Current Status of Gene Expression Profiling to Assist Decision Making in Stage II Colon Cancer

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ABSTRACT

The decision regarding adjuvant therapy for patients with stage II colon cancer remains a challenge. In contrast to stage III colon cancer, for which compelling clinical data support the use of adjuvant chemotherapy, the clinical benefit of systemic therapy in unselected patients with stage II disease is modest at best. Risk stratification based on clinicopathologic features and DNA mismatch repair status is commonly used in adjuvant therapy

decisions, but these factors do not have a desired level of precision in identifying patients at high risk. Recently, gene expression platforms have been developed to further define risk and to assist in therapeutic decision making for patients with stage II disease. This review describes those platforms that are furthest along in clinical development, in an effort to place their potential clinical application in context. *The Oncologist* 2014;19:704–711

Implications for Practice: The selection of patients to receive adjuvant therapy in stage II colon cancer remains one of the greatest clinical challenges for medical oncologists. Currently, clinicopathologic characteristics and microsatellite instability status are used to help define risk and guide decisions about adjuvant therapy. In the past few years, several genomic profiling platforms have been developed, with the hope that they could add precision to this risk assessment. This review provides an overview of the gene expression platforms that are furthest along in development and discusses their potential role in making decisions about adjuvant therapy in stage II disease.

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer mortality in the U.S., with approximately 143,000 new cases and 51,000 deaths expected in 2013 [1]. At diagnosis, 25% of colon cancer cases are classified as stage II, for which surgical resection remains the mainstay of treatment, with a 5-year survival rate of 80% [2]. Several studies have analyzed the impact of adjuvant therapy in stage II disease. A meta-analysis of five studies with 1,016 patients from the IMPACT B2 investigators [3], a pooled analysis of seven trials with 3,302 patients [4], and an analysis of the Surveillance, Epidemiology, and End Results database of 3,151 stage II patients [5] have shown no benefit with adjuvant therapy in stage II disease. This is in contrast to the Quick and Simple and Reliable (QUASAR) study, which showed a modest (~4%) absolute benefit despite a 20% proportional reduction in the risk of recurrence and death [6]. Given excellent outcomes with surgery alone, in unselected patients, the justification for adjuvant chemotherapy for a small benefit, as noted in one study [6]—weighed against toxicities, inconvenience, cost, and psychological distress—creates one of the most challenging decisions in the management of patients with colon cancer.

Currently, clinicopathologic characteristics (e.g., less than 12 lymph nodes [LNs] identified at surgery [7, 8], T4 tumor, poorly differentiated tumor, presence of bowel obstruction or perforation [4, 9, 10], and presence of lymphovascular invasion [5, 11]) are considered in selecting high-risk stage II patients for adjuvant treatment. The consideration is based on extrapolating the relative benefits of adjuvant therapy in stage III disease as indirect evidence of benefit in those with high-risk stage II disease [11]. However, evidence suggests that there may be no benefit for adjuvant therapy in patients who are aged ≥ 65 years with or without these poor prognostic features [12]. Microsatellite instability (MSI) is used clinically as a molecular marker for screening of Lynch syndrome and has a role as a prognostic and predictive marker in stage II disease. MSI characterizes tumors with deficient DNA mismatch repair (dMMR) associated with loss of function (due to mutation or silencing) of one of the four DNA mismatch repair genes: *MLH1* (most commonly), *MSH2*, *MSH6*, and *PMS2*. Assessment of MMR status using immunohistochemistry to detect expression of mismatch repair proteins is highly concordant (>95%) with MSI using

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polymerase chain reaction (PCR), in which dMMR corresponds to high-degree MSI (MSI-H). It has been shown that colon cancers with dMMR have favorable outcomes and receive no benefit from fluoropyrimidine-based adjuvant therapy and a lower 5-year survival rate [13, 14]. We also know that capecitabine appears to be equivalent to 5-fluorouracil (5-FU) and leucovorin (LV) in the adjuvant setting for stage III disease [15]. There is no improvement in survival with the addition of oxaliplatin to 5-FU/LV (FOLFOX4) compared with 5-FU/LV (MOSAIC study) [16] in stage II patients and in elderly stage II patients [17]. Recently, analysis of tumors from the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07 clinical trial showed that MMR status was not predictive for oxaliplatin benefit [18]. An exploratory analysis assessing the predictive value of MSI phenotype in hereditary versus sporadic stage II and III CRC showed that the benefit from adjuvant 5-FU-based chemotherapy might be different according to the underlying molecular mechanism. Treatment benefits were observed in MSI germline tumors (disease-free survival [DFS]: hazard ratio [HR]: 0.31; 95% confidence interval [CI]: 0.14–0.70; $p = .006$) but not in those with MSI sporadic tumors (DFS: HR: 1.50; 95% CI: 0.82–2.74; $p = .182$) [19]. *BRAF* mutation in colon cancer has been associated with MSI and portends worse survival in most studies [20, 21]. Several studies have reported the independent, opposing prognostic effects of *BRAF* mutation and MSI [21–23]. In CALGB 89803, a randomized study comparing postoperative adjuvant 5-FU/LV with irinotecan, 5-FU, and LV (IFL) in stage III colon cancer [24], the combined *BRAF* and MSI subtyping analysis suggested that *BRAF*-mutated MSS tumor is an unfavorable subtype, whereas *BRAF* wild-type MSI-H tumor is a favorable subtype, and *BRAF*-mutated MSI-H and *BRAF* wild-type MSS tumors are intermediate subtypes [21]. A nonsignificant trend toward improved overall survival was also observed for IFL versus 5-FU/LV (HR: 0.52; 95% CI: 0.25–1.10) in *BRAF*-mutated tumors, but the sample size was small and no stage II tumors were studied [21].

Because there is minimal benefit from adjuvant therapy in unselected stage II colon cancer patients, improved risk stratification, identification of those tumors most likely to be responsive to treatment, and accurate identification of patients who may not benefit from treatment remain unmet needs. Gene expression profiling offers a potential prognostic and predictive molecular classification tool in stage II colon cancer. It promises greater discrimination and more reliable interpretation than clinical parameters. This review assesses several platforms that are in development and currently available for clinical use, including their validation techniques and their prognostic and predictive roles in the clinical setting.

GENE EXPRESSION PLATFORMS IN STAGE II COLON CANCER

Oncotype DX Colon Cancer Assay

The Oncotype DX Colon Cancer assay (Genomic Health, Redwood City, CA, <http://www.genomichealth.com>) is a 12-gene expression assay based on reverse transcription-PCR (RT-PCR) and performed on archival formalin-fixed and paraffin-embedded (FFPE) tumor tissue specimens [25]. The assay uses a recurrence score (RS) as a continuous variable to

predict the recurrence risk of colon cancer in both stage II and III disease. The development strategy for Oncotype DX was analogous to the development of the Oncotype DX breast cancer assay [26, 27]. In the training set, expression levels of 761 candidate genes were measured on samples from four separate studies involving patients with resected stage II and III colon cancer (NSABP C-01 and C-02, Cleveland Clinic observational series, NSABP C-04 and C-06) [28]. The final gene list associated with recurrence and a distinct set of genes associated with clinical benefit from adjuvant FU/LV chemotherapy was then selected on the basis of strength of association with patient outcome in development studies, representation of key biologic pathways, consistency of performance across studies and patient subsets, and analytic performance [28]. RS and treatment score were calculated from prespecified algorithms and cut points for low, intermediate, and high recurrence risk groups (i.e., $RS < 30$, $30-40$, and ≥ 41 , respectively) and low-, intermediate-, and high-risk treatment groups were defined in the study [25]. A validation set used 1,436 stage II colon cancer patients from the QUASAR study who were assigned randomly between adjuvant FU/LV chemotherapy and surgery and for which 1,307 samples were subject to tissue microarray analysis [6, 25]. The primary outcome was recurrence, rather than DFS or overall survival (OS), because tumor gene expression was anticipated to predict risk of recurrence but not death as a result of other causes. Secondary endpoints included DFS and OS. When T stage, MMR status, number of nodes examined, tumor location, tumor grade, lymphovascular invasion, and age were entered into a multiple-covariate regression model, the continuous RS was significantly associated with recurrence risk (HR: 1.43; 95% CI: 1.11–1.83; $p = .006$) [25]. Tumor obstruction or perforation, however, was not included. The 3-year risk of recurrence of the low-, intermediate-, and high-RS groups were 12%, 18%, and 22%, respectively, in the surgery-only arm and 8.9%, 13.3% and 16.3%, respectively, in the chemotherapy arm [25]. Because the proportional reductions in risk of recurrence with FU/LV chemotherapy were similar across the range of RSs, larger absolute improvement in 3-year recurrence with adjuvant FU/LV treatment was observed in the high-risk group (6%) compared with the low-risk group (3%).

Which subgroup of high-risk stage II patients can benefit from the use of the RS? In the validation study [25], the prognostic implications of T4 tumors (higher risk) and those with MMR deficiency (low risk) were reaffirmed as the strongest predictors of recurrence; therefore, the RS is less likely to play a role in treatment decisions. Limitations of the Oncotype validation study included a tissue block retrieval rate of 68%, which compares favorably with other recently reported trials, and a 62% rate of cases with fewer than 12 LNs examined. Furthermore, none of the pathologic or molecular biomarkers were associated with treatment efficacy and neither was the RS; consequently, the test is prognostic but not predictive, limiting the ability to identify patients expected to have very high absolute benefit from adjuvant treatment. Finally, the magnitude of benefit from adjuvant treatment is likely to be smaller when considering OS rather than the study endpoint of 3-year recurrence-free survival.

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The RS was also validated in another large prospective study (NSABP C-07) of stage II and III colon cancer patients who received FU/LV or FU/LV plus oxaliplatin (FOLFOX) as adjuvant therapy [29, 30]. The Cox model for 5-year recurrence risk in FU-treated patients by RS group (low, intermediate, and high) for stage II patients showed rates of 9% (95% CI: 6%–13%), 13% (95% CI: 8%–17%), and 18% (95% CI: 12%–25%), respectively [30]. Again, it is observed that in stage II patients, the absolute difference in the 5-year recurrence risk between the high- and low-risk groups that received adjuvant chemotherapy was only 9%. Relative benefit of oxaliplatin was similar across a range of RSs (interaction $p = .48$); accordingly, in the Cox model and in Kaplan-Meier analyses, absolute benefit of oxaliplatin increased with higher RS [29, 30]. RS was not predictive of relative benefit of oxaliplatin added to adjuvant FU but enabled better discrimination of absolute oxaliplatin benefit as a function of risk.

Oncotype DX Colon Cancer recurrence score is clinically validated as a prognostic gene signature in its association with recurrence risk in patients with stage II colon cancer in independent prospective studies. Among the large proportion (~70%) of T3, MMR-proficient tumors in stage II disease, the risk of 3-year recurrence ranged from 12% to 22% in the QUASAR validation trial and the risk of 5-year recurrence ranged between 9% and 18% in the NSABP C-07 validation study. Clinical utility of this assay depends on whether this information would assist the decision making of physicians and individual patients, given that the potential benefit of adjuvant treatment in the high-risk group still remains modest. We suggest that the use of Oncotype DX must be based on preference and not routinely applied to all patients with T3, MMR-intact stage II colon cancers. This test may be most useful in patients who would consider adjuvant therapy for modest benefit but would defer treatment if risk of relapse were very low.

ColDx

ColDx (Almac, Craigavon, U.K., <http://www.almacgroup.com/coldx/>) is a prognostic microarray-based 634-transcript gene signature that identifies high-risk versus low-risk stage II colon cancer patients for recurrence after surgery using FFPE tumor samples [31]. Stage II tumor samples were collected retrospectively from 12 institutions, and total RNA was extracted, amplified, and hybridized to the Almac Colorectal Cancer DSA [31]. The primary end-point was DFS at 5 years, and low-risk patients were followed for a minimum of 5 years. A training set consisted of 215 patients (66% low-risk and 34% high-risk patients; T4 tumors: 13%). Low-risk patients were defined as those with no cancer recurrence within 5 years of primary

surgery, and high-risk patients were defined as those with metastatic cancer recurrence within 5 years of primary surgery. No pre- or postoperative cancer therapy within 1 year of surgery was allowed. The prognostic signature was applied to an independent validation set of 144 patients that was enriched for recurrence (59% low-risk and 41% high-risk patients; T4 tumors: 17%) using the threshold score identified in the training set. The signature predicted 5-year recurrence-free survival of approximately 70% in the low-risk group and 40% in the high-risk group (HR: 2.53; 95% CI: 1.54–4.15; $p < .001$) [31]. The assay was assessed against known clinical prognostic factors such as tumor stage, grade and location, patient age and sex, mucinous and nonmucinous subtypes, lymphovascular invasion, and number of LNs retrieved but not for MSI status. It was shown that the signature provides prognostic information in addition to the conventional risk factors (HR: 2.55; 95% CI: 1.47–4.42; $p < .001$) [31]. Functional analysis was also performed whereby the signature was analyzed against biologic pathways known to be relevant to colon cancer recurrence and the most significant pathway identified was the *IGF-1* signaling pathway in addition to *TGF- β* and *HMGB1* signaling pathways [31]. All of these have been previously reported to confer a poor prognosis in colon cancer through promoting tumor growth, invasion, and metastasis and preventing apoptosis [32–34].

Similar to the Oncotype DX Colon Cancer assay, the ColDx assay uses FFPE tissue, which has the benefit of having genomic testing performed on stored rather than fresh tissue. Limitations of this study included the small number of patients studied and the retrospective nature of the validation study whereby the low- and high-risk groups were defined retrospectively based on recurrence status. It also lacks information on MSI status and absence of integration of its results with standard clinical risk criteria [11] (e.g., a minimum of six LNs was used as an inclusion criterion compared with the standard criterion of 12 LNs). Further study is needed to determine the prognostic value in an unselected population and the benefit of adjuvant treatment in the high-risk population.

ColoPrint

The ColoPrint assay (Agendia, Irvine, CA, <http://www.agendia.com>) uses oligonucleotide microarray technology on fresh tissue RNA based on the expression of 18 unique genes [35]. The classifier identifies a sample as either low-risk or high-risk for development of distant metastasis in stage II or III CRC patients [35, 36]. For stage II disease, the training set consisted of 100 prospectively collected CRC samples from a Dutch institution, and the validation set consisted of 114 prospectively collected CRC samples from a Spanish institution. Adjuvant chemotherapy was allowed (19.1% and 37.4% of patients in the training and validation sets, respectively). Gene expression analysis consists of hybridization of the samples against a colon cancer reference pool, and a supervised training approach was performed to identify a prognostic CRC signature with a significant 5-year distant metastasis-free survival (DMFS) association. For the analysis of the validation set, the primary endpoint was relapse-free survival (RFS) and median follow-up was 55 months [35].

In the subgroup analysis of patients with stage II disease ($n = 114$), 63.2% were classified as low-risk and 36.8% were classified as high-risk, with 5-year RFS rates of 90.9% and 73.9%, respectively ($p = .017$) [35]. ColoPrint was the strongest predictor for RFS and performed independently of the American Society of Clinical Oncology (ASCO) risk criteria [3], regardless of chemotherapy administration. Chemotherapy was administered to 36% of stage II patients, with equal distribution between both risk groups, and had no impact on RFS or OS. The authors concluded that ColoPrint identifies two-thirds of patients with stage II CRC who are at sufficiently low risk of recurrence (91% of whom did not experience relapse) and who may be managed safely without adjuvant chemotherapy. This conclusion should be interpreted cautiously because many patients in the analysis received adjuvant treatment, and the study permitted inclusion of rectal cancer as well as colon cancer patients. Nevertheless, the potential for a diagnostic test to identify a large proportion of stage II colon cancer patients with excellent prognosis ($>90\%$ cure), sparing the need for adjuvant treatment, would be a significant clinical advance.

It is uncertain whether the low-risk profile identified by ColoPrint was confounded by MSI-H status. MSI-H was found in a small number of stage II patients (21 of 29 patients) in the first validated set of patients and was not significant in the multivariate analysis in the pooled analysis of stage II patients. The reported rate of 72% of MSI-H status in stage II patients the study by Salazar et al. [35] is very high when compared with the reported alteration rates of 12%–22% in larger studies [37, 38]. A similar finding was also observed in the high reported rate of *BRAF* mutations in stage II patients in this study, at 52% compared with less than 10% in other larger studies [37, 38]. An additional limitation of this study is the need for fresh frozen tissue for the assay; this need would impede its widespread adoption.

ColoPrint was tested in a second validation cohort that consisted of 233 patients who underwent curative resection (R0) for stage II or III colon cancer [39]. In 135 stage II patients, ColoPrint identified most patients (73.3%) as low-risk [40]. The 5-year DMFS was 95% for low-risk patients and 81% for high-risk patients [40]. Only 3 patients (2.2%) received adjuvant chemotherapy and MSI status was known in 132 patients [40]. The good prognosis of the MSI-H patients was recognized by ColoPrint; most patients (86%) were classified as ColoPrint low risk. Interestingly, the results for recurrence risk for ColoPrint and the clinical factors were discordant in 50% of patients, likely from the small sample size [40]. In a pooled analysis of 320 stage II patients from independent validation cohorts from five European hospitals, ColoPrint classified two-third of patients as low risk [41]. Preliminary results identified the 3-year RFS as 94% for low-risk patients and 79% for high-risk patients, with an HR of 2.74 (95% CI: 1.54–4.88; $p = .006$) [41]. Using ASCO risk criteria [11], the identification of high-risk patients (chemotherapy information unknown) was not significant (HR: 1.43; 95% CI: 0.81–2.55; $p = .22$), and no clinical parameter added power to the ColoPrint classification in the multivariate analysis [41]. A phase III clinical trial is currently recruiting patients for assessment of the ColoPrint test in 785 patients with stage II CRC (PARSC study, ClinicalTrials.gov identifier NCT00903565).

The primary objective of the study is to validate the performance of ColoPrint in estimating the 3-year relapse rate in patients with stage II CRC. The first part of the study is a retrospective analysis of the impact of ColoPrint on adjuvant treatment decision. Patients with stage II CRC are treated with surgery and adjuvant therapy based on the investigator's discretion without knowledge of the ColoPrint test results. The investigator is then provided with baseline clinical characteristics, adjuvant treatment information, and the ColoPrint result for each patient and is asked what the recommended treatment for the patient would have been if the ColoPrint result were known at diagnosis and whether it would have affected the confidence level of the treatment decision. In the extension study, the impact of ColoPrint on adjuvant treatment in stage II CRC patients will be evaluated prospectively by collecting baseline clinical data and the patient's and physician's chemotherapy intentions, the patient's perceived recurrence risk, and the decisional conflict before and after knowing the ColoPrint result.

Previstage

Previstage guanylyl cyclase C (GCC) colorectal staging test (DiagnoCure, Quebec City, QC, Canada, <http://www.diagnocure.com>) uses quantitative RT-PCR to quantify GCC mRNA in formalin-fixed LN tissue. The GCC mRNA encodes for a human receptor for the paracrine hormones guanylin and uroguanylin, which is ubiquitously expressed in gastrointestinal epithelium and preserved in primary and metastatic CRC cells [42–45]. Studies have shown that molecular detection of LN metastases have impact on patient prognosis [46–48]. The presence of GCC mRNA in pericolic LNs, for example, has been demonstrated as a prognostic factor in patients with node-negative (pN0) colon cancer [49–51], and recurrence rates in GCC-positive LNs have been reported to be similar to those observed in stage III disease [49]. The prognostic significance of GCC expression in LNs of patients with stage II colon cancer not treated with adjuvant chemotherapy has been reported [42]. The training set consisted of 241 patients with at least 10 LNs examined. The lymph node ratio (LNR), the number of GCC positive LNs divided by the total number of informative LNs, was used to categorize patients as low risk (LNR: 0–0.1) or high risk (LNR: >0.1) for recurrence. This was a prospectively designed retrospective study, and patients were followed for a minimum of 36 months. Independent prognostic information such as MMR status and pathologic features (T stage, tumor location, bowel perforation or obstruction, number of LNs assessed, and presence of lymphovascular invasion) was available for the majority of patients. The LNR significantly predicted higher recurrence risk for 35% of patients classified as high risk (HR: 2.38; $p = .02$) [42]. The estimated 5-year recurrence rates were 10% and 27% for the low- and high-risk groups, respectively [42]. After adjusting for age, T stage, number of nodes assessed, and MMR status, GCC LNR remained associated with the risk of recurrence (HR: 2.61; 95% CI: 1.17–5.83; $p = .02$) but not with OS [42]. In a subgroup analysis of 181 patients with T3 tumor, ≥ 12 LNs examined, and negative margins, a strong relationship between the GCC LNR and recurrence risk was observed (HR: 5.06; 95% CI: 1.61–15.91, $p = .003$) [42]. These study results require validation (which is ongoing), and the

Table 1. Gene expression platforms in stage II colon cancer

Platforms	Oncotype [25]	ColDx [31]	ColoPrint [35]	Previstage [42]
Patients				
Training set	Stage II/III	Stage II	Stage II/III	Stage II
Validation set (no. of stage II patients)	Stage II (<i>n</i> = 1,436)	Stage II (<i>n</i> = 144)	Stage II/III (<i>n</i> = 114)	—
Tissue source	FFPE tumor tissue	FFPE tumor tissue	Fresh frozen tumor tissue	FFPE lymph nodes
Type of study	Prospective	Retrospective	Prospective	Retrospective
Colon/rectal tissue	Colon	Colon	Colon/rectal	Colon
Adjuvant chemotherapy included	Yes	No	Yes	No
MMR data	Available	Not available	Available	Available
Type of signature	Prognostic	Prognostic	Prognostic	Prognostic
Technology	RT-PCR	Microarray	Microarray	qRT-PCR
Type of gene signature	12-gene signature	634-transcript signature	18-gene classifier	GCC gene expression
Endpoints				
Training set	Recurrence risk	5-year DFS	5y-DMFS	Time to recurrence
Validation set	Recurrence risk	5-year DFS	RFS	—
Presentation of results	Continuous RS; low-, intermediate-, high-risk groups	Low risk vs. high risk	Low risk vs. high risk	Low risk vs. high risk
Validation set results	Recurrence risk at 3 years: RS low, 12%; RS intermediate, 18%; RS high, 22%	High risk: 5-year RFS, 40%; low risk: 5-year RFS, 70%	High risk: 5-year RFS, 67.2%; low risk: 5-year RFS, 87.6%	—
Follow-up	5.5 years	5 years	4.5 years	3 years

Abbreviations: —, no data; DFS, disease-free survival; DMFS, distant metastasis-free survival; GCC, guanylyl cyclase C; FFPE, formalin-fixed paraffin-embedded; MMR, mismatch repair; RFS, relapse-free survival; RS, recurrence score; RT-PCR, reverse transcription-polymerase chain reaction; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

test requires the availability of regional LNs from colonic resection. Because no patients received adjuvant chemotherapy, the predictive potential of the GCC assay was not assessable; however, if validated in the ongoing study, this test may also have clinical utility for identifying a substantial proportion of patients (i.e., low risk) who do not require adjuvant treatment.

DISCUSSION

Genomic platforms to risk-stratify stage II colon cancer patients are being developed to assist with the decision making process for adjuvant therapy. Oncotype DX and ColoPrint are commercially available. Table 1 compares the features and development strategies of the various platforms considered in this review. Direct comparison of the platforms is limited by the fact that training and validation sets consisted of different patient populations, including some patients with stage III disease and some with rectal cancer. Apart from Oncotype DX, the number of patients with stage II disease in the validation sets of the other platforms was small. Despite being an important prognostic and predictive marker, MMR status was not reported with some platforms (e.g., ColDx). Aside from ColDx, these platforms do not identify patients at very high risk of recurrence. The Oncotype DX assay, for example, stratifies recurrence risk at 3 years as 12%, 18%, and 22% in the low-, intermediate-, and high-risk groups, respectively. The absolute difference between these groups (10% between the low- and high-risk groups; 4% between the

intermediate- and high-risk groups) may not be sufficient to affect clinical decision making. We believe that future platform development should seek broader discrimination among risk categories that would more clearly identify those patients who should and should not be treated in the adjuvant setting. Furthermore, each of these platforms provides prognostic information but is not predictive in the sense of discerning differences in relative treatment benefit. Predictive information would provide enhanced value for these diagnostic platforms. That said, as noted with Oncotype DX, there is a consistent relative risk reduction across all risk levels, allowing for estimation of absolute risk reduction at each RS with chemotherapy.

We believe that absolute reduction in risk of cancer recurrence (provided that this is a validated surrogate for survival with the adjuvant treatment being considered) should be the primary consideration in deciding whether the benefit from adjuvant chemotherapy for colon cancer is clinically worthwhile. In contrast to “relative” risk reduction, which is dependent on the underlying risk and thus may overestimate clinical benefit for individuals at lower baseline risk, “absolute” risk reduction provides a clear assessment of the potential benefit for individual patients. To compare the platforms considered in this review, we calculated the relative risk reduction that would be required from adjuvant treatment to accomplish a 5% absolute risk reduction. The threshold of 5% was selected as a level that we believe most oncologists and

Table 2. Relative performance of genomic platforms in identification of patients with high-risk stage II colon cancer

Platform	Relapse rate in high-risk group (%)	Relative risk reduction needed to achieve 5% absolute reduction in relapse (%)
Oncotype [25]	22	23
ColDx [31]	60	8
ColoPrint [35]	26	19
Previstage [42]	27	19

The relapse rate in each high-risk group was recorded based on the respective studies utilizing the platforms of interest. The high-risk group was chosen as an example as this is the group that would most likely gain benefit from adjuvant therapy. The relative risk reduction of each platform is calculated by dividing the absolute reduction in relapse (in this case, 5% was used as the minimal cutoff felt to be clinically significant) by the relapse rate. By using the relative risk reduction, the efficacy of the different platforms can be compared, and it is demonstrated that the most valuable test for identifying a subgroup (that is to gain the most from the intervention) is a test that can significantly discriminate a high relapse rate in the high-risk group when compared with the low-risk group.

Figure 1. Proposed algorithm for the decision regarding adjuvant chemotherapy in stage II colon cancer patients. High-risk clinicopathologic features include T4 tumor, fewer than 12 lymph nodes identified at surgery, poorly differentiated tumor, presence of bowel obstruction or perforation, and presence of lymphovascular invasion.

Abbreviations: dMMR, deficient DNA mismatch repair; pMMR, proficient DNA mismatch repair.

patients would consider with an adjuvant therapy toxicity profile similar to fluoropyrimidines with or without oxaliplatin (Table 2). If one assumes that current adjuvant treatments provide a relative risk reduction of approximately 20% [6], a clinically useful test would identify a population with at least 25% risk of recurrence. This metric is met by several of the platforms under discussion (i.e., Oncotype DX, ColoPrint, and ColDx). Many patients may be comfortable with lower potential benefit from adjuvant treatment, and for these patients, the benefit of genomic risk stratification with a diagnostic test that provides a continuous score may be particularly useful. It is important to note, however, that the biological information and prognostic and predictive value of these genomic platforms have not been formally compared in a study.

In contrast to “relative” risk reduction, which is dependent on the underlying risk and thus may overestimate clinical benefit for individuals at lower baseline risk, “absolute” risk reduction provides a clear assessment of the potential benefit for individual patients.

Another potential benefit for use of a molecular classifier in stage II colon cancer is the ability to reduce harm and potentially save health care costs for patients who will not benefit from adjuvant therapy because their risk of relapse is very low. An economic analysis of the use of Oncotype DX in stage II, T3, MMR-proficient colon cancer predicted that the

assay would reduce the use of adjuvant chemotherapy by 17% in addition to improving quality-adjusted survival overall and saving medical costs from a societal perspective [52]. Empirical support for this analysis was provided by a prospective survey of the decision making by oncologists in 187 stage II colon cancer patients treated in a multi-institutional cancer research consortium [53]. Preliminary results found that after receiving the Oncotype DX RS, physician recommendations in favor of adjuvant chemotherapy decreased by 22%. Increased treatment intensity was more likely at higher RS values and decreased intensity at lower RS values ($p = .011$), and any change in recommendation was more likely when MMR status was unknown at baseline ($p = .041$) [53]. In terms of cost, average total direct medical costs decreased by about \$4,000, and the net effect on average patient well-being was a gain of 0.083 quality-adjusted life year [54].

We are entering an era in which genomic profiling in stage II colon cancer may be appropriately integrated into decision-making algorithms for adjuvant therapy (Fig. 1). We propose, for example, that in a newly diagnosed stage II colon cancer patient, the decision for adjuvant therapy can be stratified initially by age or comorbidities, based on evidence that older patients may not have an OS benefit from adjuvant treatment [12, 55]. This lack of benefit may be due to competing risks of death in older patients in the context of small potential benefits from adjuvant therapy. In younger patients, the next stratification factor could be MMR status, with MMR-deficient tumors (~15%) not receiving adjuvant chemotherapy because of their good prognosis and lack of benefit from 5-FU chemotherapy [13]. MMR-proficient tumors could then be stratified by T3 versus T4 tumor. Because T4 tumors are associated with poor prognosis [11], chemotherapy should be considered. The majority (85%) of stage II tumors are T3 tumors. Genomic profiling would be able to provide additional risk stratification for patients with T3 and MMR-proficient tumors who wish to consider the modest benefits from adjuvant therapy. The results of genomic analysis could then be used in discussion with these patients about the risks and benefits of adjuvant treatment.

It is important to underscore that the use of the genomic profiling tests does not circumvent the need to have a thorough discussion with the patient to ensure integration of patient preferences in the decision for adjuvant therapy. The value assigned to a particular absolute benefit differs from patient to patient, and understanding the risk of recurrence provided by a test that can account for the biology of the cancer and provide greater precision regarding a risk estimate may further assist in making judgment about adjuvant treatment. This additional information may be particularly useful in providing peace of mind when the recurrence risk is found to be very low and adjuvant treatment may be avoided.

CONCLUSION

We do not believe that there is currently a gene expression platform with sufficient performance characteristics or validation to warrant application to all patients with stage II colon cancer. Nevertheless, for patients who would value modest benefits from adjuvant treatment, the application of a diagnostic test that might indicate that adjuvant therapy is not needed could provide personal benefit. At a minimum, the assays described in this review provide proof of concept that gene expression profiling has the potential to guide patient selection. Further refinement of expression platforms to enrich the population of patients who are unlikely to relapse or to identify patients at very high risk of relapse would lead to improvements in patient selection for adjuvant treatment, enhance quality adjusted survival, and potentially save health care costs.

AUTHOR CONTRIBUTIONS

Conception/Design: Cheng E. Chee, Neal J. Meropol
Provision of study material or patients: Cheng E. Chee, Neal J. Meropol
Collection and/or assembly of data: Cheng E. Chee, Neal J. Meropol
Data analysis and interpretation: Cheng E. Chee, Neal J. Meropol
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DISCLOSURES

Cheng E. Chee: Celgene (C/A); **Neal J. Meropol:** U.S. Patent US20020031515 A1, "Methods of therapy for cancers characterized by overexpression of the HER2 receptor protein" (IP).

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013;63:11–30.
2. O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J Natl Cancer Inst* 2004;96:1420–1425.
3. Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer. International Multicentre Pooled Analysis of B2 Colon Cancer Trials (IMPACT B2) Investigators. *J Clin Oncol* 1999;17:1356–1363.
4. Gill S, Loprinzi CL, Sargent DJ et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: Who benefits and by how much? *J Clin Oncol* 2004;22:1797–1806.
5. Schrag D, Rifas-Shiman S, Saltz L et al. Adjuvant chemotherapy use for Medicare beneficiaries with stage II colon cancer. *J Clin Oncol* 2002;20:3999–4005.
6. Gray R, Barnwell J, McConkey C et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: A randomised study. *Lancet* 2007;370:2020–2029.
7. Swanson RS, Compton CC, Stewart AK et al. The prognosis of T3N0 colon cancer is dependent on the number of lymph nodes examined. *Ann Surg Oncol* 2003;10:65–71.
8. Greene FL, Stewart AK, Norton HJ. A new TNM staging strategy for node-positive (stage III) colon cancer: An analysis of 50,042 patients. *Ann Surg* 2002;236:416–421; discussion 421.
9. Sargent DJ, Goldberg RM, Jacobson SD et al. A pooled analysis of adjuvant chemotherapy for resected colon cancer in elderly patients. *N Engl J Med* 2001;345:1091–1097.
10. Compton CC, Fielding LP, Burgart LJ et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 2000;124:979–994.
11. Benson AB III, Schrag D, Somerfield MR et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *J Clin Oncol* 2004;22:3408–3419.
12. O'Connor ES, Greenblatt DY, LoConte NK et al. Adjuvant chemotherapy for stage II colon cancer with poor prognostic features. *J Clin Oncol* 2011;29:3381–3388.
13. Sargent DJ, Marsoni S, Monges G et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* 2010;28:3219–3226.
14. Ribic CM, Sargent DJ, Moore MJ et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant

chemotherapy for colon cancer. *N Engl J Med* 2003; 349:247–257.

15. Twelves C, Wong A, Nowacki MP et al. Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* 2005;352:2696–2704.

16. André T, Boni C, Navarro M et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncol* 2009; 27:3109–3116.

17. Tournigand C, André T, Bonnetain F et al. Adjuvant therapy with fluorouracil and oxaliplatin in stage II and elderly patients (between ages 70 and 75 years) with colon cancer: Subgroup analyses of the Multicenter International Study of Oxaliplatin, Fluorouracil, and Leucovorin in the Adjuvant Treatment of Colon Cancer trial. *J Clin Oncol* 2012; 30:3353–3360.

18. Gavin PG, Colangelo LH, Fumagalli D et al. Mutation profiling and microsatellite instability in stage II and III colon cancer: An assessment of their prognostic and oxaliplatin predictive value. *Clin Cancer Res* 2012;18:6531–6541.

19. Sinicrope FA, Foster NR, Thibodeau SN et al. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 2011;103:863–875.

20. Samowitz WS, Sweeney C, Herrick J et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res* 2005;65:6063–6069.

21. Ogino S, Shima K, Meyerhardt JA et al. Predictive and prognostic roles of BRAF mutation in stage III colon cancer: Results from intergroup trial CALGB 89803. *Clin Cancer Res* 2012;18:890–900.

22. Dahlin AM, Palmqvist R, Henriksson ML et al. The role of the CpG island methylator phenotype in colorectal cancer prognosis depends on microsatellite instability screening status. *Clin Cancer Res* 2010;16:1845–1855.

23. Roth AD, Tejpar S, Delorenzi M et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: Results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010;28:466–474.

24. Saltz LB, Niedzwiecki D, Hollis D et al. Irinotecan fluorouracil plus leucovorin is not superior to fluorouracil plus leucovorin alone as adjuvant treatment for stage III colon cancer: Results of CALGB 89803. *J Clin Oncol* 2007;25:3456–3461.

25. Gray RG, Quirke P, Handley K et al. Validation study of a quantitative multigene reverse transcriptase-polymerase chain reaction assay for assessment of recurrence risk in patients with stage II colon cancer. *J Clin Oncol* 2011;29:4611–4619.

26. Paik S, Shak S, Tang G et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351: 2817–2826.

27. Paik S, Tang G, Shak S et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006;24:3726–3734.

28. O'Connell MJ, Lavery I, Yothers G et al. Relationship between tumor gene expression and recurrence in four independent studies of patients with stage II/III colon cancer treated with surgery alone or surgery plus adjuvant fluorouracil plus leucovorin. *J Clin Oncol* 2010;28:3937–3944.

29. O'Connell M, Lee M, Lopatin M et al. Validation of the 12-gene colon cancer recurrence score (RS) in NSABP C07 as a predictor of recurrence in stage II and III colon cancer patients treated with 5FU/LV (FU) and 5FU/LV+oxaliplatin (FU+Ox). *J Clin Oncol* 2012;30(suppl):3512a.

30. Yothers G, O'Connell MJ, Lee M et al. Validation of the 12-gene colon cancer recurrence score in NSABP C-07 as a predictor of recurrence in patients with stage II and III colon cancer treated with fluorouracil and leucovorin (FU/LV) and FU/LV plus oxaliplatin. *J Clin Oncol* 2013;31:4512–4519.

31. Kennedy RD, Bylesjo M, Kerr P et al. Development and independent validation of a prognostic assay for stage II colon cancer using formalin-fixed paraffin-embedded tissue. *J Clin Oncol* 2011;29: 4620–4626.

32. Noshio K, Yamamoto H, Taniguchi H et al. Interplay of insulin-like growth factor-II, insulin-like growth factor-I, insulin-like growth factor-I receptor, COX-2, and matrix metalloproteinase-7, play key roles in the early stage of colorectal carcinogenesis. *Clin Cancer Res* 2004;10:7950–7957.

33. Tsushima H, Kawata S, Tamura S et al. High levels of transforming growth factor beta 1 in patients with colorectal cancer: Association with disease progression. *Gastroenterology* 1996;110: 375–382.

34. Völz K, Brezniceanu ML, Bösner S et al. Increased expression of high mobility group box 1 (HMGB1) is associated with an elevated level of the antiapoptotic c-IAP2 protein in human colon carcinomas. *Gut* 2006;55:234–242.

35. Salazar R, Roepman P, Capella G et al. Gene expression signature to improve prognosis prediction of stage II and III colorectal cancer. *J Clin Oncol* 2011;29:17–24.

36. Glas AM, Floore A, Delahaye LJ et al. Converting a breast cancer microarray signature into a high-throughput diagnostic test. *BMC Genomics* 2006;7: 278.

37. Roth AD, Delorenzi M, Tejpar S et al. Integrated analysis of molecular and clinical prognostic factors in stage II/III colon cancer. *J Natl Cancer Inst* 2012; 104:1635–1646.

38. Hutchins G, Southward K, Handley K et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011;29: 1261–1270.

39. Rosenberg R, Maak M, Simon I et al. Independent validation of a prognostic genomic profile (ColoPrint) for stage II colon cancer (CC) patients. *J Clin Oncol* 2011;29(suppl 4):358a.

40. Maak M, Simon I, Nitsche U et al. Independent validation of a prognostic genomic signature (ColoPrint) for patients with stage II colon cancer. *Ann Surg* 2013;257:1053–1058.

41. Tabernero J, Moreno V, Rosenberg R et al. Clinical and technical validation of a genomic classifier (ColoPrint) for predicting outcome of patients with stage II colon cancer. *J Clin Oncol* 2012;30(suppl 4):384a.

42. Sargent DJ, Resnick MB, Meyers MO et al. Evaluation of guanylyl cyclase C lymph node status for colon cancer staging and prognosis. *Ann Surg Oncol* 2011;18:3261–3270.

43. Winn B, Tavares R, Matoso A et al. Expression of the intestinal biomarkers guanylyl cyclase C and CDX2 in poorly differentiated colorectal carcinomas. *Hum Pathol* 2010;41:123–128.

44. Waldman SA, Cagır B, Rakinic J et al. Use of guanylyl cyclase C for detecting micrometastases in lymph nodes of patients with colon cancer. *Dis Colon Rectum* 1998;41:310–315.

45. Mejia A, Schulz S, Hyslop T et al. GUCY2C reverse transcriptase PCR to stage pN0 colorectal cancer patients. *Expert Rev Mol Diagn* 2009;9: 777–785.

46. Nicastrì DG, Doucette JT, Godfrey TE et al. Is occult lymph node disease in colorectal cancer patients clinically significant? A review of the relevant literature. *J Mol Diagn* 2007;9:563–571.

47. Iddings D, Ahmad A, Elashoff D et al. The prognostic effect of micrometastases in previously staged lymph node negative (N0) colorectal carcinoma: A meta-analysis. *Ann Surg Oncol* 2006;13: 1386–1392.

48. Liefers GJ, Cleton-Jansen AM, van de Velde CJ et al. Micrometastases and survival in stage II colorectal cancer. *N Engl J Med* 1998;339:223–228.

49. Waldman SA, Hyslop T, Schulz S et al. Association of GUCY2C expression in lymph nodes with time to recurrence and disease-free survival in pN0 colorectal cancer. *JAMA* 2009;301:745–752.

50. Schulz S, Hyslop T, Haaf J et al. A validated quantitative assay to detect occult micrometastases by reverse transcriptase-polymerase chain reaction of guanylyl cyclase C in patients with colorectal cancer. *Clin Cancer Res* 2006;12:4545–4552.

51. Haince JF, Houde M, Beaudry G et al. Comparison of histopathology and RT-qPCR amplification of guanylyl cyclase C for detection of colon cancer metastases in lymph nodes. *J Clin Pathol* 2010;63: 530–537.

52. Hornberger J, Lyman GH, Chien R et al. A multigene prognostic assay for selection of adjuvant chemotherapy in patients with T3, stage II colon cancer: Impact on quality-adjusted life expectancy and costs. *Value Health* 2012;15:1014–1021.

53. Srivastava G, Renfro LA, Behrens RJ et al. Prospective evaluation of a 12-gene assay on treatment recommendations in patients with stage II colon cancer. *J Clin Oncol* 2012;30(suppl 34):453a.

54. Alberts SR, Yu T, Behrens RJ et al. Real-world comparative economics of a 12-gene assay for prognosis in stage II colon cancer. *J Clin Oncol* 2012; 30(suppl 34):391a.

55. Meropol NJ. Ongoing challenge of stage II colon cancer. *J Clin Oncol* 2011;29:3346–3348.