In this issue of *Clinical Cancer Research*, Hyslop and colleagues show that the level of guanylate cyclase 2C (GUCY2C) mRNA that is detected by quantitative reverse transcriptase PCR (RT-PCR) in lymph nodes is highly correlated with the risk of tumor recurrence and relapse-and disease-free survival in patients with resected stage II colorectal cancer (1).

Stage II colon and rectal cancers (i.e., cancers without apparent lymph node metastases by standard histopathologic criteria) are clinically a heterogeneous disease (2, 3). Whereas most patients with this tumor stage will be cured by surgery alone, a subset of patients will experience tumor recurrence, mainly in the form of distant metastatic disease. This raises the question as to whether adjuvant therapies should be offered to all stage II patients to reduce the risk of recurrence and thereby improve overall survival outcomes (4). However, given that the majority of these patients will have already been cured by surgical resection, investigators have used various approaches in recent years to identify patients with a higher (and lower) risk of recurrence and guide postoperative adjuvant treatment decisions. Some clinical factors have been proposed to be associated with a higher risk of recurrence in stage II colon cancer, including the T4 stage [involvement of serosa (T4a) or tumor infiltration into adjacent organs (T4b)], the number of lymph nodes identified in the surgical specimen, obstruction/perforation, lymphovascular invasion, and undifferentiated histology (4). More recently, molecular parameters and signatures have been investigated as potential prognostic markers for patients with stage II colon cancer. With the exception of the deficient mismatch repair enzyme phenotype (also termed microsatellite instability), which is associated with an excellent prognosis, none of the current molecular signature assays are able to clearly separate patients into highly distinct prognostic groups (5, 6). Thus, their value for clinical decision-making with regard to adjuvant therapy has so far been limited.

GUCY2C (also known as guanylyl cyclase C, intestinal guanylate cyclase, or heat-stable enterotoxin receptor) is an enzyme that is encoded by the GUCY2C gene. It is a receptor for the gastrointestinal hormones guanylin and uroguanylin and is found exclusively in the luminal side of intestinal epithelial cells (7). It is also the key receptor for heat-stable enterotoxins produced by pathogens such as *Escherichia coli*. Knockout mice for GUCY2C do not develop secretory diarrhea upon infection with enteropathogen *E. coli* (8). Loss of GUCY2C expression has been correlated with increased incidence of intestinal tumors, particularly in the presence of *APC* mutations (9). On the other hand, GUCY2C overexpression is commonly found in metastatic colorectal cancer, which may indicate that this highly evolutionarily conserved intestinal receptor plays a pivotal role in intestinal cell homeostasis (7).

The high specificity of GUCY2C as an intestinal marker forms the basis for its utility as a marker for occult lymph node metastases in colorectal cancer. In an initial study in 257 patients with pN0 colorectal cancers, qualitative RT-PCR for GUCY2C mRNA was able to separate patients into 2 distinct groups with a tumor recurrence of 6.3% versus 20.9% based on the presence or absence of GUCY2C mRNA, with a hazard ratio of 4.66 for recurrence-free survival and high statistical significance in multivariate analysis (10).

The analysis presented in this journal goes beyond that initial report by using quantitative RT-PCR of lymph nodes from 291 prospectively enrolled patients with stage II colorectal cancer. Recursive partitioning was used to define the optimized cutoff for the quantitative RT-PCR results, and eventually 60%, 31%, and 9% of patients were classified as carrying a low, intermediate, or high molecular tumor burden, respectively. The prognostic separation obtained with these criteria is striking. Of the 176 patients with low GUCY2C mRNA levels, only 4 developed tumor recurrence (2.3%), in comparison with 33.3% and 68% for patients with intermediate and high GUCY2C mRNA levels, respectively. The hazard ratios for time to recurrence were 25.5 and 65.4 for intermediate and high mRNA levels, respectively, compared with low mRNA levels. A similar but slightly attenuated graded result was found for correlation with disease-free survival.

The potential implications of these results for clinical practice and for our understanding of the pathomechanisms...
that are involved in the process of metastasis cannot be underestimated. The data raise the question as to whether distant metastases can develop without any lymphatic involvement, that is, without leaving a molecular trace of metastasis in regional lymph nodes behind (Fig. 1). The outcomes for patients with low GUCY2C mRNA levels in the Hyslop analysis are remarkably good, with a very low risk of tumor recurrence in the low-single-digit percentage range, whereas in almost all patients who developed distant metastases, intermediate or high mRNA levels could be identified in resected lymph nodes. The results therefore call into question the relevance of a hematologic (and not lymphatic) tumor spread in early-stage colorectal cancer. It could very well be that the prognosis for a truly lymph node–negative colorectal cancer, when analyzed with novel molecular detection methods for occult tumor cells, is much better than previously assumed. This notion is supported by recent data from the Adjuvant Colon Cancer Endpoints Group, which show that patients with stage II (but not stage III) disease diagnosed in 1996–2007 had a significantly longer time to recurrence than patients whose disease was diagnosed in 1978–1995, suggesting a refined and improved definition of "lymph node negativity" in the modern era (11).

Is GUCY2C RT-PCR of resected lymph nodes ready for use in clinical practice at this point? Unfortunately, the current study has certain limitations that will need to be addressed in future trials before this assay can be established in routine practice. The study was conducted over a period of >5 years in an oligo-center setting, which means that the actual accrual rate was quite low over time. The specific quantitative RT-PCR technique used in the study requires fresh-frozen tissue, which is not established for routine surgery, although this issue could likely be addressed. The study combined rectal and colon cancers, which express different metastatic patterns (for example, more lung metastases are found in rectal cancer patients); however, the authors specifically break out the colon cancer group in their analysis.

Eventually, a prospective validation study of this assay will be essential to establish GUCY2C as a marker for occult lymph node involvement with prognostic implications in colon and rectal cancer. Such a multicenter study is currently being conducted in a more selected group of patients with colon cancers only, at least 10 lymph nodes identified, and no previous treatment with adjuvant chemotherapy. In addition, this validation study is being performed on formalin-fixed tissue. Recently presented preliminary data show the feasibility of this approach and suggest that detection of GUCY2C mRNA in lymph nodes is associated with risk of disease recurrence in stage II colon cancer (12). If confirmed, these results could have significant implications for the selection of patients with stage II colorectal cancer for adjuvant therapy.
Disclosure of Potential Conflicts of Interest

Axel Grothey is a consultant for Genentech, Bayer, and Pfizer.

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