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# GUCY2C molecular staging personalizes colorectal cancer patient management

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## Abstract

While the most significant prognostic and predictive marker in the management of colorectal cancer patients is cancer cells in regional lymph nodes, approximately 30% of patients whose lymph nodes are ostensibly free of tumor cells by histopathology ultimately develop recurrent disease reflecting occult metastases. Molecular techniques utilizing highly specific markers and ultra-sensitive detection technologies have emerged as powerful staging platforms to establish prognosis and predict responsiveness to chemotherapy in colorectal cancer patients. This review describes the evolution of the tumor suppressor GUCY2C as a prognostic and predictive molecular biomarker that quantifies occult tumor burden in regional lymph nodes for staging patients with colorectal cancer.

### Keywords

biomarker; colorectal cancer; guanylyl cyclase C; occult tumor burden; prediction; prognosis; quantitative reverse transcriptase-PCR; racial disparities; staging

Clinicopathologic staging continues to be the most significant prognostic marker of survival and predictive marker of therapeutic response in cancer patient management. However, this staging paradigm is imperfect, and identification of patients who will develop disease recurrence or who could benefit from adjuvant chemotherapy remains an unachieved goal in most cancers [1–4]. The development of technology platforms that query genomic information and postgenomic function has provided novel diagnostic markers and therapeutic targets with the potential to personalize prevention, detection and, ultimately, the

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cure of cancer [5–8]. Despite this scientific progress, the translation of the products of the new biology into clinical algorithms for patient care has lagged, reflecting the absence of the foundational evidence defining these emerging technologies in diagnostic and therapeutic management guidelines [9–11]. Using colorectal cancer as a model, this review will examine the utility of molecular staging tools for clinical prognosis and prediction to personalize patient management. Specifically, the utility of molecular staging using the tumor suppressor GUCY2C as a biomarker for quantifying occult metastases in lymph nodes, predicting disease recurrence and identifying patients who might benefit from therapy will be explored.

## Colorectal cancer

Colorectal cancer is the fourth most common malignancy, with approximately 150,000 new cases each year, and the third most common cause of cancer death [12]. It produces approximately 10% of cancer mortality in the USA, and death from colorectal cancer approaches approximately 50% [12–14]. Mortality reflects metastatic disease; approximately 20% of patients with colorectal cancer have unresectable metastases at diagnosis, while ≥30% of patients will develop metastases during their illness [1,4,13,14]. Surgery is the treatment with the greatest influence on survival. However, while surgery excises all obvious tumor, occult metastases produce recurrence and death [12–18]. Disease recurrence occurs in approximately 10% of patients whose tumors are confined to the mucosa (stage I) and to more than 50% of patients whose tumors exhibit metastases to regional lymph nodes (stage III) [12–18].

### Staging & prognosis

Tumor cells in regional lymph nodes are the most important prognostic marker of survival in colorectal cancer because they indicate the potential presence of distant metastases undetected by conventional methods [1,4,12–15,18–23]. Although staging by histopathology continues to be the standard, imprecision reflects inherent methodological limitations [4,13,23]. Histology has limited sensitivity, detecting as few as one cancer cell in 200 normal cells [24]. In addition, microscopy reviews <0.01% of available tissue thus producing a sample error since >99.9% of each tissue specimen is not examined [1,4,24]. The clinical impact of restrictions from microscopy can best be appreciated by considering the rate of recurrence of cancer. Stage I and II disease, limited to the bowel wall without involvement of regional lymph nodes (pN0), should be completely cured by surgery, but 10% of stage I and 30% of stage II patients develop recurrent disease [4,13,14,23]. Stage III patients with metastatic tumor cells in regional lymph nodes experience recurrence at a rate of up to 70% [13,14]. Differences in recurrence rates in patients with stage I and II disease reflect their heterogeneity: some are truly node-negative while others have stage III or IV disease that is inaccurately staged by histopathology [1,4,13,20,25–27].

### Staging & therapeutic prediction

In colorectal cancer, stage also predicts which patients will benefit from adjuvant chemotherapy. Indeed, adjuvant chemotherapy improves survival of stage III colon cancer patients, increasing time-to-recurrence by up to 40% and overall survival by up to 30% [15,19,28–34]. Furthermore, new biologically-targeted therapies, including monoclonal antibodies directed at mechanism-based molecular signaling components, including VEGF and EGF receptors, has increased 5-year median and overall survival in patients with metastatic disease from 7 to ≥30% [35]. However, the advantage of adjuvant chemotherapies in patients with pN0 colorectal cancer remains unclear, with only small improvements in survival in some, but not all, trials [13–15,18,19,21,22,36]. This unclear treatment benefit is reflected in modern treatment paradigms, some of which advocate

adjuvant chemotherapy for patients with poor clinicopathologic features including lymphovascular invasion, extension into the bowel wall, or penetration into surrounding anatomical structures [18,37–39]. Unpredictable responses to chemotherapy in pN0 patients probably reflects heterogeneity in occult nodal metastases [1,4, 20,23,40–42]. Methods that detect those metastases can identify pN0 patients who could benefit from chemotherapy [15,35].

## Molecular staging paradigms

Histopathology remains the most significant staging paradigm for patients with colorectal cancer, reflecting a relationship between nodal cancer cells, prognosis and prediction [1,4,12–15,18–22]. However, histopathology underestimates metastases in specimens; approximately 70% of nodes with metastases have nests of tumor cells below 0.5 cm, which escape observation [4,13,14,23]. Beyond histopathology, emerging platforms for molecular staging that pair disease biomarkers with a detection technology such as quantitative reverse transcriptase-PCR (RT-qPCR) provide a system for the sensitive detection of metastases [4,23]. Molecular staging interrogates the entire specimen, eliminating sampling errors, and can detect one tumor cell in approximately one million normal cells, providing unsurpassed sensitivity [4,23]. The utility of these molecular approaches for staging patients has remained unclear, reflecting studies with insufficient patient cohorts, inadequate longitudinal follow-up and analytic paradigms that were not appropriately validated [1–4]. However, meta-analyses suggest that these emerging molecular platforms provide a distinct advantage for staging patients with colorectal cancer [1,4,20,27,42,43].

## GUCY2C

### GUCY2C is a novel tumor suppressor

GUCY2C is one of the family of enzyme receptors synthesizing cGMP from GTP. This protein is selectively expressed by intestinal epithelial cells [44–53]. GUCY2C is the receptor for the paracrine hormones uroguanylin and guanylin produced in the small intestine and colon, respectively. Their binding to the extracellular domain of GUCY2C activates the intracellular catalytic domain, producing cGMP [49,52,54–60]. GUCY2C regulates epithelial cell proliferation, DNA damage repair, and metabolic programming regulating homeostasis along the crypt-surface axis [61–73]. Interestingly, guanylin and uroguanylin are the most commonly lost gene products in intestinal carcinogenesis [74–78]. Indeed, eliminating GUCY2C signaling increases tumors in mouse models of genetic and carcinogen-based carcinogenesis, reflecting dysregulation of proliferation and chromosomal instability [64]. Thus, GUCY2C is a tumor suppressor regulating homeostasis along the crypt-surface axis whose silencing, reflecting paracrine hormone loss, contributes to colorectal carcinogenesis [62–65,69].

### GUCY2C is a biomarker for metastatic colorectal cancer

GUCY2C exhibits expression characteristics that make it useful as a biomarker of colorectal cancer metastases in extra-intestinal tissues. GUCY2C has been identified in all samples of normal intestine, but not in extra-gastrointestinal parenchyma, including the lung and liver [40,43,45,46,54]. In addition, GUCY2C has been identified in nearly all human colorectal tumors, regardless of anatomical location or grade, but not in extragastrointestinal malignancies [40,43,45,46,54,77,79–82]. Moreover, GUCY2C is overexpressed by most colorectal tumors compared with normal intestinal epithelial cells [79,83,84]. Paradoxical overexpression of the GUCY2C tumor suppressor by primary and metastatic colorectal cancer reflects universal loss of guanylin and uroguanylin expression, silencing paracrine signaling cascades producing receptor super-sensitization. Expression is normally selectively restricted to intestinal epithelial cells, but universal overexpression by metastatic

cancer cells underscores the utility of GUCY2C as a biomarker of occult metastases in lymph nodes of colorectal cancer patients being staged [42].

## Detection of clinically significant occult nodal metastases using GUCY2C RT-qPCR

Retrospective studies suggested that GUCY2C RT-PCR identified occult nodal metastases related to clinical outcomes in colorectal cancer patients [43]. These preliminary studies provided support for an adequately powered prospective blinded multicenter clinical trial of the utility of GUCY2C, detected by RT-qPCR, to identify clinically significant occult nodal metastases. Thus, 257 stage 0–II pN0 colorectal cancer patients were enrolled at one of seven academic medical centers and two community hospitals in the USA and Canada [42]. Lymph nodes were dissected from fresh colon and rectum specimens, and half of each was used for histopathology, while the other half was used for molecular analysis by GUCY2C RT-qPCR. Remarkably, ≥85% of patients with histologically-negative lymph nodes harbored occult metastases in at least one lymph node detected by molecular staging [42]. These analyses revealed that, surprisingly, most patients considered free of metastases by histopathology have occult disease in regional lymph nodes. In that context, 20.9% (95% CI: 15.8–26.8) of patients with, but only 6.3% (95% CI: 0.8–20.8) of patients without, occult nodal metastases developed disease recurrence ($p = 0.006$) [42]. Indeed, occult metastasis detected by molecular staging was associated with poorer prognosis and reduced disease-free survival in both stage I and II patients and in patients with colon and rectal cancers [42]. Importantly, in patients with occult metastases, time to recurrence and disease-free survival were nearly identical to those of patients with stages IIIA and IIIB disease, underscoring the ability of this molecular platform to accurately stage patients [42]. Multivariate analyses revealed that molecular staging provided a powerful independent marker of risk, and patients with occult metastases had shorter times to recurrence and reduced disease-free survival [42]. This prospective study is the first to provide level 1 evidence for the utility of molecular staging of lymph nodes to personalize individual prognostic risk in cancer, employing an adequately powered, blinded, prospective multicenter clinical trial design [3]. Indeed, the absence of evidence employing this rigorous study design has been an essential element restricting the translation of molecular diagnostics into clinical management algorithms that personalize prognostic risk stratification and therapeutic response prediction [1,4]. Moreover, this approach employing GUCY2C RT-qPCR to detect occult lymph node metastases to stage patients with colorectal cancer has been independently validated across laboratories, operators and technology platforms [85–87].

## Quantity of occult metastatic tumor cells across the regional lymph node network is a prognostic marker of risk in pN0 colorectal cancer

Although a high proportion of pN0 patients harbor occult metastases according to GUCY2C RT-qPCR, most pN0 patients will not undergo recurrence [13,88,89]. Reconciliation of this apparent inconsistency relies on the recognition that the categorical (yes/no) presence of nodal metastases does not assure recurrence but, rather, indicates risk. Uncertainty of the clinical significance of nodal metastases can best be appreciated by considering that only approximately 50% of stage III patients develop recurrent disease although all have visible nodal metastases by histopathology [13,88,89]. The uncertainty of the clinical significance of occult nodal metastases highlights the limitations of qualitative RT-PCR generally, and GUCY2C RT-qPCR specifically, for categorical (yes/no) identification of occult metastases [4]. The superior sensitivity of qualitative RT-PCR, with its optimum tissue sampling and capacity for single-cell discrimination, identifies occult metastases below the threshold of prognostic risk [4,13,23,42], limiting the specificity of molecular staging. Indeed, this capacity for single-cell discrimination increases the sensitivity of this approach to false-positive signals resulting from contamination of biospecimens with normal intestinal epithelial or colorectal cancer cells.

There is an emerging paradigm that goes beyond the categorical (yes/no) presence of tumor cells, to quantify occult metastatic tumor burden (how much) across the regional lymph node network to define tumor behavior and disease risk. This paradigm has its origins in, and builds upon, two established concepts in histopathology. Thus, there is a quantitative relationship between prognostic risk and number of nodes harboring tumor cells by histology, where stage III patients with at least four involved nodes exhibit a recurrence rate greater than those with three or less involved nodes [13,88,89]. In addition, there is a quantitative relationship between the volume of cancer cells in individual nodes and prognostic risk, and metastases ≥0.2 cm are associated with increased disease recurrence while the relationship between individual tumor cells or nests <0.02 cm and risk remains undefined [13,88,89]. The emergence of RT-qPCR provides a unique opportunity to quantify occult tumor burden to assign prognostic risk and predict therapeutic benefit [3,90]. Quantitative measures of GUCY2C expression provide a molecular analog of morphological assessment of metastatic volumes in lymph nodes. This molecular quantification augments 2D morphology by quantifying metastases in a large volume of tissue, rather than a thin section, and in all available lymph nodes to estimate occult tumor burden across the regional lymph node network.

To examine the quantitative relationship between occult nodal metastases, clinical tumor behavior and prognostic risk, we designed analytic paradigms to explore the association of occult tumor burden, quantified by GUCY2C RT-qPCR, with outcomes in colorectal cancer patients [42,90]. The relationship of clinical outcomes (time to recurrence, disease-free survival) and prognostic indicators, including occult tumor burden, was established by statistical analytic paradigms integrating recursive partitioning with Cox models [90]. This analysis revealed that 176 (60%) patients harbored low tumor burden ($Mol_{Low}$), and 172 (40%) remained free of disease (recurrence rate 2.3% [95% CI: 0.1–4.5]). Furthermore, 90 (31%) patients had intermediate tumor burden ($Mol_{Int}$) and 30 (33.3% [95% CI: 23.7–44.1]) developed recurrent disease. Moreover, 25 (9%) patients demonstrated high tumor burden ($Mol_{High}$), and 17 (68.0% [95% CI: 46.5–85.1]) developed recurrent disease ($p < 0.001$). This analysis demonstrated that molecular tumor burden was an independent prognostic marker and $Mol_{Int}$ and $Mol_{High}$ patients experienced a graded risk of earlier time-to-recurrence ($Mol_{Int}$, adjusted hazard ratio [HR]: 25.52 [95% CI: 11.08–143.18]; $p < 0.001$; $Mol_{High}$, HR: 65.38 [95% CI: 39.01–676.94]; $p < 0.001$) and reduced disease-free survival ($Mol_{Int}$, HR: 9.77 [95% CI: 6.26–87.26]; $p < 0.001$; $Mol_{High}$, HR: 22.97 [95% CI: 21.59–316.16]; $p < 0.001$). These observations provide a striking enhancement over the use of GUCY2C as a categorical (yes/no) marker, where 88% of patients were GUCY2C-positive and exhibited a recurrence risk of 20% [42]. They highlight the unique clinical opportunity to utilize occult tumor burden as a diagnostic marker to assign risk in patients with pN0 colorectal cancer. Identification of cohorts ($Mol_{High}$) of pN0 patients with a mortality risk equivalent to patients with stage IV disease with disseminated metastases underscores the prognostic value of quantitative occult tumor burden analysis [90]. Moreover, it is tempting to speculate that patients with the greatest occult tumor burden might benefit from therapy. Indeed, occult tumor burden as a marker that discriminates clinically important from unimportant metastases may represent a paradigm shift in staging.

## Racial disparities in stage-specific outcomes reflect differences in occult tumor burden

Despite decreasing mortality from colon cancer, reflecting advances in screening and therapy, there is a widening racial gap in incidence and survival [91–93]. There is approximately 20% greater incidence of, and approximately 40% higher mortality from, colon cancer in black, compared with white, patients [91–94]. Disparities in outcomes reflect advanced stage at diagnosis, socioeconomic differences and differences in therapeutic management [93–99]. Beyond these factors that influence overall outcomes, there are racial

disparities in stage-specific outcomes [93,95,97,100]. Paradoxically, the greatest disparities in outcomes occur in early-stage disease, with approximately 40% excess mortality in blacks with pN0 colon cancer. These stage-specific disparities do not appear to principally reflect socioeconomic status, access to medical care, or cultural-specific customs [93,95,97,100]. Rather, they could reflect a contribution of a higher incidence and quantity of occult metastases in nodes [93,97].

In that context, prospective evaluation of the utility of GUCY2C to detect occult metastases in lymph provided a unique opportunity to explore the racial distribution of occult tumor burden and its association with prognostic risk in colorectal cancer [42,101]. Analysis of GUCY2C expression in this cohort revealed a fourfold greater level of categorical occult metastases in individual nodes in 23 black patients compared with 259 white patients ($p < 0.001$; 95% CI: 3.3–6.7). Occult tumor burden across the regional lymph node network stratified the entire cohort into categories with low (60%; recurrence rate = 2.3% [95% CI: 0.1–4.5]), intermediate (31%; recurrence rate = 33.3% [95% CI: 23.7–44.1]), and high (9%; recurrence rate = 68.0% [95% CI: 46.5–85.1]; $p < 0.001$) risk. Multivariable analysis revealed that race ($p = 0.02$), tumor stage ($p = 0.02$) and number of lymph nodes collected for histology ($p = 0.003$) were independent prognostic markers. Black patients, compared with white patients, were more likely to harbor levels of occult tumor burden associated with the highest risk (adjusted odds ratio = 5.08 [95% CI: 1.55–16.65]; $p = 0.007$). These analyses highlight the utility of occult tumor burden quantified by GUCY2C RT-qPCR as a marker of tumor metastases that estimates prognostic risk contributing to racial disparities in stage-specific outcomes in colorectal cancer [101]. Beyond prognostic risk, they suggest that if occult tumor burden predicts therapeutic benefit, it represents a detect–treat paradigm that could reduce racial disparities in mortality in colon cancer.

## Future perspectives

To date, the most powerful indicator of prognosis and response to adjuvant chemotherapy in colorectal cancer is the identification of cancer cells in lymph nodes by histopathology [1,4,12–15,18–22]. Despite its central position in all staging paradigms, approaches to detecting lymph node metastases are inadequate. Up to 30% of patients with node-negative colon cancer succumb to disease recurrence, associated with occult metastases in lymph nodes undetected by conventional methods [1,4,13,14,20,23,40,41,102]. There is an unmet clinical need for new approaches to more precisely evaluate tumor metastases in regional lymph nodes in colon cancer patients. Recently, a blinded, multicenter prospective study demonstrated the utility of molecular staging to detect occult tumor metastases in regional lymph nodes to predict risk of disease recurrence [42]. Categorical occult tumor metastases, defined by molecular staging, was a powerful independent marker of risk of disease recurrence [42]. Furthermore, occult tumor burden across the regional lymph node network stratified pN0 patients into cohorts with low, intermediate and high risk [90]. Moreover, differences in occult tumor burden contribute to racial disparities in stage-specific outcomes in colon cancer [101]. This prospective clinical trial represents the first level I evidence supporting the importance of occult metastases in regional lymph nodes in defining prognostic risk in patients with colon cancer. These data establish a framework for the application of molecular staging in lymph nodes for personalized prognostic risk assessment in patients with cancer.

While these observations are a beginning, their translation into useful staging tools in cancer will require considerable analyses in the future. These results will require confirmation in an independent cohort of patients with colorectal cancer, consistent with the emerging learn–confirm paradigm in biomarker translation, in which integration into patient management algorithms requires validation in independent populations [103–110]. Beyond prognosis,

there is an established association between metastases in regional lymph node and the efficacy of chemotherapy in patients with colorectal cancer. While adjuvant chemotherapy improves clinical outcomes in stage III patients, its impact on survival in pN0 patients remains unclear [13–15,18,19,21,22,36]. This heterogeneity of therapeutic benefit in node-negative patients may in part reflect the inherent inaccuracy of staging by histopathology [1,4,20,23,40–42]. By contrast, molecular staging has identified node-negative patients with a prognostic risk profile that closely matched stage III patients, a cohort that derives benefit from adjuvant chemotherapy [42,90,101]. These observations suggest that pN0 patients who harbor occult metastases detected by molecular staging could also benefit from adjuvant chemotherapy. In the future, studies will examine whether occult lymph node metastases defined by molecular staging predicts chemotherapeutic efficacy. These studies will assess if, in patients with occult metastases in regional lymph nodes identified by molecular staging, those treated with chemotherapy have improved clinical outcomes compared with those who are followed without treatment.

Beyond application to patients with pN0 colon cancer, occult tumor burden quantified by GUCY2C RT-qPCR is also applicable to prognosis and prediction in patients with stage III colon cancer, in which approximately 50% of patients with visible metastases remain free of disease recurrence. Similarly, these principles can be applied to rectal cancer patients, in which GUCY2C also identifies clinically significant occult metastases [42]. Furthermore, occult tumor burden may be extended to esophageal and gastric adenocarcinoma, which ectopically express GUCY2C, reflecting epithelial transformation involving intestinal metaplasia [79,111]. Moreover, this diagnostic paradigm may offer prognostic and predictive solutions to other populations beyond African–Americans with identical stage-specific racial disparities in pN0 colorectal cancer, including Hispanics, Mexicans and Hawaiians [95].

Evolving genomic platforms provide a rich source of prognostic and predictive information about primary tumors that can enhance staging algorithms, optimizing outcomes that drive patient management. Analyses of primary tumors to define gene expression and epigenetic profiles, disease-associated mutations in oncogenes or tumor suppressors and metabolomic and proteomic signatures that individualize assessments of recurrence risk, responses to adjuvant chemotherapy, and biologically-targeted treatments are enhancing the prognostic and predictive management of cancer patients [112–116]. However, defining the prognostic and predictive character of primary tumors by molecular analyses may be most relevant in the context of whether tumors have metastasized. A primary tumor with a molecular signature suggesting a poor prognosis might represent less risk to the patient if that tumor was completely resected at the time of surgery, before metastases occurred. This relationship between molecular signatures of risk in primary tumors, resection and metastases may underlie the observation that, to date, molecular signatures have had only an indeterminate impact on the management of patients with cancer [117,118]. Thus, emerging technology platforms defining prognosis and prediction for clinical management employing molecular analyses of primary tumors might produce the greatest benefit when applied to patients harboring occult nodal metastases, rather than to those free of metastatic disease. Here, molecular staging offers a unique opportunity to prioritize complex and expensive molecular analyses of primary tumors to optimize cost-effective patient management [42]. In the future, trials will examine the applicability of reflexed analytical paradigms in which all histologically node-negative patients undergo molecular staging, to determine whether there is clinically important occult lymph node metastases, followed by further molecular testing of primary tumors only for patients at increased prognostic risk, to identify therapies personalized to the biology of their individual malignancies [119].

It is important to consider that RT-qPCR is an evolving technical platform that primarily remains the domain of centralized specialty laboratories and has not yet been broadly distributed to most academic and community medical centers. These realities raise the important question concerning limitations to implementation of molecular staging as a clinical standard central to practice guidelines. In that regard, molecular diagnostics is an emerging $14 billion dollar business, which is increasing at a rate exceeding 10% annually [120,121]. Indeed, the number of esoteric molecular diagnostic tests approved by the FDA each year is growing aggressively, from 72 in 2006 to 134 in 2009. Additionally, the number of home-brew molecular diagnostic tests, developed in individual laboratories, was in excess of 1400 in 2009. These considerations suggest that molecular diagnostic tests, including molecular staging, available to clinicians and patients will grow in number. In the near-term, central laboratory performance sites provide the depth of experience and validated technology platforms that align with requirements for FDA regulatory performance and Centers for Medicare and Medicaid Services reimbursement. They will ultimately support the most informative approaches to incorporate molecular staging paradigms into patient-centered algorithms for disease management.

## References

Papers of special note have been highlighted as:

■ of interest

■■ of considerable interest


1 ■. Iddings D, Ahmad A, Elashoff D, Bilchik A. The prognostic effect of micrometastases in previously staged lymph node negative (N0) colorectal carcinoma: a meta-analysis. Ann Surg Oncol. 2006; 13(11):1386–1392. Meta-analysis demonstrating the utility of molecular techniques to stage patients with colorectal cancer. [PubMed: 17009147]

2. Mejia A, Waldman S. Previstage™ GCC test for staging patients with colorectal cancer. Expert Rev Mol Diag. 2008; 8:571–578.

3. Mejia A, Schulz S, Hyslop T, Weinberg DS, Waldman SA. GUCY2C reverse transcriptase PCR to stage pN0 colorectal cancer patients. Expert Rev Mol Diagn. 2009; 9(8):777–785. [PubMed: 19895223]

4. Nicastri DG, Doucette JT, Godfrey TE, Hughes SJ. Is occult lymph node disease in colorectal cancer patients clinically significant? A review of the relevant literature. J Mol Diagn. 2007; 9(5): 563–571. [PubMed: 17916603]

5. Clarke SJ, Chua W, Moore M, et al. Use of inflammatory markers to guide cancer treatment. Clin Pharmacol Ther. 2011; 90(3):475–478. [PubMed: 21775983]

6. Nass SJ, Balogh E. National Cancer Policy Summit: addressing opportunities and challenges in cancer care and research. Clin Pharmacol Ther. 2011; 89(6):770–771. [PubMed: 21508936]

7. Paugh SW, Stocco G, Mccorkle JR, Diouf B, Crews KR, Evans WE. Cancer pharmacogenomics. Clin Pharmacol Ther. 2011; 90(3):461–466. [PubMed: 21796115]

8. Zahedi P, De Souza R, Piquette-Miller M. Optimizing cancer care: is the future bright? Clin Pharmacol Ther. 2011; 90(3):347–350. [PubMed: 21862960]

9. Amstutz U, Carleton BC. Pharmacogenetic testing: time for clinical practice guidelines. Clin Pharmacol Ther. 2011; 89(6):924–927. [PubMed: 21508939]

10. Haga SB, Burke W. Practical ethics: establishing a pathway to benefit for complex pharmacogenomic tests. Clin Pharmacol Ther. 2011; 90(1):25–27. [PubMed: 21691271]

11. Waldman SA, Hohl RJ, Kearns GL, Swan SJ, Terzic A. Clinical pharmacology as a foundation for translational science. Clin Pharmacol Ther. 2011; 90(1):10–13. [PubMed: 21691266]

12. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin. 2007; 57(1):43–66. [PubMed: 17237035]

13■. Compton CC, Greene FL. The staging of colorectal cancer: 2004 and beyond. CA Cancer J Clin. 2004; 54(6):295–308. Definitive description for the staging and management of colorectal cancer. [PubMed: 15537574]

14. Greene, FL. AJCC Cancer Staging Manual. 6. Springer; NY, USA: 2002.

15■. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med. 2005; 352(5): 476–487. Authoritative description of the therapeutic management of patients with colorectal cancer. [PubMed: 15689586]

16. Ries LA, Wingo PA, Miller DS, et al. The annual report to the nation on the status of cancer, 1973–1997, with a special section on colorectal cancer. Cancer. 2000; 88(10):2398–2424. [PubMed: 10820364]

17. Sobrero A, Kerr D, Glimelius B, et al. New directions in the treatment of colorectal cancer: a look to the future. Eur J Cancer. 2000; 36(5):559–566. [PubMed: 10738119]

18. Wolpin BM, Meyerhardt JA, Mamon HJ, Mayer RJ. Adjuvant treatment of colorectal cancer. CA Cancer J Clin. 2007; 57(3):168–185. [PubMed: 17507442]

19. Andre T, Boni C, Mounedji-Boudiaf L, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. 2004; 350(23):2343–2351. [PubMed: 15175436]

20. Bilchik AJ, Hoon DS, Saha S, et al. Prognostic impact of micrometastases in colon cancer: interim results of a prospective multicenter trial. Ann Surg. 2007; 246(4):568–575. discussion 575–567. [PubMed: 17893493]

21. Mamounas E, Wieand S, Wolmark N, et al. Comparative efficacy of adjuvant chemotherapy in patients with Dukes' B versus Dukes' C colon cancer: results from four national surgical adjuvant breast and bowel project adjuvant studies (C-01, C-02, C-03, and C-04). J Clin Oncol. 1999; 17(5): 1349–1355. [PubMed: 10334518]

22■. Gray R, Barnwell J, et al. Quasar Collaborative Group. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 2007; 370(9604): 2020–2029. Definitive demonstration of the benefit of adjuvant chemotherapy in patients with stage II colon cancer. [PubMed: 18083404]

23. Iddings D, Bilchik A. The biologic significance of micrometastatic disease and sentinel lymph node technology on colorectal cancer. J Surg Oncol. 2007; 96(8):671–677. [PubMed: 18081169]

24. Ratto C, Sofo L, Ippoliti M, et al. Accurate lymph-node detection in colorectal specimens resected for cancer is of prognostic significance. Dis Colon Rectum. 1999; 42(2):143–154. discussion 154–148. [PubMed: 10211489]

25. Galandiuk S, Wieand HS, Moertel CG, et al. Patterns of recurrence after curative resection of carcinoma of the colon and rectum. Surg Gynecol Obstet. 1992; 174(1):27–32. [PubMed: 1729745]

26. Greenson JK, Isenhart CE, Rice R, Mojzisik C, Houchens D, Martin EW Jr. Identification of occult micrometastases in pericolic lymph nodes of Duke's B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. Correlation with longterm survival. Cancer. 1994; 73(3):563–569. [PubMed: 7507795]

27■■. Liefers GJ, Cleton-Jansen AM, van de Velde CJ, et al. Micrometastases and survival in stage II colorectal cancer. N Engl J Med. 1998; 339(4):223–228. First description of the application of real time-PCR to staging node-negative colon cancer. [PubMed: 9673300]

28. Andre T, Sargent D, Tabernero J, et al. Current issues in adjuvant treatment of stage II colon cancer. Ann Surg Oncol. 2006; 13(6):887–898. [PubMed: 16614880]

29. De Gramont A, Tournigand C, Andre T, Larsen AK, Louvet C. Targeted agents for adjuvant therapy of colon cancer. Semin Oncol. 2006; 33(6 Suppl 11):S42–S45. [PubMed: 17178286]

30. De Gramont A, Tournigand C, Andre T, Larsen AK, Louvet C. Adjuvant therapy for stage II and III colorectal cancer. Semin Oncol. 2007; 34(2 Suppl 1):S37–S40. [PubMed: 17449351]

31. Fuchs CS, Mayer RJ. Adjuvant chemotherapy for colon and rectal cancer. Semin Oncol. 1995; 22(5):472–487. [PubMed: 7570058]

32. Krook JE, Moertel CG, Gunderson LL, et al. Effective surgical adjuvant therapy for highrisk rectal carcinoma. N Engl J Med. 1991; 324(11):709–715. [PubMed: 1997835]

33. Moertel CG, Fleming TR, Macdonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med. 1990; 322(6):352–358. [PubMed: 2300087]

34. Wolmark N, Rockette H, Fisher B, et al. The benefit of leucovorin-modulated fluorouracil as postoperative adjuvant therapy for primary colon cancer: results from national surgical adjuvant breast and bowel project protocol C-03. J Clin Oncol. 1993; 11(10):1879–1887. [PubMed: 8410113]
35. Kopetz S, Chang GJ, Overman MJ, et al. improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol. 2009; 27(22):3677–3683. [PubMed: 19470929]
36. Sargent D, Sobrero A, Grothey A, et al. Evidence for cure by adjuvant therapy in colon cancer: observations based on individual patient data from 20,898 patients on 18 randomized trials. J Clin Oncol. 2009; 27(6):872–877. [PubMed: 19124803]
37. Benson AB 3rd, Schrag D, Somerfield MR, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol. 2004; 22(16): 3408–3419. [PubMed: 15199089]
38. Figueredo A, Charette ML, Maroun J, Brouwers MC, Zuraw L. Adjuvant therapy for stage II colon cancer: a systematic review from the cancer care ontario program in evidence-based care's gastrointestinal cancer disease site group. J Clin Oncol. 2004; 22(16):3395–3407. [PubMed: 15199087]
39. Winn R, McClure J. The NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2003; 1(9):5–13. [PubMed: 19764146]
40. Frick GS, Pitari GM, Weinberg DS, Hyslop T, Schulz S, Waldman SA. Guanylyl cyclase C: a molecular marker for staging and postoperative surveillance of patients with colorectal cancer. Expert Rev Mol Diagn. 2005; 5(5):701–713. [PubMed: 16149873]
41. Gelmann A, Desnoyers R, Cagir B, Weinberg D, Boman BM, Waldman SA. Colorectal cancer staging and adjuvant chemotherapy. Expert Opin Pharmacother. 2000; 1(4):737–755. [PubMed: 11249513]
42■■. Waldman SA, Hyslop T, Schulz S, et al. Association of GUCY2C expression in lymph nodes with time to recurrence and disease-free survival in pN0 colorectal cancer. JAMA. 2009; 301(7): 745–752. The only prospective blinded multicenter trial exploring the utility of real time-PCR for staging patients with colorectal cancer. [PubMed: 19224751]
43■. Cagir B, Gelmann A, Park J, et al. Guanylyl cyclase C messenger RNA is a biomarker for recurrent stage II colorectal cancer. Ann Intern Med. 1999; 131(11):805–812. First demonstration of the utility of GUCY2C for staging patients with colorectal cancer. [PubMed: 10610624]
44. Almenoff JS, Williams SI, Scheving LA, Judd AK, Schoolnik GK. Ligand-based histochemical localization and capture of cells expressing heat-stable enterotoxin receptors. Mol Microbiol. 1993; 8(5):865–873. [PubMed: 8102772]
45. Carrithers SL, Barber MT, Biswas S, et al. Guanylyl cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues. Proc Natl Acad Sci USA. 1996; 93(25):14827–14832. [PubMed: 8962140]
46. Carrithers SL, Parkinson SJ, Goldstein S, Park P, Robertson DC, Waldman SA. *Escherichia coli* heat-stable toxin receptors in human colonic tumors. Gastroenterology. 1994; 107(6):1653–1661. [PubMed: 7958675]
47. Cohen MB, Guarino A, Shukla R, Giannella RA. Age-related differences in receptors for *Escherichia coli* heat-stable enterotoxin in the small and large intestine of children. Gastroenterology. 1988; 94(2):367–373. [PubMed: 2891585]
48. Cohen MB, Moyer MS, Luttrell M, Giannella RA. The immature rat small intestine exhibits an increased sensitivity and response to *Escherichia coli* heat-stable enterotoxin. Pediatr Res. 1986; 20(6):555–560. [PubMed: 2872650]
49. Guarino A, Cohen M, Thompson M, Dharmsathaphorn K, Giannella R. T84 cell receptor binding and guanyl cyclase activation by *Escherichia coli* heat-stable toxin. Am J Physiol. 1987; 253(6 Pt 1):G775–G780. [PubMed: 2892417]
50. Guarino A, Cohen MB, Giannella RA. Small and large intestinal guanylate cyclase activity in children: effect of age and stimulation by *Escherichia coli* heat-stable enterotoxin. Pediatr Res. 1987; 21(6):551–555. [PubMed: 2885801]

51. Guarino A, Cohen MB, Overmann G, Thompson MR, Giannella RA. Binding of *E. coli* heat-stable enterotoxin to rat intestinal brush borders and to basolateral membranes. Dig Dis Sci. 1987; 32(9): 1017–1026. [PubMed: 3304888]
52. Lucas KA, Pitari GM, Kazerounian S, et al. Guanylyl cyclases and signaling by cyclic GMP. Pharmacol Rev. 2000; 52(3):375–414. [PubMed: 10977868]
53. Rao MC, Guandalini S, Smith PL, Field M. Mode of action of heat-stable *Escherichia coli* enterotoxin. Tissue and subcellular specificities and role of cyclic GMP. Biochim Biophys Acta. 1980; 632(1):35–46. [PubMed: 6106508]
54. Carrithers SL, Ott CE, Hill MJ, et al. Guanylin and uroguanylin induce natriuresis in mice lacking guanylyl cyclase-C receptor. Kidney Int. 2004; 65(1):40–53. [PubMed: 14675035]
55. Field M. Mechanisms of action of cholera and *Escherichia coli* enterotoxins. Am J Clin Nutr. 1979; 32(1):189–196. [PubMed: 32766]
56. Field M, Graf LH Jr, Laird WJ, Smith PL. Heat-stable enterotoxin of *Escherichia coli: in vitro* effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. Proc Natl Acad Sci USA. 1978; 75(6):2800–2804. [PubMed: 26915]
57. Guerrant RL, Hughes JM, Chang B, Robertson DC, Murad F. Activation of intestinal guanylate cyclase by heat-stable enterotoxin of *Escherichia coli*: studies of tissue specificity, potential receptors, and intermediates. J Infect Dis. 1980; 142(2):220–228. [PubMed: 6106030]
58. Hughes JM, Murad F, Chang B, Guerrant RL. Role of cyclic GMP in the action of heatstable enterotoxin of *Escherichia coli*. Nature. 1978; 271(5647):755–756. [PubMed: 203862]
59. Kuno T, Kamisaki Y, Waldman SA, Gariepy J, Schoolnik G, Murad F. Characterization of the receptor for heat-stable enterotoxin from *Escherichia coli* in rat intestine. J Biol Chem. 1986; 261(3):1470–1476. [PubMed: 3944095]
60. Schulz S, Green CK, Yuen PS, Garbers DL. Guanylyl cyclase is a heat-stable enterotoxin receptor. Cell. 1990; 63(5):941–948. [PubMed: 1701694]
61. Li P, Lin JE, Chervoneva I, Schulz S, Waldman SA, Pitari GM. Homeostatic control of the crypt-villus axis by the bacterial enterotoxin receptor guanylyl cyclase C restricts the proliferating compartment in intestine. Am J Pathol. 2007; 171(6):1847–1858. [PubMed: 17974601]
62. Li P, Lin JE, Snook AE, et al. Colorectal cancer as a paracrine deficiency syndrome amenable to oral hormone replacement therapy. Clin Transl Sci. 2008; 1:163–167. [PubMed: 19727435]
63. Li P, Lin JE, Snook AE, Schulz S, Pitari GM, Waldman SA. Colorectal cancer is a paracrine deficiency syndrome which can be prevented and treated by oral hormone replacement therapy. Curr Mol Pharmacol. 2008; 2:285–292. [PubMed: 20021465]
64. Li P, Schulz S, Bombonati A, et al. Guanylyl cyclase C suppresses intestinal tumorigenesis by restricting proliferation and maintaining genomic integrity. Gastroenterology. 2007; 133(2):599–607. [PubMed: 17681179]
65. Lin JE, Li P, Pitari GM, Schulz S, Waldman SA. Guanylyl cyclase C in colorectal cancer: susceptibility gene and potential therapeutic target. Future Oncol. 2009; 5(4):509–522. [PubMed: 19450179]
66. Lin JE, Li P, Snook AE, et al. GUCY2C establishes lineage dependence in intestinal tumorigenesis through AKT. Gastroenterology. 2008; 138:241–254. [PubMed: 19737566]
67. Pitari GM, Baksh RI, Harris DM, Li P, Kazerounian S, Waldman SA. Interruption of homologous desensitization in cyclic guanosine 3′,5′-monophosphate signaling restores colon cancer cytostasis by bacterial enterotoxins. Cancer Res. 2005; 65(23):11129–11135. [PubMed: 16322263]
68. Pitari GM, Di Guglielmo MD, Park J, Schulz S, Waldman SA. Guanylyl cyclase C agonists regulate progression through the cell cycle of human colon carcinoma cells. Proc Natl Acad Sci USA. 2001; 98(14):7846–7851. [PubMed: 11438734]
69■■. Pitari GM, Li P, Lin JE, et al. The paracrine hormone hypothesis of colorectal cancer. Clin Pharmacol Ther. 2007; 82(4):441–447. Reviews the role of the GUCY2C paracrine hormone system in the etiology of colorectal cancer. [PubMed: 17687268]
70. Pitari GM, Lin JE, Shah FJ, et al. Enterotoxin preconditioning restores calcium-sensing receptor-mediated cytostasis in colon cancer cells. Carcinogenesis. 2008; 29:1601–1607. [PubMed: 18566015]

71. Pitari GM, Zingman LV, Hodgson DM, et al. Bacterial enterotoxins are associated with resistance to colon cancer. Proc Natl Acad Sci USA. 2003; 100(5):2695–2699. [PubMed: 12594332]

72. Shailubhai K, Yu HH, Karunanandaa K, et al. Uroguanylin treatment suppresses polyp formation in the Apc (Min/+) mouse and induces apoptosis in human colon adenocarcinoma cells via cyclic GMP. Cancer Res. 2000; 60(18):5151–5157. [PubMed: 11016642]

73. Steinbrecher KA, Wowk SA, Rudolph JA, Witte DP, Cohen MB. Targeted inactivation of the mouse guanylin gene results in altered dynamics of colonic epithelial proliferation. Am J Pathol. 2002; 161(6):2169–2178. [PubMed: 12466132]

74. Birkenkamp-Demtroder K, Lotte Christensen L, Harder Olesen S, et al. Gene expression in colorectal cancer. Cancer Res. 2002; 62:4352–4363. [PubMed: 12154040]

75. Cohen MB, Hawkins JA, Witte DP. Guanylin mRNA expression in human intestine and colorectal adenocarcinoma. Laboratory Invest. 1998; 78(1):101–108.

76. Notterman DA, Alon U, Sierk AJ, Levine A. Transcriptional gene expression profiles of colorectal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. Cancer Res. 2001; 61(7):3124–3130. [PubMed: 11306497]

77. Steinbrecher KA, Tuohy TM, Heppner Goss K, et al. Expression of guanylin is downregulated in mouse and human intestinal adenomas. Biochem Biophys Res Commun. 2000; 273(1):225–230. [PubMed: 10873591]

78. Wilson C, Schulz S, Hyslop T, Waldman SA. Silencing of guanylin and uroguanylin expression in colon cancer. Expert Rev Mol Diagn. 2009; 9(8):777–785. [PubMed: 19895223]

79. Birbe R, Palazzo JP, Walters R, Weinberg D, Schulz S, Waldman SA. Guanylyl cyclase C is a marker of intestinal metaplasia, dysplasia, and adenocarcinoma of the gastrointestinal tract. Hum Pathol. 2005; 36(2):170–179. [PubMed: 15754294]

80. Fava TA, Desnoyers R, Schulz S, et al. Ectopic expression of guanylyl cyclase C in CD34+ progenitor cells in peripheral blood. J Clin Oncol. 2001; 19(19):3951–3959. [PubMed: 11579116]

81. Waldman SA, Barber M, Pearlman J, Park J, George R, Parkinson SJ. Heterogeneity of guanylyl cyclase C expressed by human colorectal cancer cell lines *in vitro*. Cancer Epidemiol Biomarkers Prev. 1998; 7(6):505–514. [PubMed: 9641495]

82. Waldman SA, Cagir B, Rakinic J, et al. Use of guanylyl cyclase C for detecting micrometastases in lymph nodes of patients with colon cancer. Dis Colon Rectum. 1998; 41(3):310–315. [PubMed: 9514425]

83. Schulz S, Hyslop T, Haaf J, et al. A validated quantitative assay to detect occult micrometastases by reverse transcriptasepolymerase chain reaction of guanylyl cyclase C in patients with colorectal cancer. Clin Cancer Res. 2006; 12(15):4545–4552. [PubMed: 16899600]

84. Witek ME, Nielsen K, Walters R, et al. The putative tumor suppressor Cdx2 is overexpressed by human colorectal adenocarcinomas. Clin Cancer Res. 2005; 11(24 Pt 1):8549–8556. [PubMed: 16361536]

85. Beaulieu M, Desaulniers M, Bertrand N, et al. Analytical performance of a qRT-PCR assay to detect guanylyl cyclase C in FFPE lymph nodes of patients with colon cancer. Diagn Mol Pathol. 2010; 19(1):20–27. [PubMed: 20186008]

86. Haince JF, Houde M, Beaudry G, et al. Comparison of histopathology and RT-qPCR amplification of guanylyl cyclase C for detection of colon cancer metastases in lymph nodes. J Clin Pathol. 2010; 63(6):530–537. [PubMed: 20498026]

87. Sargent DJ, Resnick MB, Meyers MO, et al. Evaluation of guanylyl cyclase C lymph node status for colon cancer staging and prognosis. Ann Surg Oncol. 2011; 18(12):3261–3270. [PubMed: 21533822]

88. Cunningham D, Atkin W, Lenz HJ, et al. Colorectal cancer. Lancet. 2010; 375(9719):1030–1047. [PubMed: 20304247]

89. Edge, SB.; Byrd, DR.; Compton, CC.; Fritz, AG.; Greene, FL.; Trotti, A. AJCC Cancer Staging Manual. 7. Springer; NY, USA: 2009. p. 143-164.

90■■. Hyslop T, Weinberg DS, Schulz S, Barkun A, Waldman SA. Occult tumor burden predicts disease recurrence in lymph node-negative colorectal cancer. Clin Cancer Res. 2011; 17(10): 3293–3303. First report of the application of occult tumor burden analysis quantified by molecular techniques to stage patients with colorectal cancer. [PubMed: 21307149]

91. Polite BN, Dignam JJ, Olopade OI. Colorectal cancer and race: understanding the differences in outcomes between African Americans and whites. Med Clin North Am. 2005; 89(4):771–793. [PubMed: 15925649]
92. Polite BN, Dignam JJ, Olopade OI. Colorectal cancer model of health disparities: understanding mortality differences in minority populations. J Clin Oncol. 2006; 24(14):2179–2187. [PubMed: 16682737]
93. Soneji S, Iyer SS, Armstrong K, Asch DA. Racial disparities in stage-specific colorectal cancer mortality: 1960–2005. Am J Public Health. 2010; 100(10):1912–1916. [PubMed: 20724684]
94. Le H, Ziogas A, Lipkin SM, Zell JA. Effects of socioeconomic status and treatment disparities in colorectal cancer survival. Cancer Epidemiol Biomarkers Prev. 2008; 17(8):1950–1962. [PubMed: 18708384]
95. Chien C, Morimoto LM, Tom J, Li CI. Differences in colorectal carcinoma stage and survival by race and ethnicity. Cancer. 2005; 104(3):629–639. [PubMed: 15983985]
96. Laiyemo AO, Doubeni C, Pinsky PF, et al. Race and colorectal cancer disparities: healthcare utilization vs different cancer susceptibilities. J Natl Cancer Inst. 2010; 102(8):538–546. [PubMed: 20357245]
97. Marcella S, Miller JE. Racial differences in colorectal cancer mortality. The importance of stage and socioeconomic status. J Clin Epidemiol. 2001; 54(4):359–366. [PubMed: 11297886]
98. Mayberry RM, Coates RJ, Hill HA, et al. Determinants of black/white differences in colon cancer survival. J Natl Cancer Inst. 1995; 87(22):1686–1693. [PubMed: 7473817]
99. White A, Vernon SW, Franzini L, Du XL. Racial disparities in colorectal cancer survival: to what extent are racial disparities explained by differences in treatment, tumor characteristics, or hospital characteristics? Cancer. 2010; 116(19):4622–4631. [PubMed: 20626015]
100. Chen VW, Fenoglio-Preiser CM, Wu XC, et al. Aggressiveness of colon carcinoma in blacks and whites. National Cancer Institute black/white cancer survival study group. Cancer Epidemiol Biomarkers Prev. 1997; 6(12):1087–1093. [PubMed: 9419408]
101■. Hyslop T, Weinberg DS, Schulz S, Barkun A, Waldman SA. Occult tumor burden contributes to racial disparities in stagespecific colorectal cancer outcomes. Cancer. 2011; 118(9):2532–2540. First definitive description of the contribution of occult tumor burden to racial disparities in outcomes in colon cancer. [PubMed: 21887684]
102. Abati A, Liotta LA. Looking forward in diagnostic pathology: the molecular superhighway. Cancer. 1996; 78(1):1–3. [PubMed: 8646703]
103. Krishna R, Herman G, Wagner JA. Accelerating drug development using biomarkers: a case study with sitagliptin, a novel DPP4 inhibitor for Type 2 diabetes. AAPS J. 2008; 10(2):401–409. [PubMed: 18686043]
104. Lee JW, Devanarayan V, Barrett YC, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006; 23(2):312–328. [PubMed: 16397743]
105. Lee JW, Weiner RS, Sailstad JM, et al. Method validation and measurement of biomarkers in nonclinical and clinical samples in drug development: a conference report. Pharm Res. 2005; 22(4):499–511. [PubMed: 15846456]
106. Wagner JA. Overview of biomarkers and surrogate endpoints in drug development. Dis Markers. 2002; 18(2):41–46. [PubMed: 12364809]
107. Wagner JA. Back to the future: driving innovation in drug development. Clin Pharmacol Ther. 2008; 83(2):199–202. [PubMed: 18202681]
108. Wagner JA. Strategic approach to fit-for-purpose biomarkers in drug development. Annu Rev Pharmacol Toxicol. 2008; 48:631–651. [PubMed: 17937595]
109. Wagner JA, Williams SA, Webster CJ. Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. Clin Pharmacol Ther. 2007; 81(1):104–107. [PubMed: 17186007]
110. Williams SA, Slavin DE, Wagner JA, Webster CJ. A cost–effectiveness approach to the qualification and acceptance of biomarkers. Nat Rev Drug Discov. 2006; 5(11):897–902. [PubMed: 17080026]

111. Park J, Schulz S, Haaf J, Kairys JC, Waldman SA. Ectopic expression of guanylyl cyclase C in adenocarcinomas of the esophagus and stomach. Cancer Epidemiol Biomarkers Prev. 2002; 11(8):739–744. [PubMed: 12163327]
112. Croner RS, Peters A, Brueckl WM, et al. Microarray versus conventional prediction of lymph node metastasis in colorectal carcinoma. Cancer. 2005; 104(2):395–404. [PubMed: 15952189]
113. Frigola J, Song J, Stirzaker C, Hinshelwood RA, Peinado MA, Clark SJ. Epigenetic remodeling in colorectal cancer results in coordinate gene suppression across an entire chromosome band. Nat Genet. 2006; 38(5):540–549. [PubMed: 16642018]
114. Jen J, Kim H, Piantadosi S, et al. Allelic loss of chromosome 18q and prognosis in colorectal cancer. N Engl J Med. 1994; 331(4):213–221. [PubMed: 8015568]
115. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifentreated, node-negative breast cancer. N Engl J Med. 2004; 351(27):2817–2826. [PubMed: 15591335]
116. Wang Y, Jatkoe T, Zhang Y, et al. Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. J Clin Oncol. 2004; 22(9):1564–1571. [PubMed: 15051756]
117. Sullivan R, Peppercorn J, Sikora K, et al. Delivering affordable cancer care in highincome countries. Lancet Oncol. 2011; 12(10):933–980. [PubMed: 21958503]
118. Wong WB, Carlson JJ, Thariani R, Veenstra DL. Cost effectiveness of pharmacogenomics: a critical and systematic review. Pharmacoeconomics. 28(11):1001–1013. [PubMed: 20936884]
119. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for *KRAS* gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol. 2009; 27(12):2091–2096. [PubMed: 19188670]
120. Wilson C, Schulz S, Waldman S. Cancer biomarkers: where medicine, business, and public policy intersect. Biotechnology Healthcare. Feb.2007 :33–42.
121. Wilson C, Schulz S, Waldman SA. Biomarker development, commercialization, and regulation: individualization of medicine lost in translation. Clin Pharmacol Ther. 2007; 81(2):153–155. [PubMed: 17259939]

## Executive summary

**Colorectal cancer staging: prognosis & therapeutic response prediction**

- The most important prognostic and predictive marker for staging colorectal cancer is the presence of tumor cells in regional lymph nodes.
- The gold standard for evaluating lymph nodes for the presence of tumor cells is histopathology.
- Histopathology has technical limitations, including sampling error and restricted sensitivity, detecting one cancer cell in 200 normal cells.
- These limitations are reflected in the under-staging of node-negative (pN0) patients, of which 30% develop recurrent disease.

**Molecular staging paradigms**

- Staging can be performed by quantifying the expression of specific disease biomarkers by quantitative reverse transcriptase-PCR (RT-qPCR).
- RT-qPCR overcomes sampling issues inherent in histopathology by interrogating the entire available specimen.
- RT-qPCR enhances the sensitivity of detection, with the ability to detect one cancer cell in one million normal cells.

**GUCY2C**

- GUCY2C is a tumor suppressor universally involved in intestinal tumorigenesis.
- GUCY2C is selectively expressed in intestinal epithelial cells.
- GUCY2C is universally overexpressed by primary and metastatic colorectal cancer cells regardless of their anatomic location or differentiation state.
- GUCY2C is not expressed by other tumors originating outside the GI tract.
- These characteristics support GUCY2C as a highly specific marker of metastatic colorectal cancer cells in extraintestinal tissues.

**Detection of clinically significant occult nodal metastases using GUCY2C RT-qPCR**

- A prospective, blinded multicenter clinical trial explored the utility of GUCY2C RT-qPCR for detecting occult metastases in pN0 colorectal cancer patients.
- More than 80% of pN0 colorectal cancer patients harbor occult metastases in at least one lymph node as detected by GUCY2C RT-qPCR.
- Patients whose lymph nodes were free of occult metastases had favorable prognostic characteristics, including a low (6%) rate of recurrence, prolonged time-to-recurrence, and extended disease-free survival.
- Conversely, patients whose lymph nodes harbored occult metastases had unfavorable prognostic characteristics, including a high (21%) rate of recurrence, shortened time-to-recurrence and restricted disease-free survival.

**Quantity of occult metastatic tumor cells across the regional lymph node network is a prognostic marker of risk in pN0 colorectal cancer**

- Beyond the categorical detection of occult metastases (yes/no), the quantitative capabilities of RT-qPCR offer an unprecedented opportunity to estimate the

amount of occult tumor burden resident across the regional lymph node network and enhance the sensitivity and specificity of molecular staging.

- Occult tumor burden stratified patients into cohorts of low-, medium- and high-risk. Low-risk patients represented 60% of the population and exhibited a risk of recurrence of approximately 2%. By contrast, high-risk patients represented 9% of the population and exhibited a risk of recurrence of approximately 70%.
- Differences in occult tumor burden contribute to racial disparities in clinical outcomes in pN0 colon cancer in African–American and Caucasian patients.

**Conclusion & future perspective**

- Validation in independent populations is required for analytical confirmation.
- This approach can be extended to other populations beyond pN0 colon cancer patients including those with stage III colon cancer, rectal cancer, esophageal adenocarcinoma and gastric cancer, all of which express GUCY2C.
- This approach can be extended to other racial cohorts with established disparities in colorectal cancer, including Hispanics, Mexicans and Hawaiians.
- This approach has the potential to identify pN0 patients who could benefit from adjuvant chemotherapy.
- This approach can complement and enhance molecular analyses of primary tumors to provide the greatest prognostic and predictive information to guide the clinical management of colorectal cancer patients.