tumor cells by histopathology (pN0) have a 25–30% risk of developing recurrent disease, reflected by the presence of occult nodal metastases. These observations underscore the unmet clinical need for molecular approaches to accurately detect metastatic disease and identify patients at risk for disease relapse that could benefit from adjuvant chemotherapy. Detection of disease-specific mRNA targets as prognostic and predictive markers employing quantitative reverse transcription (qRT)-PCR is an emerging technology that has become a benchmark for individualization of patient management. However, to date, applications of qRT-PCR to detecting occult nodal metastases in colorectal cancer have been equivocal, reflecting markers with suboptimal sensitivity and specificity; limitations of utilizing qualitative, rather than quantitative, RT-PCR; and underpowered study designs based on inadequate patient populations. In that context, guanylyl cyclase C (GCC) is the most sensitive and specific biomarker for metastatic colorectal cancer in extra-intestinal tissues. GCC qRT-PCR detects occult metastases in lymph nodes, providing the most powerful independent prognostic information for predicting disease recurrence in pN0 patients in prospective multicenter clinical trials. This technology forms the basis for the Previstage™ GCC Colorectal Cancer Staging Test encompassing a proprietary multiplex qRT-PCR assay compatible with formalin-fixed, paraffin-embedded lymph nodes for detecting occult metastases. Previstage GCC is a new diagnostic tool that may improve the accuracy of staging,

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prognosis of clinical outcomes and prediction of therapeutic responses to adjuvant therapy, representing a key advance in the management of patients with colorectal cancer.

Keywords

colorectal cancer; guanylyl cyclase C; lymph node; metastatic disease; predictive marker; prognostic marker; qRT-PCR; quantitative reverse transcriptase-polymerase chain reaction; staging

Management of patients with colorectal cancer depends on the ability to predict a patient's prognosis and response to adjuvant chemotherapy. Metastases to lymph nodes represent the single most important prognostic factor related to disease recurrence and predictive markers driving therapeutic decisions [1]. The emergence of molecular diagnostics provides an opportunity to individualize therapy for patients to optimize disease outcomes. Moreover, quantification of disease-associated mRNA by real-time quantitative reverse transcription PCR (qRT-PCR) can estimate tumor burden, with the goal of becoming an essential analytical tool for the detection of mRNA targets in tissue biopsies. Colorectal cancer provides an instructive example for the application of this, enabling technology for disease detection. Thus, qRT-PCR in conjunction with a highly specific marker for metastatic colorectal cancer, such as guanylyl cyclase C (GCC), should provide a more accurate estimate of metastatic cancer cells and, consequently, disease stage compared with traditional approaches including standard histopathology. Limitations of this standard paradigm are underscored by the considerable prognostic heterogeneity of patients with pN0 colorectal cancer and 25–30% ultimately die of recurrent disease, reflecting occult metastases overlooked by standard histopathology. This review provides an assessment of the practical application of GCC qRT-PCR-based molecular diagnostics for prognostic and predictive staging of patients with colorectal cancer.

Colorectal cancer

Colorectal cancer continues to be the second leading cause of death from cancer and is the third most common cancer in men and women, after cancers of the lung and prostate and lung and breast, respectively [2]. Approximately 150,000 new cases of invasive colorectal cancer are expected among men and women in the USA leading to almost 50,000 deaths [3], representing 10% of cancer-related deaths. The risk of developing colorectal cancer is influenced by both environmental and genetic factors and it affects approximately 1 million people worldwide [4].

Pathologic stage represents the most important prognostic factor influencing management decisions [5]. The tumor–node–metastasis system is the most commonly used staging system and is based on clinicopathological features such as depth of invasion of the bowel wall, extent of regional lymph node involvement and presence of distant sites of disease [6]. The depth of tumor invasion defines the T stage, increasing from T0 (confined to the epithelium) to T4 (full-thickness penetration of the bowel wall). As the depth of tumor invasion increases, the risk for regional nodal and distant spread also grows. Examination of local lymph nodes by standard histopathology categorizes the N stage, represented by N0 (no lymph nodes involved), N1 (1–3 lymph nodes involved) and N2 (>3 lymph nodes involved), while M stage addresses the presence of distant metastases. This staging system further stratifies colon cancer into clinicopathologic stages, including stage I (T1–T2, N0), stage II (T3 or T4, N0, M0) and III (any T, N1–4, M0). Mortality is closely related to clinicopathologic stage in these patients, with lymph node status being the most important prognostic marker [7]. Moreover, adjuvant chemotherapy is recommended for patients with lymph node metastases (stage III) and for selected patients without lymph node metastases (stage II) but with adverse prognostic features such as tumors with poorly differentiated histology or lympho-vascular or perineural invasion

by tumor cells. However, clinical staging often fails to discriminate the biologic behavior of a number of tumors resulting in systematic over- or undertreatment with adjuvant chemotherapy [8]. Thus, adequate lymph node staging of patients with colorectal cancer is important for determining prognosis and planning therapeutic management.

The accuracy of staging and overall survival in colorectal cancer increases proportionally with the number of lymph nodes examined, and numerous studies support the conclusions of American Joint Committee on Cancer guidelines that the minimum number of lymph nodes for adequate staging should be 12–18 [9]. Indeed, examination of fewer lymph nodes has been linked with poorer outcome in patients with node-negative and -positive disease. Examination of fewer lymph nodes may reflect a less complete surgical procedure or an inadequate inspection of the pathologic specimen, erroneously leading to understaging of the tumor and the subsequent omission of potentially beneficial adjuvant therapy.

Surgical resection is the primary treatment modality for colorectal cancer and its outcome is most closely related to the extent of disease at presentation. Approximately 20% of patients initially present with distant metastatic lesions [10] and another 30% of patients with no metastases detected preoperatively or intra-operatively eventually recur after curative surgery, reflecting the presence of occult metastases [4]. Adjuvant chemotherapy is regularly offered to patients with lymph node metastasis but, despite an overall better prognosis, patients with lymph node-negative colorectal cancer also have a significant risk of relapse, with a recurrence rate of approximately 25% in patients with lymph nodes free of tumor cells by histopathology (pN0) [10,11]. Despite this substantial risk of disease recurrence, chemotherapy in pN0 colon cancer is neither mandated nor routine and many variables are considered before offering adjuvant therapy to this cohort of patients, including the choice of treatment and the potential for short- and long-term toxicities [9].

Given the established relationship between lymph node metastases and prognosis, the risk of recurrence in pN0 patients suggests the presence of occult metastases not initially identified by histopathology at the time of surgical resection. Thus, it is important to optimize lymph node sampling and laboratory techniques to accurately assess occult metastases in regional lymph nodes. Beyond enhancing the accuracy of prognosis and risk stratification, precise evaluation of lymph node metastases could identify pN0 patients who would most benefit from adjuvant chemotherapy.

Current molecular technologies for colorectal cancer staging

Traditional histopathology remains the mainstay of colorectal cancer staging and its accuracy depends on adequate lymph node harvesting, in the context of the established prognostic relationship between number of lymph nodes analyzed and clinical outcomes [12].

Unfortunately, prognostic lymph nodes in resection specimens may be overlooked, resulting in underestimation of micrometastatic foci. Up to 70% of tumor-containing lymph nodes harbor metastases that are less than 0.5 cm in diameter, which often escape detection by surgeons and standard pathology dissection [1]. Furthermore, small deposits of tumor cells within lymph nodes escape detection, reflecting limitations in the volumes of tissue sampled, where staining of only a few 10- μ m sections omits from inspection most of the material for review, and sensitivity, where traditional histopathology can detect one tumor cell in 200 normal cells [13]. The importance of these limitations is brought into specific relief by considering the pathology categories of lymph node colonization by tumor cells, including metastases, with diameter greater than 0.2 cm, micrometastases, with implants from 0.02 to 0.2 cm in diameter, and isolated tumor cells, with implants less than 0.02 cm in diameter [14].

Beyond histopathology, more recent enabling technologies permit detection of occult tumor cells by immunohistochemistry [pN0(i+)] or by molecular techniques such as RT-PCR [pN0

(mol+)) [15]. In this context, molecular staging provides the most sensitive and specific assessment of metastatic disease [7,8,15]. Indeed, molecular staging offers a technological advancement, enhancing the resolution of occult metastases with the ability to sample the entire available tissue specimen and detect one tumor cell in approximately 10 million normal cells [7,16,17]. However, it should be noted that the relationship between occult metastasis detected by these techniques and the prognosis of patients has yet to be established.

Immunohistochemistry-based lymph node analysis for staging

Immunohistochemical staining using antibodies to antigens expressed by epithelial cells identifies tumor cells overlooked by standard histopathology to add prognostic and predictive value to current staging paradigms. Proteins most commonly employed as markers to identify micrometastases in colorectal cancer patients include cytokeratins (CKs) and carcinoembryonic antigen (CEA) [12]. In other studies, immunohistochemistry employed VEGF [18], EGF receptor [19], β -catenin, adenomatous polyposis coli [20], a variety of SMAD proteins [21], and the TGF β receptors TGF β RI and TGF β RII [22,23]. Interestingly, while immunohistochemical analyses of CKs suggest that micrometastases in regional lymph nodes are an important prognostic factor in pN0 gastric cancers [24], the prognostic value of immunohistochemistry in pN0 colorectal cancer remains controversial. In some series, CK-positive cells within lymph nodes predicted poorer prognosis [25] while other studies have failed to corroborate that relationship [26–29]. Of 15 reported studies in which patients were staged employing CEA or CK immunohistochemistry, 11 suggested that the immunohistochemical identification of occult micrometastases did not provide prognostic information useful for patient management [12]. Heterogeneous results across studies could reflect antibody selection, which defines sensitivity and specificity [30] essential for identification of occult metastases [15]. Similarly, selection of the marker is critical and the absence of prognostic utility in previous studies could reflect limitations in marker specificity, yielding false-positive results [12]. Moreover, variations in techniques, including tissue sampling, sectioning techniques and volumes of tissue analyzed can impact immunohistochemistry sensitivity and specificity [31]. Although immunohistochemistry in the context of serial sectioning can detect occult micrometastases in up to 25% of node-negative patients, this approach is too resource- and time-intensive to be practical for routine analysis of all lymph nodes in the anatomical pathology setting [32].

Nucleic acid-based amplification of molecular markers & staging

RT-PCR to detect disease-specific mRNA in histologically negative lymph nodes can identify a single tumor cell in up to approximately 10 million normal cells [17], detecting occult metastases in up to 40–50% of patients [33]. Until recently, these techniques have required fresh or frozen tissue, but with the availability of techniques for formalin-fixed, paraffin-embedded tissue, these analyses have become more tractable for routine use. Application of RT-PCR to colorectal cancer patients have primarily used CKs, CEA, CDX1, TAC-STD1 and villin [15]. Early studies using CEA RT-PCR in stage II colorectal cancer patients detected occult metastases in lymph nodes of 54% of patients, who exhibited an adjusted 5-year survival rate of 50%. By contrast, patients without occult metastases exhibited a survival rate of 91%, suggesting that molecular detection of occult metastases is a prognostic tool in stage II colorectal cancer [34]. Similar results were obtained in a study of 64 colorectal cancer patients employing paraffin-embedded lymph nodes suggesting that those expressing CEA mRNA in histopathology-negative nodes had a significantly worse prognosis than those who were RT-PCR-negative with respect to disease-free and overall survival [30]. However, in other studies, CEA RT-PCR exhibited low sensitivity and specificity and was without prognostic value for detecting micrometastases by RT-PCR [35]. CEA is expressed by fewer than 80% of colorectal

cancers, limiting its sensitivity and it is expressed by normal intestinal tissues and other tissues involved in pathologic processes such as inflammation, contributing to a low specificity [12].

Other studies of RT-PCR in colorectal cancer have employed CK8, CK18 and CK19, which were unsuitable because of low specificity [36]. By contrast, detection of CK20 by RT-PCR correlated closely with the risk of developing recurrent disease [37] in histopathology-negative lymph nodes but with the restriction that a laborious qRT-PCR procedure was required to achieve diagnostic specificity [38]. While these observations suggest that CK20 RT-PCR may be useful for identifying clinically significant occult metastases in colorectal cancer [39], other studies have not confirmed these results [40]. However, these latter studies are limited by small numbers of patients, the absence of clinical follow-up, or both [36].

Of significance, meta-analyses support the prognostic significance of occult metastases in histopathology-negative lymph nodes detected by RT-PCR in patients with colorectal cancer [7,15]. However, it is important to recognize the potential pitfalls of this approach, which include high, but variable, amplification rates, which may produce false-positive results, technical challenges that can be overcome by employing markers highly specific for the presence of occult metastases in a quantitative format (qRT-PCR). The potential utility of combining a specific molecular marker and a powerful amplification technique such as RT-PCR to identify clinically important occult metastases in the management of patients with colorectal cancer will not only be more informative than qualitative data, but will contribute to assay standardization and quality management.

Guanylyl cyclase C is a selective marker for metastatic colorectal cancer

GCC is a receptor for the paracrine intestinal hormones guanylin, uroguanylin and the bacterial heat-stable enterotoxin (ST), a principal mediator of endemic secretory diarrhea worldwide. It is selectively expressed in apical membranes of intestinal mucosal cells, from duodenum to rectum [41,42]. GCC and its paracrine hormone ligands organize and maintain the homeostasis of the crypt-villus axis, coordinating and integrating proliferation, genomic integrity, metabolic programming and mesenchymal remodeling essential for normal intestinal function [42]. Selectivity in expression in intestinal mucosal cells persists upon neoplastic transformation [43] and GCC is universally overexpressed, at protein and mRNA levels, by primary and metastatic colorectal tumors, regardless of stage, grade or anatomic location, but not by extragastrointestinal tissues or tumors [44–46].

Universal selective overexpression of GCC by colorectal tumors provides the opportunity for broad diagnostic coverage with unique characteristics of sensitivity and specificity, which can be translated to identify metastatic cancer cells in extra-intestinal sites. Circulating metastatic colorectal tumor cells were detected in blood from some patients with stage II colorectal cancer and all patients with stage III and IV colorectal cancer, but not in blood from normal subjects or patients with stage I colon cancer or other nonmalignant intestinal pathologies [12,45,47, 48]. In addition, occult metastases were detected in up to 25% of histologically negative lymph nodes tested for GCC [49]. Moreover, in patients with stage II colorectal cancer, who by definition are ostensibly free of tumor cells by histopathology, GCC expression was detected in up to 18% of lymph nodes, suggesting that the clinical staging of patients could be enhanced by employing GCC RT-PCR [44].

Colorectal cancer staging employing GCC RT-PCR

Considering the established relationship between lymph node metastasis, prognosis and disease recurrence, there is an unmet clinical need to more accurately stage patients with colorectal cancer. RT-PCR amplifies specific transcripts encoding disease-specific markers which can detect a single tumor cell in approximately 1×10^7 normal cells [50], approximately three-

times the sensitivity of immunohistochemistry [36]. RT-PCR is a sensitive and specific method to quantify the expression of specific disease-associated mRNA transcripts in nucleic acids extracted from tissue samples [51]. Moreover, current meta-analyses support the detection of occult metastases in lymph nodes from patients with colorectal cancer using RT-PCR, suggesting that this technology is a more accurate approach for identifying pN0 patients who are at higher risk for disease recurrence and might benefit from adjuvant therapy [7,15]. In that context, although amplification technologies provide a highly sensitive method for cancer cell enumeration, the availability of tumor markers with sensitivities and specificities sufficient to reliably quantify occult colorectal cancer cells have lagged [40].

Selective overexpression of GCC by human colorectal tumors, but not by other tissues and tumors, suggests that this receptor may fulfill criteria for a highly sensitive and specific molecular marker for occult metastases [51]. Indeed, GCC RT-PCR detected occult metastases in lymph nodes of pN0 patients undergoing staging for colorectal cancer [44]. Moreover, in a retrospective analysis, detection of GCC expression by qualitative RT-PCR in lymph nodes from pN0 patients was highly associated with the development of recurrent colorectal cancer [43].

These data suggest that qRT-PCR can be employed to detect and quantify GCC mRNA in lymph nodes, to better assess tumor stage and risk of disease recurrence, with the ability to detect a single cancer cell in 10^6 – 10^7 normal cells, improving detection sensitivities compared with histopathology, which detects a single cancer cell in approximately 10^2 normal cells [12,52]. Thus, an assay to quantify GCC mRNA in lymph nodes was developed and analytically validated [51]. The assay was robust, with a dynamic range over six orders of magnitude; a high analytic sensitivity, with a lower limit of quantification of 25 copies of GCC mRNA; and low variability, with a coefficient of variation of less than 5% [51]. Moreover, this assay exhibited a sensitivity and specificity of 93 and 97%, respectively, when applied to lymph nodes harboring colorectal metastases identified by histopathology (true-positives) and to those from patients who did not have colorectal cancer (true-negatives) [51]. Importantly, GCC qRT-PCR revealed that 76 of 367 (~21%) lymph nodes free of tumor cells by histopathology from six of 23 (26%) patients with colorectal cancer harbored occult metastases [53].

Employing that analytically validated assay, a prospective multicenter clinical trial assessed the utility of GCC, quantified by the qRT-PCR, as a prognostic marker of occult metastases in lymph nodes of pN0 colorectal cancer patients [54]. In this study, lymph nodes were harvested from fresh specimens from pN0 colorectal cancer patients, bisected and half of each lymph node was frozen for subsequent analyses. The other half of each lymph node was fixed in formalin and subjected to routine histopathology. In this study of approximately 250 pN0 patients, involving the analysis of approximately 2500 lymph nodes, patients whose lymph nodes were free of occult tumor cells by molecular analysis [pN0(mol–)] exhibited a three- to fourfold more favorable recurrence rate, compared with patients whose lymph nodes harbored occult metastases detected by GCC qRT-PCR [pN0(mol+)]. Furthermore, recurrence rates in pN0(mol+) patients were nearly identical to those of stage III N1 patients, in which tumor cells could be detected by histopathology in up to three lymph nodes. Importantly, multivariate analysis revealed that GCC qRT-PCR was the most powerful independent prognostic marker for recurrence in pN0 patients. This is the first blinded, adequately powered, prospective multicenter clinical trial to demonstrate that molecular staging employing qRT-PCR and a specific molecular marker improves prognostic stratification of the pN0 population in cancer. They suggest that GCC qRT-PCR identifies occult metastases in lymph nodes that independently predict time to recurrence in pN0 colorectal cancer patients. Thus, GCC may serve as a prognostic and predictive marker, identifying pN0 patients at minimum risk for disease recurrence and, conversely, who might benefit from adjuvant chemotherapy, respectively.

Previstage™ GCC Colorectal Cancer Staging Test

DiagnoCure Oncology Laboratories (West Chester, PA, USA), a biotechnology company committed to providing innovative diagnostic, prognostic and predictive molecular assays for managing patients with cancer, is commercially developing GCC qRT-PCR for the diagnostic management of patients with gastrointestinal malignancies. The first of these products, Previstage GCC Colorectal Cancer Staging Test, will apply GCC qRT-PCR to improve the accuracy of the staging of patients with colorectal cancer. Commercial development has focused on expanding GCC qRT-PCR from a diagnostic test in fresh lymph nodes to one generally applicable to formalin-fixed, paraffin-embedded specimens, a requirement to improve accessibility in clinical practice. Indeed, a robust proprietary multiplexed assay has been developed incorporating target analytes and housekeeping genes with the detection of GCC in formalin-fixed, paraffin-embedded lymph nodes over a dynamic range of six orders of magnitude, with an analytical specificity of approximately 100%. Moreover, this test identifies occult metastases in lymph nodes from patients staged as pN0 by traditional histopathology. These results compare favorably to those obtained with lymph nodes harvested from fresh specimens and suggest that the analytical platforms are essentially comparable. Initial commercial launch of Previstage for identifying occult metastases in formalin fixed, paraffin-embedded lymph nodes from patients with pN0 colorectal cancer is expected in the third quarter of 2008. The test will be provided through a central Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory.

Expert commentary

Tumor dissemination to lymph nodes remains one of the most important prognostic and predictive risk factors for many neoplastic diseases. In colorectal cancer, traditional histopathologic staging is not sufficient to accurately estimate lymph node metastases, reflecting limitations in both tissue volumes assessed and analytic sensitivity. Indeed, up to 30% of pN0 patients develop recurrent disease, likely reflecting the presence of occult metastases in lymph nodes [7,15]. In this context, qRT-PCR overcomes these limitations by permitting sampling of the entire tissue specimen, with an analytic sensitivity approaching one cancer cell in 10^7 normal cells. RT-PCR has been applied to the identification of occult metastases in lymph nodes of patients with colorectal cancer, most notably employing CKs and CEA as molecular markers. However, results with these approaches have been equivocal, in part reflecting the choice of markers with suboptimal sensitivities and specificities; analytic techniques, employing qualitative, rather than quantitative RT-PCR; inadequate study design, with insufficient lymph node and tissue volumes sampled; and underpowering of studies, employing insufficient patient populations.

GCC is one of the most sensitive and specific markers for metastatic colorectal cancer with utility for detecting clinically important occult metastases in lymph nodes [12]. Thus, qRT-PCR for GCC detects occult metastases in lymph nodes [44] that correlate closely with clinical outcomes in retrospective analyses [43]. Moreover, prospective, blinded, adequately powered multi-institutional clinical studies have provided level 1 evidence that GCC qRT-PCR detects occult metastases in lymph nodes that identifies patients with pN0 colorectal tumors who are at increased risk for recurrent disease [54]. Indeed, multivariate analysis revealed that GCC qRT-PCR was the most powerful independent prognostic indicator of disease recurrence in the pN0 population [54].

Taken together, these considerations suggest that GCC qRT-PCR may be useful as a diagnostic test for staging patients with colorectal cancer. Indeed, this technology is undergoing analytical validation by DiagnoCure Oncology Laboratories, who have developed the Previstage GCC Colorectal Cancer Staging Test encompassing a proprietary multiplex qRT-PCR assay

compatible with formalin-fixed, paraffin-embedded lymph nodes for detecting occult metastases, scheduled for commercial launch in the third quarter of 2008. This diagnostic test provides robust quantification of GCC mRNA, with a broad dynamic range, detecting the presence of tumor cells in lymph nodes with an analytical specificity of approximately 100%. This diagnostic test will have utility as an adjunctive prognostic test to enhance current staging paradigms, including traditional histopathology, in more precisely identifying patients who may be at increased risk for developing recurrent disease. It is anticipated that this successful application of molecular diagnostics to lymph node analyses will ultimately drive revision of current clinical staging classifications to include a new categorical stage of histopathology, negative but molecular positive [pN0(mol+)], that recognizes the considerable clinical prognostic information provided by this analysis. Beyond prognosis, this test could provide enhanced predictive information, identifying a cohort of patients who might benefit from receiving adjuvant chemotherapy.

Five-year view

The enabling technology of molecular analysis employing qRT-PCR of disease-specific transcripts is emerging as a key analytical tool for individualization of clinical patient management. In that context, during the next 5 years, prognostic and predictive molecular markers will be identified and developed for broad clinical application across the spectrum of neoplastic diseases, evolving into a standard of care in the community. The Previstage GCC Colorectal Cancer Staging Test will expand from the fundamental detection of the presence of occult metastases, to capturing the full diagnostic potential of this technology for quantifying cancer cells in individual lymph nodes, adding a new dimension to disease staging of pN0 and pNx patients by correlating these estimates of tumor burden with disease prognosis and prediction. Beyond prognosis, it is expected that level 1 evidence will be acquired in the form of prospective, randomized, multicenter therapeutic trials defining the utility of Previstage GCC as a predictive marker for identifying pN0 and pNx colorectal cancer patients who will most benefit from adjuvant chemotherapy. Furthermore, the utility of Previstage GCC lymph node staging will be extended to the prognostic and predictive subsetting of patients with esophageal and gastric adenocarcinomas, in the context of the unique ectopic expression of GCC by these tumors [53]. Moreover, Previstage GCC offers a unique opportunity to prioritize future complex resource-intensive analyses of primary tumors that will optimize patient management in the future. Thus, analyses of primary tumors to define mutations, gene expression and epigenetic profiles and proteomic signatures to stratify risk, predict responses to chemotherapy and personalize interventions, may best be applied to pN0(mol+), rather than pN0(mol-), patients. Taken together, these considerations underscore the importance of integrating molecular approaches incorporating disease-specific markers, like Previstage GCC, into analytical paradigms directing the management of patients with colorectal cancer.

Key issues

- Traditional paradigms for staging patients with colorectal cancer incorporating standard histopathological assessment of regional lymph nodes underestimate the extent of metastatic disease, reflected by 25–30% of pN0 patients developing recurrent disease.
- Limitations of traditional staging paradigms, including volume of tissue assessed and analytic sensitivity, can be eliminated by employing disease-specific markers and a powerful molecular amplification technology such as quantitative reverse transcriptase (qRT)-PCR.
- Guanylyl cyclase C (GCC) is the most sensitive and specific marker described to date for identifying metastatic colorectal cancer cells in extra-intestinal tissues and occult

lymph node metastases detected by GCC qRT-PCR. GCC is the most powerful prognostic indicator for risk of disease recurrence in pN0 colorectal cancer patients.

- The Previstage™ GCC Colorectal Cancer Staging Test will identify occult metastatic tumor cells in formalin-fixed, paraffin-embedded lymph nodes, identifying patients who may be at increased risk of developing recurrent disease who could most benefit from adjuvant chemotherapy.

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