Summary: Detection of guanylyl cyclase C mRNA in lymph nodes of resected stage II colorectal cancer is highly correlated with the risk of tumor recurrence. If validated, these results could have significant implications for the selection of patients for adjuvant therapy in this disease.
In this issue of *Clinical Cancer Research*, Hyslop et al demonstrate that the level of Guanylate cyclase 2C (GUCY2C) mRNA detected by quantitative RT-PCR in lymph nodes is highly correlated with the risk of tumor recurrence, relapse-free 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) While most patients with this tumor stage will be cured by surgery alone, a subset of patients will experience tumor recurrence, mainly in form of distant metastatic disease. This raises the questions, if adjuvant therapies should be offered to all stage II patients to reduce the risk of recurrence and thereby improve overall survival outcomes.(4) Since the majority of these patients, however, will already be cured by surgical resection, various approaches have been utilized in recent years and decades to identify patients with higher (and lower) risk of recurrence to guide postoperative adjuvant treatment decisions. Some clinical factors have been cited to be associated with a higher risk of recurrence in stage II colon cancer, including T4 stage (involvement of serosa – T4a, or tumor infiltration into adjacent organs – T4b), number of lymph nodes identified in the surgical specimen, obstruction/perforation, lympho-vascular 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 (dMMR) phenotype, also termed microsatellite instability (MSI-H), which is associated with excellent prognosis, all current molecular signature assays have not been able to clearly separate patients out into highly distinct prognostic groups.(5, 6) Thus, their value for clinical decision-making with regard to adjuvant therapy has so far been limited.

Guanylate cyclase 2C, also known as guanylyl cyclase C (GCC), intestinal guanylate cyclase, or the heat-stable enterotoxin receptor (hSTAR) is an enzyme 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 also forms 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, in particular, in the presence of *APC* mutations.(9) On the other hand, *GUCY2C* overexpression is commonly found in metastatic colorectal cancer, which could indicate a pivotal role of this highly evolutionary conserved intestinal receptor for intestinal cell homeostasis.(7)

The high specificity of *GUCY2C* as intestinal marker forms the basis for using it 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 current analysis in this journal goes beyond this initial report by utilizing quantitative RT-PCR of lymph nodes from 291 prospectively enrolled patients with stage II colorectal cancer. Recursive partitioning was used to define optimized cut-off for the quantitative RT-PCR results and eventually classified 60%, 31%, and 9% of patients as carrying low, intermediate, and high molecular tumor burden, respectively. The prognostic separation using these criteria was striking. Of the 176 patients with low *GUCY2C* mRNA levels, only 4 developed tumor recurrence (2.3%), compared with 33.3% for intermediate, and 68% for patients with high *GUCY2C* mRNA levels. Hazard ratios for time to recurrence were 25.5 and 65.4 for intermediate and high 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 involved in the process of metastasis...
cannot be underestimated. The data raise the question if distant metastases can develop without any lymphatic involvement, meaning without leaving a molecular trace of metastasis in regional lymph nodes behind (Figure 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 question the relevance of a hematologic (and not lymphatic) tumor spread in early stage colorectal cancer. It could well be that the prognosis of 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 ACCENT (Adjuvant Colon Cancer Endpoints) group which demonstrated that the time to recurrence of patients with stage II, but not of stage III disease diagnosed 1996-2007 was significantly longer than of patients diagnosed 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, there are certain imitations of the current study which will need to be addressed in future trials before this assay can potentially be established in routine practice. The study was conducted over more than 5 years in an oligo-center setting, meaning the actual accrual rate was quite low over time. The specific quantitative RT-PCR technique utilized in the study required the use of fresh-frozen tissue, which is not established in routine surgery, although this issue could likely be addressed. The study combined rectal and colon cancers, which do express a different metastatic pattern with more lung metastases found in rectal cancer patients, although 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 implication in colon and rectal cancer. Such a multicenter study is ongoing in a more selected group of patients with colon cancers only, at least 10 lymph nodes identified and not treated with adjuvant chemotherapy. In addition, this validation study is performed on formalin-fixed tissue. Recently presented preliminary data demonstrated the feasibility of this approach and suggested 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.

Figure 1. Guanylate cyclase C (GUCY2C), is a receptor for the gastrointestinal hormones guanylin and uroguanylin, and is found exclusively in the luminal side of intestinal epithelial cells. The high specificity of GUCY2C as intestinal marker forms the basis for using it as a marker for occult lymph node metastases in colorectal cancer. Very few, if any patients, develop distant metastasis without detectable GUCY2C mRNA levels in regional lymph nodes.
References

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