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Technical Brief
Number 13

Gene Expression Profiling for Predicting Outcomes in Stage II Colon Cancer

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Gene Expression Profiling for Predicting Outcomes in Stage II Colon Cancer

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Prepared by:
Blue Cross and Blue Shield Association Technology Evaluation Center
Evidence-based Practice Center
Chicago, IL

Investigators:
Edgar R. Black, M.D.
Louise Falzon, P.G.DipInf
Naomi Aronson, Ph.D.

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The information in this report is intended to help health care decisionmakers—patients and clinicians, health system leaders, and policymakers, among others—make well-informed decisions and thereby improve the quality of health care services. This report is not intended to be a substitute for the application of clinical judgment. Anyone who makes decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent information, that is, in the context of available resources and circumstances presented by individual patients.

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies and strategies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

This EPC evidence report is a Technical Brief. A Technical Brief is a rapid report, typically on an emerging medical technology, strategy or intervention. It provides an overview of key issues related to the intervention – for example, current indications, relevant patient populations and subgroups of interest, outcomes measured, and contextual factors that may affect decisions regarding the intervention. Although Technical Briefs generally focus on interventions for which there are limited published data and too few completed protocol-driven studies to support definitive conclusions, the decision to request a Technical Brief is not solely based on the availability of clinical studies. The goals of the Technical Brief are to provide an early objective description of the state of the science, a potential framework for assessing the applications and implications of the intervention, a summary of ongoing research, and information on future research needs. In particular, through the Technical Brief, AHRQ hopes to gain insight on the appropriate conceptual framework and critical issues that will inform future comparative effectiveness research.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this Technical Brief. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by email to epc@ahrq.hhs.gov.

Carolyn M. Clancy, M.D.
Director, Agency for Healthcare Research and Quality

Jean Slutsky, P.A., M.S.P.H.
Director, Center for Outcomes and Evidence
Agency for Healthcare Research and Quality

Stephanie Chang, M.D., M.P.H.
Director, EPC Program
Center for Outcomes and Evidence
Agency for Healthcare Research and Quality

Supriya Janakiraman, M.D., M.P.H.
Task Order Officer
Center for Outcomes and Evidence
Agency for Healthcare Research and Quality

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Gene Expression Profiling for Predicting Outcomes in Stage II Colon Cancer

Structured Abstract

Background. While adjuvant chemotherapy is recommended in patients with stage III colon cancer, its role in stage II disease is unclear. In treating 100 stage II patients with adjuvant chemotherapy, three or four will benefit, while others will suffer significant adverse effects. Research is underway to improve this decisionmaking. Gene expression profiling (GEP) is one of the techniques being studied.

Purpose. The objective of this Technical Brief is to provide a summary of the state of the science on use of GEP in predicting outcomes, including benefit from adjuvant chemotherapy, in patients with stage II colon cancer. This Brief also summarizes key uncertainties.

Methods. Four guiding questions were used to frame this Technical Brief. A scan of the published literature through May 2012 identified studies describing the relationship between GEPs and outcomes in stage II colon cancer. Other sources included the U.S. Food and Drug Administration Web site, Key Informants, clinical trial Web sites, and scan of the grey literature.

Findings. Results have been published for GEP assays in stage II colon cancer; 13 GEP assays for prognosis, one GEP for prediction (reduced tumor recurrence from adjuvant therapy), and one microRNA profile for prognosis. Five GEP assays are available commercially. Published studies have not provided information related to clinical utility, the effect that using the GEP result in patient care has on net health outcome. Limited information was found for analytic validity. The current evidence does not provide the type of information needed to answer major questions about use of GEP assays in these patients.

Conclusion. Although information is emerging about use of GEP assays to inform the decision about use of adjuvant chemotherapy in patients with stage II colon cancer, studies to date have not provided the type of information needed to address major uncertainties.

Contents

Tables

Boxes

Figures

Appendixes

Background

Stage II Colon Cancer

Colon cancer is a common malignancy affecting both women and men. In 2012, it is expected to be the fourth most commonly diagnosed cancer (after prostate, breast, and lung cancer) with an estimated 103,170 new cases and, combined with rectal cancer, is the second most common cause of cancer deaths (after lung cancer) with 51,690 deaths.[1]

The most important prognostic factor for colon cancer is stage at diagnosis. About 40 percent of patients with colon cancer are initially diagnosed with stage I disease (these localized tumors do not invade through the muscularis propria). Stage I disease has a 5-year survival rate of over 95 percent.[2] Five-year survival rates for patients diagnosed with stage II disease are between 58 and 83 percent. Stage II colon cancer is characterized by full-thickness tumor invasion of the bowel wall and the absence of lymph node and distant metastases. Stage II disease is now subdivided into IIA (T3 tumors that invade through the muscularis propria into the pericolorectal tissues), IIB (T4a tumors that directly penetrate to the surface of the visceral peritoneum), and IIC (T4b tumors where tumor directly invades or is adherent to other organs or structures).[3] The relative 5-year survival rate for stage II T4a tumors is higher than for T4b lesions.[4] Stages III and IV have a worse prognosis.

A number of negative prognostic factors, that is, factors associated with increased risk of recurrence, have been identified in stage II disease including T4 tumors (which tend to be large); obstruction or bowel perforation at initial diagnosis; an inadequately low number of assessed lymph nodes from surgery (12 or fewer); poorly differentiated histology, vascular, lymphatic, and perineural invasion; a high preoperative level of carcinoembryonic antigen; and the presence of indeterminate or positive resection margins.[2, 5]

Most patients with stage II colon cancer are cured with surgery alone; however, 25 to 30 percent of those with resected stage II disease will develop recurrence or die from their disease. In this group, adjuvant chemotherapy may produce a small improvement in overall survival when compared with surgery alone, and it may afford a small reduction in the risk of disease recurrence. In a 2010 review, Midgley et al. commented, "[W]e have to treat 100 stage II patients to cure 3 or 4, while accepting that up to 40 percent of those treated will suffer significant toxicity…."[6] Thus, routine use of adjuvant therapy is usually recommended only in subgroups with stage II disease that may be at higher than average risk for recurrence, such as those whose initial diagnosis is complicated by bowel perforation. For patients where a decision is made to administer adjuvant chemotherapy, an additional decision has to be made about the specific adjuvant regimen that will be used. Identifying the individuals in whom the potential benefits of chemotherapy outweigh the risks is challenging. The current system to assess recurrence risk using the prognostic factors noted above (e.g., a T4 tumor) may be inadequate for determining individual risk. To that end, advances in molecular and genomic medicine, such as gene expression profiling, may improve the clinician's ability to assess individualized risk and predict response to adjuvant therapy, as long as the test results provide new information that is clinically valid and useful.

Gene Expression Profiles

Gene expression profiles (GEPs), also known as gene expression patterns or signatures, measure the activity of "expression" of multiple genes using a single sample. Gene expression results from DNA transcription into messenger RNA (mRNA); mRNA then serves as the template for protein synthesis. Gene expression is determined by analyzing RNA in the sample, generally using either reverse transcription quantitative polymerase chain reaction or DNA microarrays. GEP tests use defined protocols to evaluate the specimens to be analyzed: preparing the RNA samples, copying into DNA, normalizing the raw expression measurements, and computing summary results (summary indices). Data from a GEP test can provide information about a cell's type, its current state of activity, and its local environment.

This Brief will focus on the use of GEP tests for both prognostic and predictive outcomes. For the purposes of this Technical Brief, prognostic outcomes relate to disease prognosis such as recurrence of tumor or survival. The prognostic outcome often assesses disease recurrence over time (usually over 3 to 5 years) and is generally measured by disease-free survival. In contrast, use of GEP assays for predictive outcomes is different. This use correlates the GEP result with benefit (reduced recurrence rate and improved survival) from adjuvant chemotherapy. Predictive assays are especially important in that their use could result in improved survival.

Predicting the two types of outcomes may require different GEP signatures. A GEP signature that is found to be accurate for identifying prognosis may not be accurate for predicting benefit from adjuvant therapy. Similarly, an accurate predictive GEP signature may not provide accurate prognostic information. In addition, predictive GEP signatures may only apply to the adjuvant regimen for which they were studied.

According to Midgley et al. "There are good examples of gene expression profiles being used to improve disease classification in lymphoma and breast cancer and aid in determination of prognosis in breast cancer."[6] Using GEP tests could improve the current staging system for colon cancer. However, in a 2009 publication, based on eight cohorts from six studies, Lu et al. noted that existing GEP tests produced sufficiently high false-positive and false-negative rates to preclude routine use and commented that additional validation studies were needed.[7] GEP tests were found to be in various stages of development for use in colon cancer. Given the additional studies on tests reported in that publication and the emergence of new tests, followup of the Lu publication with a Technical Brief is warranted.

Confounding Factors

There are other factors that can impact disease outcomes and test interpretation that will be mentioned briefly. First, a number of publications and reviews, for example a recent review by Vilar and Gruber,[8] provide information on the implications of microsatellite instability, also known as mismatch repair (MMR) deficiency, in colon cancer. The data indicate that MMR deficiency (high microsatellite instability, MSI-H) may identify a small (15–20 percent) population of patients with stage II disease who may derive no benefit or may even experience deleterious effects from adjuvant fluorouracil/leucovorin (FU/LV) -based chemotherapy.[8] Colonic malignancies with these characteristics, that is, MSI-H, often demonstrate improved disease-free survival. MSI testing is widely available and used along with clinical risk factors to help make decisions about adjuvant chemotherapy in patients with stage II colon cancer. Thus, it is important that published reports on GEP tests in colon cancer treatment decisionmaking specifically comment on whether (and how) they measured MSI status and how they took this

into account in their analysis. As an example, the GEP test might be studied only after patients with MSI-H tumors are excluded.

The adjuvant chemotherapy regimen used when studying GEP assays is another confounding factor. Over the last decade, the schedule and duration of adjuvant chemotherapy in treating colon cancer has changed from a 12-month course of bolus FU and levamisole combination to a 6-month course of either infusion FU or an oral fluoropyrimidine such as capecitabine with or without oxaliplatin. Thus, it will be important to note, as suggested by Tabernero and Baselga[9] the chemotherapy regimen(s) for which response was predicted. At present, few studies have addressed use of GEP assays for predicting response to adjuvant therapy. If predictive GEP assays are developed, their use should not be generalized to other regimens.

A recent publication by Gerlinger and colleagues[10] reported intratumor heterogeneity and underestimation of the tumor genomic landscape from analysis of multiple samples from patients with primary renal carcinomas and metastatic sites. The heterogeneity included finding gene-expression signatures of both good and poor prognosis in different regions of the same tumor. The possibility of having similar findings of heterogeneity in stage II colon cancer may need to be studied, especially if results from initial GEP studies are not successfully validated.

Scope of Report

Because the topic of this Brief is GEP testing and GEP is based on results from expression of multiple genes, this Brief does not include reports for expression of single genes. This report also excludes studies of single or multiple genetic mutations. Furthermore, given current knowledge and the complexity of this clinical situation, it seems unlikely that a single gene marker would provide accurate prediction. These single-gene markers may be used to predict response to chemotherapy, prognosis, potential for chemotherapy-related adverse events, or chemotherapy-related dose adjustments, but are not included in the scope of this Brief. New assays that measure RNA to detect tumor cells are also beyond the scope of this Brief. Since the topic of this Brief is patients with stage II colon cancer, publications on the use of GEP for other stages of colon cancer, or for both colon and rectal cancer, will not be included, unless the publication presents information on patients with stage II colon cancer separately. While the term “colorectal cancer” is often used and reports may combine these cases, because of the different approach to surgical and adjuvant therapy of rectal tumors, this Brief on GEP in colon cancer excluded studies with combined analysis of colon and rectal malignancies.

Thus, GEP testing of patients following surgical resection of stage II colon cancer has potential benefit related to prognosis (recurrence) and also related to prediction (benefit from adjuvant therapy), but the magnitude is unknown. Identifying patients who do not need adjuvant chemotherapy because of very low risk of recurrence or predicted lack of benefit from adjuvant chemotherapy would improve the net health outcome by avoiding treatment-related adverse effects. On the other hand, introducing routine testing without a clear understanding of benefits and risks could result in those who would benefit from adjuvant therapy not receiving it. This Technical Brief provides an overview of the “state of the science” for the use of GEP testing in patients with stage II colon cancer. This Brief will not list or synthesize study results. However, the conceptual framework and uncertainties identified in this Brief can inform future comparative effectiveness research.

Guiding Questions

Guiding Question 1. What are important aspects of GEPs?

- What GEP products are available for clinical use in patients with stage II colon cancer?
- What types of tissue specimens (e.g., frozen tissue) are analyzed for the GEP?
- What is the test turn-around time, that is, how long does it take to obtain test results?
- What are the potential benefits and harms of this testing compared with current practice?
- Is GEP testing a replacement or add-on technology?

Guiding Question 2. What is the clinical approach for using GEP assays?

- Are all patients with stage II colon cancer included in studies of GEPs?
- What is the U.S. Food and Drug Administration (FDA) status of these tests?
- Currently, how widely used are these tests?

Guiding Question 3. What is the current evidence for the technology/intervention?

- What published and unpublished studies, including both derivation and validation studies, have reported on the clinical validity and clinical utility for each of the GEPs for potential use in patients with stage II colon cancer?
- How extensively have the clinical validity and clinical utility of these tests been validated?
- Was a reclassification analysis performed with risk stratification using GEP when compared with that obtained with standard risk factors?
- What information is available regarding analytic validity?

Guiding Question 4. What important issues are raised by using GEP testing for stage II colon cancer?

- What are the key unresolved or controversial issues with using GEP testing in patients with stage II colon cancer?
- What are the implications of the current level of diffusion and/or further diffusion of this technology/intervention given the current state of the evidence?
- What key studies are currently underway? What are the important questions for future research?

Methods

Several sources were used to inform this Technical Brief. Information was collected from a review of published medical literature, narrative review articles, a search of the grey literature, and discussions with Key Informants. In addition, the Agency for Healthcare Research and Quality's (AHRQ's) Scientific Resource Center requested Scientific Information Packets from test manufacturers in March and April 2012.

Guiding Questions 1 and 2 above rely on information from published narrative reviews and clinical guidelines and information in the grey literature. The latter included information culled from manufacturers, patient advocacy groups, and other sources as identified in an Internet search (described in the following sections).

Guiding Question 3 was addressed through a review of the peer-reviewed literature. Key Informants provided input on the potential clinical outcomes of interest and the potential benefits and harms of GEP testing.

Guiding Question 4 relies on integrating information from Key Informants, grey literature, narrative reviews, and review of the literature.

Given the current role of GEP testing in patients with breast cancer, questions formulated in a report for the AHRQ on GEP testing in patients with breast cancer[11] were used to inform this Technical Brief. In developing the framework for review of the published literature, this Brief used prior publications that discussed approaches for use of archived specimens and assessment of tumor-marker utility.[12, 13]

Data Sources

Discussions With Key Informants

The Key Informants included clinical experts, payers, and patients. The clinical experts were from the disciplines of medical oncology, surgical oncology, laboratory medicine, epidemiology, and clinical genetics and had expertise in colon cancer and/or genetics.

One group conference call was held with the Key Informants. During the first call, the Key Informants provided input on the literature review, for example, key outcomes, potential tests, and proposed inclusion/exclusion criteria for the literature search. As followup to the group conference call, the Key Informants were interviewed individually by telephone, using a semi-structured interview outline that provided the content experts the opportunity to share their experiences with GEP testing in patients with colon cancer and their opinions on unresolved or controversial issues related to GEP testing.

Grey Literature Search

Internet search was conducted at the FDA Web site concerning GEP tests in stage II colon cancer. Additional searches were conducted as noted below. This information was used in answering Guiding Questions 1, 2, and 4.

Web sites of companies who are developing and currently offering GEP tests for colon cancer were searched to inform Guiding Questions 1 and 2. Examples include Agendia, Inc. (www.agendia.com/pages/coloprint/), Precision Therapeutics (www.precisiontherapeutics.com), Everist genomics (www.everistgenomics.com), Genomic Health (www genomichealth.com), Signal Genetics, LLC (www.signalgenetics.com and https://www.chipdx.com/colon-

module.aspx), Signature Diagnostics (www.signature-diagnostics.de/predictor.htm), and Affymetrix, Inc. (www.affymetrix.com).

Clinical guidelines for colon cancer were reviewed for recommendations on using GEP testing for stage II colon cancer. Guidelines reviewed were from the American Society of Clinical Oncology (www.asco.org and www.cancer.net) and the National Comprehensive Cancer Network (www.nccn.org).

Patient-oriented and patient-advocacy Web sites for colon cancer were searched. Sites reviewed included the American Cancer Society (www.cancer.org), American Society of Clinical Oncology (www.asco.org), CancerColon Cancer Alliance (www.ccalliance.org), Fight Colorectal Cancer (http://fightcolorectalcancer.org), and NCCN (National Comprehensive Cancer Network) (www.nccn.com).

Published Literature Search

A scan of the published medical literature was conducted to address Guiding Question 3. Searches were performed in MEDLINE®, Embase®, and the Cochrane Library (specifically CENTRAL, DARE, and the HTA Database). The search strategy for MEDLINE® (start date of 1946) is shown in Appendix A.

The DistillerSR® Systematic Review Tool (Evidence Partners Inc., Manotick, ON, Canada) was used to facilitate the screening and study selection process, as follows. Titles and abstracts were screened independently by two reviewers to detect potential articles relevant to the topic. Full-text articles of those marked as potentially relevant were retrieved and screened independently by two reviewers for inclusion or exclusion in the Brief. Disagreements were reviewed and resolved by consensus. Reference lists of included studies were reviewed to identify additional studies.

Published studies, with English-language abstracts, were included in this Technical Brief if they described analytic validity, clinical validity, or clinical utility for GEP test results in patients with stage II (or Duke B) colon cancer. The included studies used GEP assays analyzing RNA (e.g., cDNA or oligonucleotide microarrays) and reported clinical outcome (e.g., cancer recurrence or death) with at least 2 years of followup, as 2 years is the minimum followup time needed to adequately assess whether or not a patient is free of recurrence. In addition, benefit from adjuvant chemotherapy was used as another outcome marker. The data elements abstracted from included articles is listed in Appendix B.

Studies were excluded if they did not describe use of a GEP (multiple genes) or did not provide information that correlated the GEP result with a clinical outcome. Studies that used GEP results for other purposes, such as in the diagnosis of colon cancer or determining cancer stage at the time of surgery were also not included. Studies that did not provide results specific for stage II colon cancer, because they included various stages of cancer or both colon and rectal cancers) were not included in this Brief. However, citations for these studies were noted and are listed in Appendix C.

Findings

Description of Proposed Intervention

Guiding Question 1. What are important aspects of GEPs?

What GEP Products are Available for Clinical use in Patients With Stage II Colon Cancer?

As of July 2012, in the U.S., five gene expression profile (GEP) assays are commercially available; one of these GEP assay may also be available through a clinical trial and one is noted for nondiagnostic or research use only (Table 1). These GEP assays are all prognostic indicators.

Table 1. Gene expression assays available for clinical use in stage II colon cancer

Assay	Company	Gene Signature	Specimen Used
ColoPrint® Colon Cancer Recurrence Assay*	Agendia, Inc	18 Gene Expression Profile (using Agilent microarray)	Fresh tissue or fresh, frozen tissue
ColonPRS®+	Signal Genetics, L.L.C.	163 Genes	Fresh, frozen biopsies
GeneFx® Colon	Precision Therapeutics	634 Probe-set signature	Formalin-fixed paraffin embedded tissue
OncoDefender-CRC (colon and rectal cancer)	Everist Genomics	5 Genes	Formalin-fixed paraffin embedded tissue
Oncotype DX® Colon Cancer Assay	Genomic Health, Inc.	12 Genes (Seven prognostic and five reference genes)	Formalin-fixed paraffin embedded tissue

*May also be available for use through PARSC Clinical Trial
+Currently (July 2012) available for nondiagnostic or research use only

These commercially available assays use proprietary approaches to measure RNA using either reverse-transcriptase polymerase chain reactions (PCR) or a gene chip microarray system to determine the GEP result.

What Types of Tissue Specimens (e.g., Frozen Tissue) are Analyzed for the GEP?

Two sources of tissue are being used for these assays; either fresh-frozen tissue or formalin-fixed, paraffin-embedded (FFPE) tissue. Either approach is acceptable. Assays that use the FFPE-tissue are generally viewed as more convenient because the FFPE-specimens use tissue from the process used to prepare surgical specimens for routine histological examination. Because RNA is unstable, it degrades in the FFPE-specimens, and therefore these specimens contain less RNA. This approach uses reference genes to adjust for the RNA degradation. Other GEP assays, and especially GEP assays being developed, use fresh, frozen tissue or fresh tissue kept in media such as RNARetain because there is less degradation of the RNA. This approach results in a non-standard approach to specimen-handing, especially for frozen samples, and can lead to problems with obtaining or storing the specimens. As noted in Table 1, three of the available GEP assays use FFPE-tissue. In addition, in many cases, the tissue specimen undergoes microdissection to obtain specimens that have a high proportion of tumor cells (e.g., 80 percent or more). Very little information is provided about tissue handling and processing or about freezing and storage of tumor tissue that is subsequently used for the GEP assay.

What is the Test Turnaround Time, That is, how Long Does it Take To Obtain Test Results?

Minimal data were found concerning this question. Information on three of the GEP assays: ColoPrint® Colon Cancer Recurrence Assay and the OncoDefender-CRC assay, indicated (www.agendia.com, and www.everistgenomics.com respectively) that the results of its assay are available within 7 to 10 days following receipt of the tumor sample and Oncotype DX® Colon Cancer assay indicated results are available in 10-12 days (www.oncotypedx.com).

In considering this question about turn-around time, the clinical context for use of the result needs to be considered. The results of the GEP assays in this Technical Brief may provide additional information concerning the decision about administering adjuvant chemotherapy in patients with stage II colon cancer. The discussion with a patient about the benefits and risks of adjuvant chemotherapy usually occurs 3 to 6 weeks after surgery (once final pathology results are known) and the chemotherapy is generally initiated between 4 to 8 weeks after surgery. Thus, test turn-around time of one to two weeks would seem to be acceptable.

Therefore, if the GEP assay is to be useful in clinical care, the turn-around time has to provide results within the time between when a patient with stage II disease is presented with options regarding adjuvant therapy and the time when the adjuvant therapy needs to be started (generally no later than 8 weeks after surgery).

What are the Potential Benefits and Harms of This Testing Compared With Current Practice?

The key research questions that must be addressed are whether use of GEP results lead to improved net health outcome and whether use of GEP assays is better than use of alternative prognostic and predictive markers, including both clinical findings as well as other candidate biomarkers, such as microsatellite instability.

As noted in the Background section, currently the decision about administering adjuvant chemotherapy in patients with stage II colon cancer is based on the presence or absence of risk factors, many of which come from detailed histological examination of the tumor. These risk factors help to predict the likelihood of recurrent (metastatic) disease and thus impact the decision regarding adjuvant chemotherapy. Other factors, including the patient's co-morbid conditions and functional status can impact the recommendations. As also noted previously, with current practice we cannot identify the exact subset of patients with stage II disease who will benefit from adjuvant chemotherapy.

Thus, there are several potential benefits for use of GEP in patients with stage II colon cancer. If the GEP assay can accurately identify stage II patients who are at very low risk (prognostic marker) for a future recurrence of colon cancer; use of adjuvant chemotherapy would likely not be needed. This benefit would be particularly important if these patients are incorrectly classified with current methods as high risk and are given adjuvant chemotherapy.

Another potential benefit for use of a GEP assay is to identify those with stage II colon cancer at high risk of recurrence. Adjuvant chemotherapy may then be recommended in these patients. This may be most useful if the GEP result accurately identified patients who are considered to be at low risk for recurrence with currently used measures and thus do not receive adjuvant chemotherapy. While use of GEP in this way as a prognostic marker does not indicate likelihood of response to adjuvant chemotherapy, this is similar to how decisions about adjuvant chemotherapy are made currently using clinical markers.

An important potential benefit for use of a GEP assay in these stage II patients is to identify those for whom adjuvant chemotherapy using a specific therapeutic regimen provides substantial reduction in the risk of future recurrence of colon cancer and leads to improved survival. This use as a predictive marker is important in that the outcome is improved survival from a specific regimen of adjuvant chemotherapy. Note that a predictive GEP assay might have a different genetic signature than a prognostic GEP assay. If so, the predictive assay would be used if the prognostic result indicated high risk of recurrence. In this situation, the GEP result is correlated with the benefit from adjuvant chemotherapy.

The primary potential harm for use of GEP in these patients is similar to harms with most testing; that is, making decisions about treatment for patients who have false-positive or false-negative results. In particular, incorrectly withholding adjuvant chemotherapy because of the result of a GEP assay in a patient with stage II colon cancer who would benefit from treatment would result in harm. Thus, it is imperative to understand the accuracy (clinical validity) of the GEP assays being evaluated.

Is GEP Testing a Replacement or add-on Technology?

While the final answer to this question requires additional research, at the present time this test likely will be an "add-on" technology. That is, the GEP assays are being studied as a better way to identify risk of disease recurrence and/or benefit from adjuvant therapy than existing approaches. The existing approaches generally use information that is routinely obtained from the surgical procedure (presence of bowel perforation) and histological examination of the tumor (perivascular invasion by tumor). Use of the GEP assays will not replace these standard procedures.

With additional research the GEP assays could replace determination of microsatellite instability as a risk marker in stage II cancer. However, this is a theoretical possibility at this time, and this possibility would likely vary among different GEP assays.

Guiding Question 2. What is the clinical approach for using GEP assays?

Are all Patients With Stage II Colon Cancer Included in Studies of GEPs?

There is variability in the clinical studies of GEP assays regarding which patients with stage II colon cancer are included. Some studies include all stage II patients, while others only include those tumors without microsatellite instability.

In addition, GEP assays to predict response to adjuvant chemotherapy should be assumed to apply only to patients receiving the specific drug regimens that were used in deriving and validating the GEP assay. That is, the predictive GEP assays should be assumed to be regimen specific.

What is the U.S. Food and Drug Administration (FDA) Status of These Tests?

A search of the U.S. Food and Drug Administration (FDA) Web site did not provide any information regarding the use of the commercially available GEP assays for use in stage II colon cancer.[14] Thus, none of these GEP assays has specific approval from the FDA.

However, regulatory oversight for genetic tests is not straightforward. The primary regulatory bodies overseeing genetic tests such as GEPs, in the U.S. are the FDA and the Centers

for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). The FDA addresses the safety and effectiveness of diagnostic tests and the quality of the design and manufacture of these tests, whereas CLIA regulates laboratory personnel qualifications, quality-control procedures, and proficiency testing programs. The FDA has jurisdiction over both laboratory developed tests (LDTs) for use in an in-house laboratory and tests classified as "in-vitro devices" that are distributed to other laboratories as test kits. Historically, the FDA has subjected test kits to premarket review of safety and effectiveness, but not most LDTs. Thus, GEPs marketed as LDTs may enter the U.S. market with analytical validation under laboratory regulations imposed by CLIA but without evidence of clinical validity or utility. If a specific test were to be reviewed by the FDA, the review would evaluate its safety and effectiveness, which implies review of information on at least clinical validity. The GEP assays currently (July 2012) available are being marketed as LDTs.

Currently, how Widely Used are These Tests?

Input from Key Informants indicated that currently there is little use of GEP assays in the clinical care of patients with stage II colon cancer; clinical use would be to assist in the decision regarding adjuvant chemotherapy. Key Informants also noted this is an area of great clinical interest and active research with many research presentations at major scientific meetings. Some of the Key Informants expect use rates to increase. The interest in this topic is also likely related to the use of GEP assays in cases of early stage breast cancer. The current availability of five GEP assays (Table 1) seems to also indicate interest in their use for patients with stage II colon cancer.

Input from Key Informants indicated that current standard of care for stage II patients does not include GEP testing. Guidelines of professional societies (National Comprehensive Cancer Network and American Society of Clinical Oncology) have reached a similar conclusion.[5, 15] The patient-oriented Web sites of the American Cancer Society, American Society of Clinical Oncology, Cancer.Net, Colon Cancer Alliance, Fight Colorectal Cancer, and NCCN (National Comprehensive Cancer Network) contained variable information about use of GEP assays. While not every site had information, some provided general information about GEP testing while others provided information about specific tests. A summary of the information from those Web sites is found in Table 2.

Table 2. Information about gene expression profile assays in colon cancer from patient-oriented Web sites

Organization	Information Noted
American Cancer Society	No specific information on GEP assays in colon cancer
ASCO (American Society of Clinical Oncology)	Has links to abstracts of ASCO annual meetings No specific information found on GEP assays
Cancer.Net	Item that indicates gene test may predict recurrence risk for colon cancer
Colon Cancer Alliance	Has page on "Oncotype DX® For Stage II Colon Cancer" that includes/links to Resources and Information
Fight Colon Cancer	Lists several news items on developments in use of GEP assays in colon cancer
NCCN (National Comprehensive Cancer Network)	Has Guidelines for Patients: Colon Cancer Does not mention GEP assays

Evidence Map

Guiding Question 3. What is the cCurrent evidence for the technology/intervention?

Published Literature

The search strategy used to identify published studies for MEDLINE® (start date of 1946 through May 2012) is shown in Appendix A. This strategy was adapted for EMBASE®, and the Cochrane Library (specifically CENTRAL, DARE, and the HTA Database). A total of 1,363 citations were identified in the searches. Combined review of the titles and abstracts excluded 1, 154 of the citations. The principal reasons for exclusion at this stage were that a study enrolled only patients with metastatic colon cancer; the patients did not have colon cancer (publications dealing with only rectal cancer were excluded at this stage); the testing involved only a single gene (and thus was not a gene-expression profile); the testing did not analyze RNA; or the testing was chromosome or mutation analysis (rather than gene-expression). Publications that used GEP results for other purposes, such as the diagnosis of colon cancer or determining cancer stage at the time of surgery, were also excluded at this time. Review of the 209 full-text articles identified 20 studies related to the analytical validity, clinical validity, and/or clinical utility in patients with stage II colon cancer. The major reasons for exclusion during full-text review were that specific outcome data for patients with stage II colon cancer were not presented or that the test being studied was not a GEP assay, either because it was a single-gene marker or an assay that did not measure RNA. Therefore, this Brief includes data from 20 studies[16-33] that provided primary information on the relationship between GEP results and clinical outcomes, along with information on analytic validity, in patients with stage II colon cancer. Of note, two of these publications[19, 28] include datasets with combined information on Stage II colon and rectal cancer; these studies are included here because subsequent validation study[19, 26] involved only patients with stage II colon cancer. A list of data elements abstracted from these included studies can be found in Appendix B.

What Published and Unpublished Studies, Including Both Derivation and Validation Studies, Have Reported on the Clinical Validity and Clinical Utility for Each of the GEPs for Potential use in Patients With Stage II Colon Cancer? How Extensively Have the Clinical Validity and Clinical Utility of These Tests Been Validated?

GEP Assays as Prognostic Markers

The majority of the published studies evaluated use of the GEP assays as a prognostic marker, that is, determining the relationship, clinical validity, between the GEP test result and outcomes such as recurrence of cancer or survival in the population studied. Based on these published studies, information about the state of the science for use of GEP assay as a prognostic marker, that is, relationship between the marker and disease recurrence, in stage II colon cancer is shown in Table 3. The included studies[16-20, 22, 23, 25-28, 30-33] described thirteen different GEP assays for this application. Four of the commercially available GEP assays are listed in the first rows in Table 3; the peer-reviewed publication[24] for the fifth commercially available assay

(OncoDefender-CRC) includes data for patients with both stage I and II colon cancer and is thus not included in Table 3; summary information for that study is provided in the next section.

Table 3. Summary of scientific evidence for gene expression profiles in stage II colon cancer: prognostic—clinical validity and clinical utility

GEP: Name and/or Gene Expression	Initial Derivation: No. CC Pts.	Initial Published Validation	Subq Val: No. Studies	Subq Val: No. Patients	Subq Val: No. Dataset	Subq Val: Study Design	Recurrence	Overall Survival	Re-class Done?	Re-Class Comparison	Clinical Utility	Discrimination Discussed	Account for MSI	Ethnicity
Oncotype DX® Colon Cancer Assay: 12-Gene Prognostic (7 Prognostic and 5 Reference Genes) Derivation: (O'Connell 2010)[27] Validation: (Gray 2011)[20]	1,851	LO	1	1,436	1	RP	•V	•V					Yes	U.K. sites for validation
ColonPRS®: 163-Gene Prognostic Gene Expression Signature (VanLaar 2010)[31]	232	DS 33(II) 60(T)					•V							
ColoPrint® Colon Cancer Recurrence Assay 18-Gene Prognostic Classifier Derivation:* (Salazar 2011)[28] Validation: (Nitsche 2012) [26]	188	LO, DS 114(II), 206(T)	1	135	1	RP	•V		Y N	ASCO			Yes	
Gene Fx® Colon634 Probe set signature (Kennedy 2011)[23]	215	LO,DS 144(II)					•V	•V						
23-Gene Signature (Wang 2004)[33] Validation: (Barrier 2006)[16]	38	DS 36(II)	1	50	1	RP	•V							
42 Core Gene Classifier* (Eschrich 2005)[19]	78	LO,DS 32(II), 95(T)						•V 26 genes						
30-Gene Prognosis Prediction (Barrier 2006)[16]	50	LO					•						Yes, MSS only	
70-Gene Prognostic Predictor (Barrier 2007)[17]	24	LO					•							
Map7/B2M Gene Expression Ratio (Blum 2008)[18]	22	DS					•	•						
25 Genes w/in 28 Gene Breast Cancer Prognostic Signature (Wan 2010)[32]	50	LO,DS 24(II)					•V							

Table 3. Summary of scientific evidence for GEPs in stage II colon cancer: prognostic—clinical validity and clinical utility (continued)

GEP: Name and/or Gene Expression	Initial Derivation: No. CC Pts.	Initial Published Validation	Subq Val: No. Studies	Subq Val: No. Patients	Subq Val: No. Dataset	Subq Val: Study Design	Recurrence	Overall Survival	Re-class Done?	Re-Class Comparison	Clinical Utility	Discrimination Discussed	Account for MSI	Ethnicity
34 Gene Recurrence Classifier (Smith 2010)[30]	55	DS 57(II) 177(T)					•V							
9 Genes within 12 Gene genomic instability signature (Mettu 2010)[25]	50	LO					•							
54-Gene Metastasis-prone Signature (Hong 2010)[22]	70	LO					•						Yes, MSS only	Han Chinese

DS = Different Set of patients; GEP = Gene expression profiles; LO = Leave-out one validation technique; MSI = Microsatellite instability; MSS = Microsatellite stable; Re-class = reclassification; RP = Retrospective-Prospective; Y = Yes; N = No; ASCO = American Society of Clinical Oncology

*Derivation set involved patients with both colon and rectal cancers

Notes: For validation, 32(II) means 32 patients with stage II colon cancer and 90(T) means 90 patients total with various stages of colon cancer

"•" indicates outcome measured; V:Validated with different set of patients; Blank (empty) cells indicate not measured or not addressed

A subsequent, independent validation study was performed on three of the GEP assays shown in Table 3. The twelve-gene Oncotype DX® colon cancer assay was validated in a study of 1,436 patients,[20] the 18-gene ColoPrint® assay was validated in a study with 135 patients with stage II colon cancer,[26] and the 23-gene signature was validated in a study of 50 patients.[16]

In evaluating GEP assays, clinical utility is critically important because it shows how use of the assay with individual patients impacts net outcome; that is, it addresses the question "Does use of the GEP assay in clinical care improve overall patient outcome?" None of the studies in Table 3 provide information on the clinical utility with use of the GEP result for prognosis. That is, no studies showed the impact on net health outcome. The change in net outcome with use of the GEP assay could be shown with use of the GEP assay in a prospective clinical trial. Change in net outcome might also be shown with a net reclassification analysis; that is, how use of the GEP to classify the patients in the study with and without the outcome compares with conventional approaches along with linking the changes in classification to the impact on net health outcome. However, just showing that results of a GEP assay can stratify patients into risk groups and that the GEP result is an independent predictor of outcomes does not demonstrate clinical utility. Finally, none of the publications discussed the ability of the GEP result to show discrimination, that is, to classify patients into outcome groups that are clinically distinct.

As shown in Table 3, the first publication was in 2004, the most recent in 2012. The GEPs varied from using 9 genes[25] to one that used 634 probes.[23] The GEPs were derived from tissue samples from colon cancer patients ranging from 22[18] to 1,851[20, 27] (median=55). The potential role of microsatellite instability (MSI) in the study was addressed for four of the profiles (six studies).[16, 20, 22, 26-28] This is problematic because MSI status is used in the risk-stratification of patients with stage II colon cancer. Thus, MSI-status needs to be measured and/or taken into consideration in the analysis.

In 2011, Gröne[21] reported on the inability to validate the GEP results of Wang (2004)[33] and Barrier (2006)[16] with an independent sample of 53 patients with stage II colon cancer. However, because of the limited data provided in the publication (only p values from the analysis were provided), this information is not included in Table 3.

In 2009, Jorissen[34] reported on a 128-gene signature that was derived using a unique approach and thus this study is not included in Table 3 or Table 4. Instead of determining the GEP from patients with stage II cancer that did or did not develop recurrent disease, this study determined the GEP comparing fresh-frozen tissue specimens from stage A (Stage 1) and stage D (Stage IV) colorectal cancer. The GEP assay was then applied to samples from patients with stages B and C (stages II and III) colorectal cancer to determine if individual tumors were more like stage A or stage D. Validation included applying the GEP assay to an independent set of 99 patients with colon cancer (33 with stage B); the results of the assay did identify two risk groups. No information was provided on clinical utility. In a subsequent publication, Thorsteinsson[35] reported that a group of 20 patients with stage II colon cancer were not stratified with use of this GEP assay.

The information presented in Table 3 shows that numerous GEP assays have been reported. The small number of patients used to derive many of the GEP assays as well as the large number of genes contained in the assay raises questions about whether subsequent study(s) would validate the initial findings.

Was a Reclassification Analysis Performed With Risk Stratification Using GEP When Compared With That Obtained With Standard Risk Factors?

As noted above, none of the studies described the important issue of clinical utility with use of the GEP result for prognosis. No studies showed the change that use of the GEP result would have on overall patient outcomes with a net reclassification analysis; that is, how use of the GEP to classify the patients in the study with and without the outcome compares with standard approaches to classifying these stage II patients and how the change in classification impacts net outcome.

The derivation study for the 18-gene ColoPrint® prognostic classifier reported the net changes in classification of 114 patients with stage II colon and rectal cancer with use of the GEP assay compared to ASCO risk criteria.[28] In this study, the GEP assay changed risk groups for 55 patients. No additional detail was provided about the characteristics of patients whose risk category changed. The published validation study of this GEP did not provide data on net reclassification.[26]

What Information is Available Regarding Analytic Validity?

Information related to the analytic validity as well as about the type of tissue used and sample preparation for these 16 GEP assays used for prognostic outcomes is shown in Table 4. Of note, there was limited information provided about the important topic of reproducibility; how repeat assays for the same tumor compare. Clark-Langone et al., reported on the analytic validity of the Oncotype DX® Colon Cancer Assay.[36] The authors reported a number of measurements including PCR amplification efficiency, linearity over a range of concentrations, and reproducibility. Precision using two RNA pools for the recurrence score (RS) was 23.6 $\pm$ 1.18 (SD: standard deviation) for low RS and 43.6 $\pm$ 1.38 (SD) for high RS. As noted earlier, the type of tissue used varies; some used fresh-frozen tissue and other assays used formalin-fixed paraffin-embedded (FFPE) tissue. While it is important to determine the similarities between the assay techniques used in research studies and those that might be used in commercial applications, it is likely too early to make this determination.

Information was not found about the composition of all the commercially available GEP assays. However, there was no overlap in the genes comprising the ColoPrint® assay,[28] the OncoDefender-CRC assay,[24] and the Oncotype DX® colon cancer assay.[36]

GEP Assays as Predictive Markers

There were few studies on use of GEP assays as predictive markers, that is, relationship between the GEP assay and reduced tumor recurrence (or improved survival) with adjuvant chemotherapy. As noted earlier, this type of marker is very important in identifying improvement from use of adjuvant treatment. Deriving and validating a GEP assay for prediction would require data from comparative studies on use of adjuvant chemotherapy in patients with stage II colon cancer. Information about the state of the science for use of GEP assay as a predictive marker, that is, benefit from adjuvant chemotherapy, in stage II colon cancer is shown in Table 5. No GEP assays are commercially available for this application. A unique 11-gene (6 predictive and 5 reference genes) predictive assay was derived in a group of 816 patients with stage II colon cancer; however, the predictive marker did not validate in an independent cohort of 1,436 patients. As shown in Table 6, this assay used microdissection of fixed, paraffin-embedded tissue during sample preparation, such an approach is often used for GEP assays.

Table 4. Summary of scientific evidence for analytic validity of prognostic gene expression profiles in stage II colon cancer

GEP: Name and/or Gene Expression	Type of Tissue Used	Sample Handling	Technique for GEP	Reliability (Reproducibility)	Time to Report Results
Oncotype DX® Colon Cancer Assay: 12-Gene Prognostic (7 Prognostic and 5 Reference Genes) Derivation: (O'Connell 2010)[27] Validation (Gray 2011)[20] Analytic Performance (Clark-Langone)[36]	Fixed, paraffin-embedded tissue	Microdissected	Reverse transcription-quantitative PCR (RT-qPCR)	23.6 ± 1.18 (SD) 43.6 ± 1.38 (SD)	10-12 days
ColonPRS®: 163 Gene Prognostic Gene Expression Signature (VanLaar 2010)[31]	Fresh-frozen biopsies		Hybridization with Affymetrix U133 Plus 2.0	Replicate hybridization done to study inter-laboratory variability	?
ColoPrint® 18-Gene Signature Derivation: (Salazar 2011)[28] Validation: (Nitsche 2012) [26]	Fresh, frozen tissue		Agendia customized whole-genome high-density microarrays		7-10 days
GeneFx® Colon 634-Probe set signature (Kennedy 2011)[23]	FFPE Tissue	Tissue section with >50% tumor cells	Amplified product hybridized to Almac Colorectal Cancer DSA on Affymetrix 7G scanner.		?
23-Gene Signature (Wang 2004)[33]	Fresh, frozen specimens	Total cell population >85% tumor cells	Hybridized to U 133a GeneChip (Affymetrix, Santa Clara, CA)		?
42 Core Gene Classifier (Eschrich 2005)[19]	Frozen samples	Microdissected so >80% tumor cells	Profiled on The Institute for Genomic Research cDNA arrays. Validation with Affymetrix U133A platform		?
30-Gene Prognosis Prediction (Barrier 2006)[16]	Fresh tissue, stored in liquid nitrogen	Reviewed for >80% tumor cells	Hybridized to Affymetrix HGU133A Gene Chips		?
70-Gene Prognostic Predictor (Barrier 2007)[17]	Fresh specimens, stored in liquid nitrogen; Non-neoplastic mucosa		Hybridized to Affymetrix HGU133A GeneChip		?
Map7/B2M Gene Expression Ratio (Blum 2008)[18]	Formalin-fixed Paraffin-embedded (FFPE) tissue		Real-time RT-PCR using PE Biosystems Gene Amp® 7300 or 7500 Sequence Detection System		?

Table 4. Summary of scientific evidence for analytic validity of prognostic GEP in stage II colon cancer (continued)

GEP: Name and/or Gene Expression	Type of Tissue Used	Sample Handling	Technique for GEP	Reliability (Reproducibility)	Time to Report Results
25 Genes w/in 28 Gene Breast Cancer Prognostic Signature (Wan 2010)[32]	Cases from prior reported series		Affymetrix U133A array		?
34 Gene Recurrence Classifier (Smith 2010)[30]	Fresh tissue, flash frozen, stored -80°C		Hybridized to U133 Plus 2.0 GeneChip Expression Array		?
9 Genes w/in 12 Gene genomic instability signature (Mettu 2010)[25]			Hybridized to Affymetrix U133A arrays		?
54 Gene Metastasis-prone Signature (Hong 2010)[22]	Fresh tissue, flash frozen w/in 30 minutes, stored at -80°C	Microdissected for tumor cells >90%	Hybridized to Affymetrix U133 Plus 2 Array		?

DSA = Disease-specific array; GEP = Gene expression profiles; PCR = Polymerase chain reaction; RT-PCR = real-time polymerase chain reaction; RT-qPCR = real-time quantitative polymerase chain reaction; SD = Standard deviation
Note – Blank indicates not done or not addressed, and "?" indicates uncertain.

Table 5. Summary of scientific evidence for gene expression profiles in stage II colon cancer: predictive—clinical validity and clinical utility

GEP: Name and/or Gene Expression	Initial Derivation# CC Pts	Initial Pub Validated[a]	Subq Val # Studies	Subq Val # Patients	Subq Val # Dataset	Subq Val Study Des	Response to Treatment	Chemotherapy	Re-Class Done?	Re-Class Comparison	Clinical Utility	Discrimination	Account for MSI	Ethnicity
11 Gene Predictive (6 Predictive and 5 Reference Genes) Derivation: (O'Connell 2010)[27] Validation: (Gray 2011)[20]	816	LO	1	1,436	1	RP	•NV	FU and LV					Yes	U.K. sites for validation

GEP = gene expression profiles; LO = leave-out; NV = not successfully validated; subq val = subsequent validation; MSI = microsatellite instability; FU = fluorouracil; LV = leucovorin; RP = retrospective-prospective
[a]For example, if recurrence rate, how was it determined? (was tissue obtained?)
Note : "•" indicates Yes or included, blank indicates not done or not addressed, and "?" indicates uncertain.

Table 6. Summary of scientific evidence for analytic validity of predictive gene expression profiles in stage II colon cancer

GEP: Name and/or Gene Expression	Type of Tissue Used and Sample Handling	Sample Handling	Technique for GEP	Reliability (Reproducibility)	Time to Report Results
11 Gene Predictive (6 Predictive and 5 Reference Genes) Derivation: (O'Connell 2010)[27] Validation: (Gray 2011)[20]	Fixed, paraffin-embedded tissue	Microdissected	Reverse transcription-quantitative PCR (RT-qPCR)		?

GEP = Gene expression profiles; PCR = Polymerase chain reaction

Note – Blank indicates not done or not addressed, and "?" indicates uncertain.

GEP Using MicroRNA

Information about the state of the science for use of a gene expression profile using microRNAs (assays in Table 3 use messenger RNA [mRNA]) as a prognostic marker in stage II colon cancer is shown in Table 7. A profile using 17 microRNAs was derived in a group of 49 patients[29]; no independent validation has been reported. As shown in Table 8, this assay used fresh, frozen tissue.

Table 3 through Table 8 only include information from studies that provided specific results for patients with stage II colon cancer. In some cases, the relationship between the GEP result and clinical outcome(s) was presented only for patients with multiple stages of colon cancer or colon and rectal cancer. One example of publications combining multiple stages of cancer is a study on the five-gene prognostic assay (OncoDefender-CRC).[24] In this study by Lenehan and colleagues, a five-gene expression classifier was derived using formalin-fixed paraffin-embedded tissue from 74 patients with colorectal cancer and then validated using an independent sample of 251 patients with stage I and II colon cancer. No data were presented for clinical utility.

A listing of studies that did not provide specific results for stage II colon cancer is provided in Appendix C. Given the clinical differences between patients with various stages of colon cancer and between colon and rectal cancer, this Brief provides no summary information for these studies.

What Key Studies are Underway?

- Ongoing clinical trials on the topic from clinicaltrials.gov and other clinical trial registries or systematic reviews.

The list of clinical trials of potential relevance to this topic is provided in Table 9. These trials were identified by searching clinicaltrials.gov (www.clinicaltrials.gov) and the World Health Organization International Clinical Trials Registry platform (www.who.int/ictrp/en).

The PARSC (Prospective Study for the Assessment of Recurrence Risk in Stage II Colon Cancer Patients using ColoPrint®) study seems of particular interest to this Brief. The study began in September 2008. The enrollment period will be 4 years. It is expected that 1,800 to 2,400 patients will be enrolled in order to obtain 575 analyzable samples from eligible patients with stage II colon cancer. Approximately 25 to 35 sites will be involved worldwide. The aim of the study is to validate the performance of ColoPrint® in estimating 3-year relapse rate. Secondary objectives include comparing the objective risk assessment results from the prognostic profile (ColoPrint®) to both the risk assessment based on the American Society of Clinical Oncology criteria, as well as the investigator's independent assessment. The study will also address the logistics and quality assurance of using ColoPrint® in clinical practice.

- *Abstracts published at recent scientific meetings for potential breaking scientific developments.*

The literature review identified eleven published abstracts from recent meetings that are relevant to this topic that were not identified in our search of the published peer-reviewed literature.

Table 7. Summary of scientific evidence for MicroRNAs in stage II colon cancer: prognostic—clinical validity and clinical utility

MicroRNA Profile: Name and/or Gene Expression	Initial Derivation: No. CC Pts.	Initial Publ. Validation	Subq Val: No. Studies	Subq Val: No. Patients	Subq Val: No. Dataset	Subq Val: Study Des	Recurrence	Overall Survival	Re-class Done?	Re-Class Comparison	Clinical Utility	Discrimination Discussed	Account for MSI	Ethnicity
17 MicroRNA Expression Profile (Schepeler 2008)[29]	49	LO					•						Yes	

GEP = Gene expression profiles; LO = Leave-out one validation; No. CC Pts = Number of colon cancer patients; Subq val = Subsequent validation;
MSI = Microsatellite instability
Note – "•" indicates Yes or included, blank indicates not done or not addressed

Table 8. Summary of scientific evidence for analytic validity of MicroRNA in stage II colon cancer

MicroRNA: Name and/or MicroRNA Expression	Type of Tissue Used	Sample Handling	Technique for MicroRNA	Reliability (Reproducibility)	Time to report results
17 MicroRNA Expression Profile (Schepeler 2008)[29]	Fresh tissue, frozen in liquid nitrogen		Real-time reverse transcriptase-PCR and in situ hybridization		?

PCR = Polymerase chain reaction
Note – "•" indicates Yes or included, blank indicates not done or not addressed, and "?" indicates uncertain.

Table 9. Ongoing trials for gene expression profiling in colorectal cancer

Name of Study	Status	Sponsor	Trial Number
A Prospective Study for the Assessment of Recurrence Risk in Stage II Colon Cancer Patients Using ColoPrint (PARSC)	Currently recruiting	Agendia	NCT00903565
Study of Biomarkers Using Blood and Tissue Samples From Patients With Colorectal Cancer or Colorectal Polyps and From Patients Without Polyps (Primary objective is to predict risk of colorectal cancer [CRC]; a secondary objective is to predict response to therapy for various stages of CRC.)	Currently recruiting	Indiana University National Cancer Institute (NCI)	NCT00898378
A Prospective Study of Pharmacogenetic Factors and Gene Expression Profile (This is a parallel study to an RCT of modified FOLFOX-6 as adjuvant therapy in stage II/III colon cancer. This might provide predictive information.)	Currently recruiting	Samsung Medical Center, Seoul	NCT01472601

RCT = Randomized controlled trial

In 2010, Adams and colleagues reported on a 32-probe set signature (Predictor C) derived from a cohort of 55 patients with colorectal cancer.[37] This GEP was then evaluated on another set of 164 patients, 90 of whom had stage II cancer. The GEP result was associated with

recurrence. Many additional details need to be ascertained for this study, including whether or not patients with rectal cancer were included in this cohort.

In 2011, Venook et al., reported the second independent validation of the twelve-gene signature (Oncotype DX® colon cancer assay) with samples of patients from the CALGM 9581 study of adjuvant chemotherapy in stage II colon cancer.[38] The GEP results were validated for predicting risk of recurrence, including in patients with T3 tumors who did not have microsatellite instability. No additional information was provided on clinical utility.

In 2012, Cartwright and colleagues presented preliminary results from a survey of practicing oncologists on recommendations for adjuvant chemotherapy with use of the Oncotype DX® colon cancer test.[39] These results were noted as preliminary and additional details may become available. Data from this descriptive, survey-based approach is not sufficient to demonstrate the clinical utility of GEP testing.

In 2012, Tabernero and colleagues presented results of a validation study using ColoPrint® with a pooled set of 320 patients with stage II cancer.[40] The abstract discussed reproducibility and stability of the assay as well as clinical validity. Additional details are needed about this study, including whether or not this was only patients with colon cancer. (The initial ColoPrint® studies included stage II patients with both colon and rectal cancer.[28]) In addition, details about potential overlap between these 320 patients and patients reported in other publications and presentations with ColoPrint® need to be clarified.

In 2012 at the ASCO Annual Meeting, O'Connell et al., reported another independent validation of the twelve-gene signature (Oncotype DX® colon cancer assay) with samples of patients from the NSABP C-07 study of fluorouracil (FU) and FU plus oxaliplatin in stage II/III colon cancer.[41] The GEP results were validated for predicting risk of recurrence, including in patients with stage II disease. No information was provided on clinical utility.

In 2012 at the ASCO Annual Meeting, Roth et al., reported results for validation of both the Genomic Health and Veridex risk scores with gene expression data from 580 stage III and 108 stage II colon cancer patients from the PETACC-3 trial.[42] Risk scores from both profiles were significantly associated with relapse-free survival in both the stage III and stage II cohorts. For stage III, the highest effect size was a combined model that included both risk scores as well as T-stage, N-stage and MSI status. No information was included related to clinical utility.

In 2012 at the ASCO Annual Meeting, Budinska reported on the identification and validation of five sub-types of colorectal cancer based on clinic-pathological variables and molecular (expression) markers.[43] The subtypes had significant differences in survival. Additional detail is needed about the classification as well as specific information for Stage II colon cancer patients.

In 2012 at the ASCO Annual Meeting, Mambo et al., reported on using a set of 30 microRNAs to discriminate among 118 stage IIA colon cancers who did and did not have a recurrence.[44] No further validation was reported. Many additional details need to be ascertained for this study, including the composition of the profile.

Also at the 2012 ASCO Annual Meeting, Salazar et al., presented a validation study using the 18 gene ColoPrint® with a pooled set of 320 patients with stage II colon cancer.[45] The abstract discussed the relationship between the GEP result and relapse-free survival for both all stage II patients and for the subset of stage II patients with T3 tumors and without MSI-H status. No information was provided about clinical utility. Details about potential overlap between these

320 patients and patients reported in other publications and presentations with ColoPrint need to be clarified.

At this 2012 ASCO Annual Meeting Salazar et al., presented a status report on the PARSC trial using ColoPrint® discussed above.[46] The abstract noted that 340 eligible stage II colon cancer patients had been enrolled; the target enrollment for this group is 575 patients.

Summary and Implications

Guiding Question 4. What important issues are raised by using GEP testing for stage II colon cancer?

Colon cancer is a common malignancy in both women and men. Improving the decisionmaking about adjuvant chemotherapy for patients with stage II, that is, node-negative, colon cancer is an important clinical question. The majority of stage II patients do well with surgery alone, but some patients do develop recurrent disease and show improved survival from adjuvant chemotherapy. Gene expression profile (GEP) assays are one potential novel approach to determine risk of recurrence and benefit of adjuvant therapy and thus improve this decisionmaking by either avoiding chemotherapy and medication-related adverse effects in those unlikely to have a recurrence or identifying those most likely to show benefit from adjuvant chemotherapy.

However, in order to assure that use of GEP assays improves clinical care of patients, the GEP assays need to demonstrate clinical validity and clinical utility. Clinical validity is necessary but not sufficient; it demonstrates the relationship between the GEP result and clinical outcome in a population. Clinical utility is evidence that use of the GEP assay improves net health outcome.

What Are the Key Unresolved or Controversial Issues With Using GEP Testing in Patients With Stage II Colon Cancer?

While the potential advantages of using the GEP assays seem promising, the clinical utility is uncertain. No prospective studies have reported on what happens to net health outcome, considering both benefits and harms, when GEP results are used in managing patients. Data are also very limited for net reclassification analysis; that is, how the overall risk classification provided by GEP results compares with risk classification using other predictors. Reclassification analysis might be used in assessing clinical utility by analyzing how the changes in patient classifications impact net health outcome.

For some GEP assays, there is also uncertainty about the clinical validity, that is, the initial results have not been evaluated (validated) using a large number of samples from different representative populations. Also, most of the studies have evaluated the GEP assays as a possible prognostic marker for disease recurrence. There is much less information regarding use as a predictive marker for response to adjuvant chemotherapy, which would impact patient management. To date, information is lacking about the extent to which GEP results do, or do not, classify patients into distinct groups that have clinical relevance.

Indications for use of the GEP assay have not been defined. Which patients with stage II colon cancer will be tested is an open question: will the GEP be used with all stage II patients or just those with T3 tumors that are microsatellite stable (do not have microsatellite instability) or some other subgroup of patients? Another concern is that there is little information about reproducibility of the GEP test results. Test variability can result from different approaches to sample preparation, analysis, and perhaps heterogeneity of the tumor. In addition, factors such as tissue ischemia at surgery, use of certain medications, and nutrition that have been noted to impact gene expression need consideration.

The publications describing GEP assays also varied considerably in methods and definition. For example, recurrence was sometimes considered only as cancer metastatic to liver or lung

while in other cases local recurrence was also included. The rigor with which recurrence was defined and verified also varied among studies. This topic needs more attention in future studies. The impact of surgical technique was given some consideration, but could have further implications. In stage II patients, an indeterminate or positive resection margin is a clinical marker for high risk of recurrence. So, one could assume that the results of a GEP assay would not predict future events related to the surgical procedure, for example, a recurrence related to a resection margin that showed cancer cells. An inadequate number of assessed lymph nodes (12 or fewer) is also a marker for high-risk patients, likely because some of these patients have stage III disease (positive lymph nodes). Inadequate assessment can occur because of either incomplete sampling at surgery or incomplete pathological evaluation. Adequate lymph node sampling (sampling and reporting) is now recognized as an important quality measure in colon cancer surgery. However, in older series inadequate assessment occurred more often. Thus, GEP assays derived from these older series may not apply to current patients where sufficient numbers of nodes are typically obtained and analyzed. Thus, it is important to note the adequacy of lymph node assessment for both studies of GEP derivation and validation. One important aspect of the outcome of recurrence that was not found to be problematic was duration of followup; this was typically assessed with a minimum followup of 3 years.

While at the time of this report there are five commercially available GEP assays, this is an area of active research and it seems likely that additional assays will become available in the next 1 to 2 years. Currently available GEP assays are being marketed as laboratory-developed tests (LDT); currently, none of the commercially available GEP assays has specific FDA approval for use in stage II colon cancer.

What are the Implications of the Current Level of Diffusion and/or Further Diffusion of This Technology/Intervention Given the Current State of the Evidence?

While results of both false-positive and false-negative tests raise concerns, incorrectly identifying someone as low-risk with the GEP result and not administering adjuvant chemotherapy, especially in a patient with clinical risk factors, may lead to significant consequences. Thus, the performance of these assays needs to be evaluated in multiple patients from diverse settings. While there is some use of these tests at present, given the major unanswered questions noted above, further diffusion of GEP in making treatment-related decisions, such as not giving adjuvant chemotherapy, raises concern. In addition, the lack of data related to GEP assays for predicting improved survival with adjuvant chemotherapy is also of concern.

While specific rates of use for the commercially available GEP assays are not known, our Key Informants felt there is limited current use of GEP assays in the care of patients with stage II colon cancer based on their clinical experience and/or discussions with colleagues. While Key Informants agreed this was both an important clinical issue and an active research question, they differed in their opinions about future trends in use of the GEP assays in light of the current evidence. Key Informants noted that additional information related to clinical utility, such as prospective trials and/or net reclassification analysis, was needed.

Since only a few of the GEP tests that have been reported in the literature are available commercially, the state of the evidence for commercially available GEP assays is presented in Table 10. This table does not compare results for each of the assays nor comment on the quality

of the studies; this table only indicates whether or not a type of evidence is available, including whether or not there has been successful validation of the GEP assay, in the peer-reviewed literature. Additional information about each of these assays, including more detail about individual studies, is presented in Table 3 and Table 4.

Table 10. Summary of peer-reviewed studies for available gene expression assays for use in the decision about adjuvant chemotherapy in stage II colon cancer

Assay	Validated for Recurrence (Stage II)	Validated for Treatment Benefit	Net Reclassification Analysis	Clinical Utility
ColoPrint®*	Yes	No	For derivation study only	No
ColonPRS®+	Yes	No	No	No
GeneFx® Colon	Yes	No	No	No
OncoDefender-CRC	No, Combined validation for stage I/II colon cancer	No	No	No
Oncotype DX® Colon Cancer Assay	Yes	No	No	No

*May also be available for use through PARSC Clinical Trial
+Currently (July 2012) available for nondiagnostic or research use only

As shown in the Evidence Map section of this Brief and Table 10, the clinical utility for use of GEP assays is not known. We did not identify any prospective studies that assessed change in net health outcome with use of a GEP assay, nor did we identify studies that used an overall reclassification analysis and analyzed the impact of the reclassification from use of a GEP assay on net health outcome as an approach to assess clinical utility. There also was limited information on the reproducibility of test findings. It also is not certain which stage II patients, all or a subset, might be tested using the GEP assay. The publications also do not discuss whether or not results of the GEP assays stratify patients into clinically meaningful groups. As noted above in Table 9, trials are in progress but they may not address or provide results for all the major issues noted in this section. The PARSC study using the ColoPrint® assay appears designed to answer a number of key questions for this one assay. Box 1 summarizes the key decisionmaking uncertainties we identified for use of GEP assays in stage II colon cancer.

Box 1. Key decisionmaking uncertainties for gene expression profiling assays in stage II colon cancer

Analytic Validity

What is the reproducibility of the assay results?

Is there an effect of sample preparation, analysis, and/or tumor heterogeneity?

Clinical Validity (Diagnostic Accuracy)

How is recurrence defined, e.g. local disease, distant disease, or both?

Are prognostic (recurrence) and/or predictive (benefit from therapy) uses considered?

How extensively has the gene expression profiling (GEP) assay been tested (validated) with additional patients from different populations?

Patients

Which patients (which subsets) with stage II colon cancer should receive GEP testing?

Might this only be used in T3 tumors with without microsatellite instability?

Clinical Utility

What is the clinical utility for use of the GEP results?

How does use impact net outcome, such as survival or recurrence-free survival?

This can be measured through prospective studies.

Reclassification analysis (net reclassification) linked with change in net health outcome might also provide information.

How does use of GEP compare with other approaches to risk stratification (classification)?

Next Steps

Conceptual Framework and Future Research

In this Technical Brief we have organized the emerging gene expression profile (GEP) tests according to their unique signatures and have also noted which GEP assays are commercially available. Regardless of specific aspects of a GEP assay, the assessment of diagnostic tests typically follows a stepwise approach.[47] This approach progresses from establishing the technical (analytic) and clinical validity to assessing the impact of the test result on clinical decisionmaking and then patient outcome. The potential adverse effects from use of the test are also considered. This step-wise approach is applicable to use of GEP testing in stage II colon cancer (Figure 1).

What Are the Important Questions for Future Research?

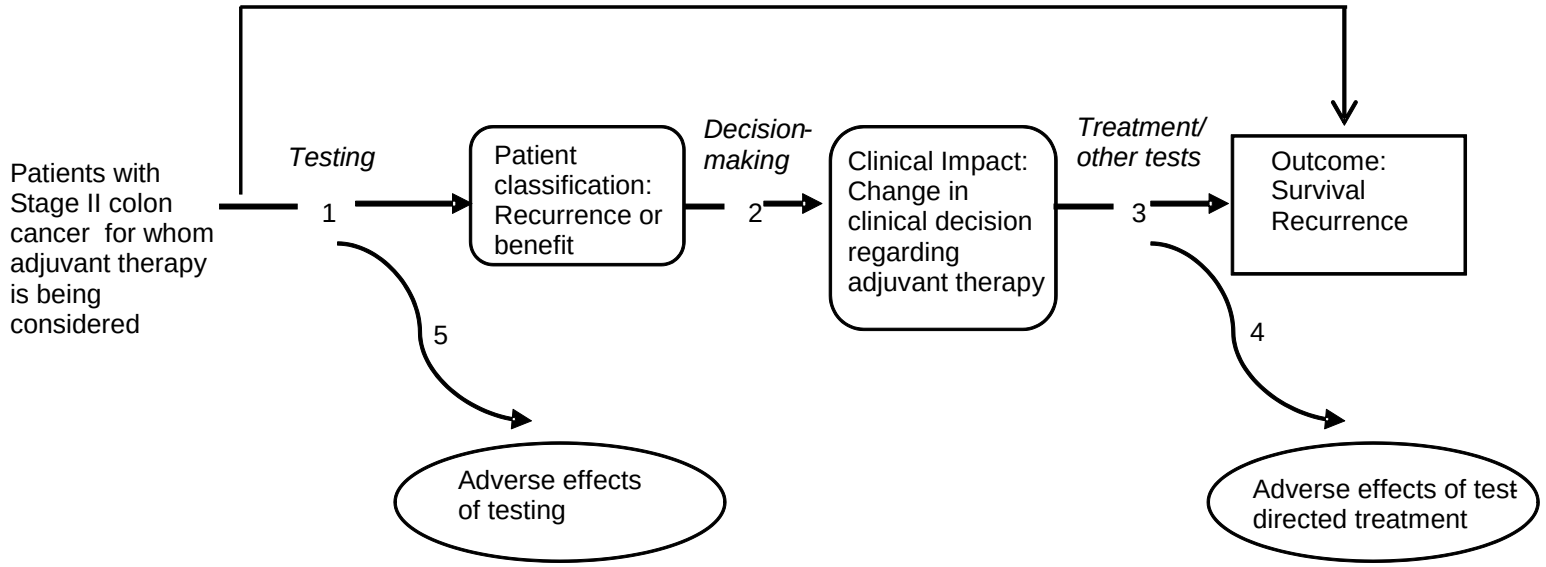
Based on this conceptual framework, findings from the evidence map, and input from Key Informants, we suggest that future research on the various GEP assays, whether used for prognostic or predictive measures, include the following:

- Clinical Utility. Does use of the GEP assay for treatment management improve net health outcome (considering benefits and risks) in patients with stage II colon cancer? (Research questions 2, 3, 4, and 5). Studies should be prospective trials using GEP results or the studies should compare classification with use of GEP results to results with use of conventional risk stratification and link the changes in classification to their impact on net health outcome. Can prognostic GEP assays identify individual patients at low risk of recurrence who can safely avoid adjuvant chemotherapy? Can GEP assays identify individual patients who will show improved survival with adjuvant chemotherapy or particular adjuvant regimens?

- Clinical Validity. How strongly is the GEP assay result correlated with the outcome of interest? Development of evidence on clinical utility requires robust evidence on clinical validity. (Research question 1).
- Patients. Which patients with stage II colon cancer should be tested using the GEP assay? (Research question 2). Should the GEP test is applied to all stage II colon cancer patients or a subset such as those whose tumors do not have microsatellite instability?
- What is the analytic validity of the GEP assay? (Research question 1). Are results reproducible?

While information is emerging about use of GEP assays to inform the decision about use of adjuvant chemotherapy in stage II colon cancer, studies to date have not provided the type of information needed to address major uncertainties.

Figure 1. Analytic Framework



Research Questions

1. Test accuracy (clinical validity, analytic validity)
2. Impact of test on management?
3. Impact of management on health outcomes?
4. Adverse events of test-directed treatment?
5. Adverse events of testing?

Definition of Terms

Biomarker:

The Biomarkers Definitions Working Group defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[48]

Gene expression profile (GEP or "signature"):

A GEP is one type of biomarker. According to Subramanian and Simon,[49] a GEP is a biomarker in which the expression levels of multiple genes are combined in a defined manner to provide a score or a classifier. GEPs measure the activity or "expression" of multiple genes in a single RNA sample, which may reflect both normal and malignant cellular function. Various methods exist to measure gene expression, including the reverse transcriptase polymerase chain reaction and DNA microarrays. GEPs may have an important role to play in determining prognosis and guiding treatment decisions if found to be reliable, valid, and clinically useful.

Clinical validity:

How consistently and accurately the test detects or predicts the intermediate or final outcomes of interest.[50]

For this Brief, data for clinical validity is defined as the relationship (e.g., sensitivity, specificity, accuracy, etc.) of the GEP results to the risk of disease recurrence and/or death after surgery for stage II colon cancer. Whether or not data are available about the incremental information provided by GEP testing when compared with standard clinical and pathological risk factors will be reported. Clinical validity is also defined as the relationship (e.g., sensitivity, specificity, accuracy, etc.) of the GEP results to the response to adjuvant chemotherapy currently used after surgery for stage II colon cancer. Again, the availability of data on the incremental information provided by GEP testing when compared with standard clinical and pathological risk factors will be noted.

Clinical utility:

How likely use of the test is to significantly improve patient outcomes.[50]

Clinical utility is defined as the balance of benefits and harms when GEP testing is used to impact clinical decisionmaking, that is, the decision about using adjuvant chemotherapy. Clinical utility should reflect patient outcomes such as improved survival. As noted by Simon et al.,[13] clinical utility requires that a test be actionable, that is, the medical indication for using the test is clear and the magnitude of outcomes or treatment effects associated with different test results are sufficiently great as to influence treatment decisions.

Analytic validity:

How accurately and reliably the test measures the genotype of interest.[50]

The analytic validity of the GEP test is also important, and information about the extent of information on analytic validity will be assessed. Analytic validity relates to the reliability and validity of the test itself, that is, does it measure the genes that are part of the assay, and are the test results reproducible? Lack of reproducibility could result from both preanalytic factors (e.g., sample preparation) as well as analytic factors (such as reagents used.)

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Appendix A. Search Strategy for MEDLINE®

1. exp Colorectal Neoplasms/
2. ((colorectal or colon$ or rectal or Sigmoid) adj3 (neoplasm$ or cancer$ or carcino$ or tumo?r$)).tw.
3. 1 or 2
4. Gene Expression Profiling/
5. (gene expression adj (profil$ or monitor$ or pattern$ or signature$ or predictor$ or test$ or chip$ or regulation)).tw.
6. transcript$ expression analys$.tw.
7. transcriptom$.tw.
8. ((DNA or cDNA or tissue) adj (fingerprint$ or microarray$)).tw.
9. ((mrna or mirna or Microarray or MicroRNA) adj2 (profil$ or expression or signature$)).tw.
10. array sequence analysis.tw.
11. oncotype dx.tw.
12. Affymetrix.tw.
13. Coloprint.tw.
14. (Mismatch repair deficiency status or dmmr).tw.
15. microsatellite instability/
16. Microsatellite Repeats/
17. replication error phenotype$.tw.
18. or/4-17
19. recurrence/
20. (recur$ or relaps$).tw.
21. exp treatment outcome/
22. (respons$ or outcome$ or react$ or eligibl$).tw.
23. Chemotherapy, Adjuvant/
24. (adjuvant or chemotherap$).tw.
25. or/19-24
26. incidence/
27. exp mortality/
28. follow up studies/
29. prognos$.tw.
30. predict$.tw.
31. course$.tw.
32. or/26-31
33. and/3,18,25,32
34. (animals not humans).sh.
35. 33 not 34

Appendix B. Elements Abstracted From Included Articles

For all articles:

- Article (details regarding author(s), institutions, funding)
- Which test(s) were used?
  - Type(s) of tissue used (e.g., fresh tissue)
  - Time for analysis (turnaround time)
- Inclusion and exclusion criteria
  - Was only a subset of patients with stage II disease used? If so, which one?
  - For adjuvant chemotherapy, which regimens were used?
    - Sample size (number of patients/specimens)
  - If samples are from previous studies, which studies?
    - Is there information to assess the completeness of sampling from previous studies?

For articles that describe analytic validity:

- Was information provided about preanalytic factors (e.g., handling of specimens)?
- What aspects of analytic validity were measured (e.g., accuracy, reproducibility)?
- Does it provide narrative summary of results related to analytic validity?

For articles that describe clinical validity and clinical utility:

- What outcomes were measured, for example, recurrence rates, response to adjuvant chemotherapy (regimen specific), avoidance of chemotherapy (with reduction in medication side effects), et cetera?
- What was the study design?
  - Prospective, retrospective, or prospective-retrospective
  - Randomized trial, controlled trial, cohort study, or case (convenience) series
  - Derivation and/or validation (For validation, when was the method of analysis and interpretation determined?)
- Was a reclassification analysis performed (to determine if the GEP test results provide new (additional) information when compared with that obtained with usual predictors)?
- Was MSI (MMR) status determined?
  - If so, by which method?
- Was MSI (MMR) status considered in the overall analysis?
  - If so, how?
- Was the GEP performed (analysis, calculation, interpretation) as described in initial studies for derivation and/or studies of analytic validity?
- Does the final classification provide discrimination among groups of patients; that is, are the patients grouped into a specific category that is distinct and different from other categories? (In addition, when the GEP predicts risk groups, how were the cut-points for various groups determined?)
- Do the authors comment on factors such as race and ethnic background that could impact the generalizability of the findings?
- For blinding, were determinations of the outcomes and the GEP result made independently, that is, did those assessing outcomes know the results of GEP testing?

The following additional information will be obtained from these articles (related to the validity of the outcomes):

- For recurrence of disease, how was it measured (duration of followup, biopsy result, imaging finding, level of carcinoembryonic antigen, etc.)?
- For response to adjuvant chemotherapy, how was it measured?
- For disease-free survival, how was it determined?

Note: Study results will NOT be abstracted.

Appendix C. Publications That Did Not Provide Specific Results for Stage II Colon Cancer

1. Ågesen TH, Sveen A, Merok MA, et al. ColoGuideEx: a robust gene classifier specific for stage II colorectal cancer prognosis. Gut. 2012 Jan 2. PMID: 22213796.
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