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ORIGINAL REPORT

# 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

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See accompanying editorial on page 3904

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## ABSTRACT

### Purpose
These studies were conducted to determine the relationship between quantitative tumor gene expression and risk of cancer recurrence in patients with stage II or III colon cancer treated with surgery alone or surgery plus fluorouracil (FU) and leucovorin (LV) to develop multigene algorithms to quantify the risk of recurrence as well as the likelihood of differential treatment benefit of FU/LV adjuvant chemotherapy for individual patients.

### Patients and Methods
We performed quantitative reverse transcription polymerase chain reaction (RT-qPCR) on RNA extracted from fixed, paraffin-embedded (FPE) tumor blocks from patients with stage II or III colon cancer who were treated with surgery alone (n = 270 from National Surgical Adjuvant Breast and Bowel Project [NSABP] C-01/C-02 and n = 765 from Cleveland Clinic [CC]) or surgery plus FU/LV (n = 308 from NSABP C-04 and n = 508 from NSABP C-06). Overall, 761 candidate genes were studied in C-01/C-02 and C-04, and a subset of 375 genes was studied in CC/C-06.

### Results
A combined analysis of the four studies identified 48 genes significantly associated with risk of recurrence and 66 genes significantly associated with FU/LV benefit (with four genes in common). Seven recurrence-risk genes, six FU/LV-benefit genes, and five reference genes were selected, and algorithms were developed to identify groups of patients with low, intermediate, and high likelihood of recurrence and benefit from FU/LV.

### Conclusion
RT-qPCR of FPE colon cancer tissue applied to four large independent populations has been used to develop multigene algorithms for estimating recurrence risk and benefit from FU/LV. These algorithms are being independently validated, and their clinical utility is being evaluated in the Quick and Simple and Reliable (QUASAR) study.

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## INTRODUCTION

Although adjuvant chemotherapy is the standard of care in stage III colon cancer, its routine use in patients with stage II colon cancer is controversial.[1-10] The Quick and Simple and Reliable (QUASAR) study[11] showed that adjuvant chemotherapy with fluorouracil (FU) plus leucovorin (LV) produces a small (approximately 3%) survival benefit in stage II colon cancer, which must be balanced with its toxicity, including toxic deaths (approximately 0.5%). This narrow therapeutic index underscores the importance of selecting the appropriate patients for adjuvant treatment.

In current practice, clinical and pathologic markers (ie, intestinal perforation/obstruction, pathologic stage T4, presence of lymphatic/vascular invasion, high tumor grade, < 12 nodes examined) can identify a minority of patients with stage II disease who have higher recurrence risk, but they do not adequately assess recurrence risk for individual patients. To address this issue, the use of molecular markers, such as microsatellite instability (MSI)/mismatch repair (MMR), LOH 18q, and levels of expression of individual genes or groups of genes[12-21] has been investigated. Some recent studies suggest MMR deficiency (ie, MSI high) may identify a small

percentage (approximately 15%) of patients with stage II disease who receive little benefit from FU/LV.[22] However, the clinical utility of these markers remains under study.[23]

Here, we report the application of the quantitative reverse transcription polymerase chain reaction (RT-qPCR) platform developed for the Onco*type* DX Breast Cancer Assay (Genomic Health, Inc, Redwood City, CA)[24-26] in four independent colon cancer studies to generate the 12-gene recurrence score and 11-gene treatment score algorithms that, if validated, will quantify the risk of recurrence as well as the likelihood of differential treatment benefit of FU/LV adjuvant chemotherapy for individual patients with stage II colon cancer.

## PATIENTS AND METHODS

### Patients and Samples

Samples from four independent cohorts of patients with stage II or stage III colon cancer treated with surgery alone (National Surgical Adjuvant Breast and Bowel Project [NSABP] C-01/C-02 or Cleveland Clinic [CC] study) or surgery plus FU/LV (NSABP C-04, NSABP C-06) were studied (Appendix Table A1, online only; Appendix Fig A1, online only).[27-30] Prespecified criteria for being evaluable were as follows: eligibility for the parent clinical studies; availability of the fixed, paraffin-embedded (FPE) tumor block from initial diagnosis; presence of sufficient tumor (ie, $\geq$ 5% of tissue area occupied by invasive cancer cells in the guide hematoxylin and eosin slide); pathology diagnosis of colon adenocarcinoma (excluding signet ring carcinoma); adequate RNA to perform quantitative RT-qPCR analysis ($\geq$ 1,069 ng for C-01/C-02 and C-04 and $\geq$ 587 ng for CC and C-06); and sufficient RNA quality by predefined metrics.

### Sample Preparation

For each patient, RNA was extracted from three pooled 10-$\mu$m sections obtained from archived FPE colon tumor tissue. Nontumor elements were commonly identified on the guide hematoxylin and eosin slide reviewed for each patient and were removed by manual microdissection before transfer to the extraction tube.

### Pathology, Assay Methods, Gene Selection, Reference Gene Normalization

Assessment of tumor grade was performed according to WHO criteria[31] by an academic surgical pathologist with sub-specialty expertise in gastrointestinal pathology. The extracted RNA was quantified and then analyzed by RT-qPCR.[32] For the C-01/C-02 and C-04 cohorts, two 384 well plates, which contained a total of 761 unique assays (ie, 761-gene panel), were used for each sample. With the exception of four assays (three *K-ras* mutations and one *BRAF* mutation), all assays were designed to detect the expression levels of wild-type genes. The panel of 761 candidate genes (Appendix Table A2, online only) was constructed from published gene expression profiling data and from biologic pathways identified as functionally important in colon cancer.[17-21] For the CC and C-06 cohorts, one 384-well plate, which contained 375 unique assays (ie, 375-gene panel), was used for each sample. The genes for the 375-gene panel were chosen from the 761-gene panel on the basis of the strength of the association of their level of expression with recurrence risk and chemotherapy benefit in the C-01/C-02 and C-04 studies. Gene expression measurements were normalized relative to five reference genes. Among the available samples across the four cohorts, only eight were excluded because of inadequate RT-qPCR expression.

MMR status was assessed by immunohistochemistry for *MLH1* and *MSH2* (which identify > 90% of the MMR-deficient tumors) on fixed, primary colon tumor tissue in the CC study.[33]

### Blinding and Data Preparation

FPE tissue sections were prepared by either NSABP or CC personnel and were shipped to Genomic Health, Inc (Redwood City, CA), where the expression profiling was performed, blinded to the clinical data. The expression data and the clinical/pathology data were independently locked and then merged to construct the analysis data set for each study.

### Study Design, Objectives, and End Points

The primary objective of all four studies was to identify genes associated with recurrence-free interval (RFI), defined as the time from surgery to first colon cancer recurrence. Deaths before recurrence were considered censoring events. Second primary cancers were considered neither events nor censoring events. Secondary end points were disease-free survival and overall survival.

### Analysis Methods

Prespecified univariate (primary analysis) and multivariate relationships between clinical outcomes and categorical or continuous variables (eg, gene expression) were modeled using Cox proportional hazards regression.[34] All baseline patient characteristics related to RFI ($P < .20$) were included in the multivariate analysis for a given study. Hazard ratios (HRs) were tested for significance using the likelihood ratio test.[35] For univariate models of gene expression and RFI, an unadjusted $P$ value less than .05 was considered significant. A test of interaction was used to identify genes that predict treatment benefit; because such tests have lower power compared with the main effect tests, an unadjusted $P$ value of less than .10 was considered significant. No adjustment for multiplicity was applied. To estimate the false discovery rate (FDR), the Benjamini-Hochberg method was used within each study,[36] and a permutation-based method[37] was used across studies.

For each of the 375 genes assessed in the four studies, univariate $t$ tests were performed to identify mean differences in gene expression between patients with stage II and stage III disease in each study. Additionally, Cox proportional hazards regression models of gene expression, stage, and the interaction of gene expression and stage, stratified by study, were examined, and a $P$ value of less than .10 for interaction was considered significant. In the absence of strong evidence of stage differences, data across stages were combined for gene discovery and algorithm development.

To identify clusters of coexpressed genes and to facilitate the understanding of important biologic pathways, unsupervised hierarchical clustering of genes was performed using Pearson $r$ as the distance measure for gene expression and the unweighted pair-group average as the amalgamation method.[35] Similar results were obtained using other methods, such as principal component analysis.

A smaller subset of genes significantly and consistently related to risk of recurrence was identified by examining the results across studies. Multiple factors were considered for gene inclusion in algorithm development, including, but not limited to, the known role of the genes in important biologic pathways, analytic performance, and range of expression. The final gene panels and algorithms for prediction of recurrence risk (ie, recurrence score; Table 1) and chemotherapy benefit (ie, treatment score; Table 2) were derived as described in the text of the Appendix (online only).

Bootstrap methods[38,39] were used to evaluate the extent to which recurrence risk differed among the recurrence risk groups defined by recurrence score for patients with stage II disease. A total of 1,000 bootstrap samples were drawn randomly with replacement from the pooled data set, taking variability between studies into account. Kaplan-Meier estimates of recurrence risk at 3

**Table 1.** Prediction of Recurrence Risk: Kaplan-Meier Estimates of Recurrence Risk at 3 Years and Associated 95% CIs from Bootstrap Analysis for Patients With Stage II Disease in Surgery-Alone Studies

| Recurrence Risk Group | Patients (median %) | Risk of Recurrence at 3 Years (%) | 95% CI |
|---|---|---|---|
| Low (RS < 30) | 25 | 8 | 5 to 12 |
| Intermediate (RS 31-40) | 39 | 11 | 7 to 15 |
| High (RS $\geq$ 41) | 37 | 25 | 18 to 32 |

Abbreviation: RS, recurrence score.

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**Table 2.** Prediction of Chemotherapy Benefit: Kaplan-Meier Estimates of Recurrence Risk at 3 Years by Treatment and FU/LV Benefit and Associated 95% CIs From Bootstrap Analysis for Patients With Stage II Disease

| | Recurrence at 3 Years | | | | Result at 3 Years | |
|---|---|---|---|---|---|---|
| | Surgery Alone | | Surgery + FU/LV | | | |
| Chemotherapy Benefit Group* | Risk (%) | 95% CI | Risk (%) | 95% CI | Benefit (%) | 95% CI |
| Low | 16 | 11 to 23 | 19 | 9 to 31 | −3 | −16 to 9 |
| Intermediate | 10 | 6 to 14 | 7 | 2 to 14 | 3 | −5 to 10 |
| High | 15 | 10 to 20 | 7 | 2 to 13 | 8 | 0 to 15 |

Abbreviations: FU, fluorouracil; LV, leucovorin.
*The chemotherapy benefit groups are defined using the recurrence score and treatment score calculated as described in Figures 3 and 4. The definition of the chemotherapy benefit groups is provided in Appendix Table A3 (online only).

years were obtained for each recurrence risk group. Median recurrence risk estimates across all bootstrap samples and percentile CIs were reported. A similar approach was used to assess the results of the final multigene algorithm to predict FU/LV benefit, in which patients were divided in chemotherapy benefit groups on the basis of both their recurrence scores and treatment scores (Appendix Table A3, online only). Data were analyzed independently by the NSABP Biostatistical Center, Cleveland Clinic, and Genomic Health, Inc for individual studies. Analyses across four studies were conducted by Genomic Health.

## RESULTS

The final numbers of evaluable patients were 270 in the C-01/C-02, 765 in the CC, 308 in the C-04, and 508 in the C-06 cohort. The outcomes and clinical/demographic characteristics of evaluable patients with tumor blocks were similar to those observed in the parent NSABP studies.

The baseline characteristics of the three NSABP cohorts were generally similar; patients from CC differed in age, percentage of right-sided tumors, number of lymph nodes examined, and percentage of stage II versus stage III disease (Appendix Table A1). Univariate Cox proportional hazards regression identified nodal status (0 positive nodes and $\geq$ 12 nodes examined, 0 positive nodes and $<$ 12 nodes examined, 1 to 3 or $\geq$ 4 positive nodes) as the most significant clinical/pathologic predictor of RFI ($P < .001$) in all studies (Appendix Tables A4, A5, A6, and A7, online only; Appendix Figs A2A, A2B, A2C, and A2D, online only). T stage, available in adequate numbers of patients in CC, was associated with RFI (T4 *v* other; $P = .003$; Appendix Table A5). MMR was not associated with RFI in CC ($P = .27$; Appendix Table A5).

Univariate analysis identified 143 genes as significantly related to RFI in the C-01/C-02 cohort, 119 in the CC cohort, 143 in the C-04 cohort, and 169 in the C-06 cohort; 27%, 16%, 27%, and 11% of these genes, respectively, were expected to be false discoveries. When studies were pooled, the FDR was markedly lower. In the multivariate analysis, 43%, 74%, 50%, and 84% of the genes identified in the univariate analysis retained significance in C-01/C-02, CC, C-04, and C-06, respectively, and had similar HRs in both analyses (Appendix Figs A3 and A4, online only, for surgery-alone studies; similar results for studies of surgery + FU/LV not shown), which suggests that gene expression contributes information about recurrence beyond standard clinical and pathologic covariates. In these multivariate analyses, the contribution of nodal status was consistently statistically significant.

The relationship between gene expression, tumor stage, and RFI was investigated across studies. In univariate analyses, six of the 375 genes had significant ($P < .05$) mean differences in expression between patients with stage II and stage III disease in all four studies. Thirty-three genes had a significant interaction of gene expression and stage ($P < .1$) in Cox proportional hazards models stratified by study; 32 of these 33 genes were potential false positives (FDR = 97%), which suggests that interaction between gene expression and stage was weak. The coexpression of genes examined using cluster analysis was virtually identical in patients with stage II and stage III disease. Agreement between univariate HRs for patients with stage II and stage III disease is illustrated in Figure 1 for genes significantly associated with RFI in both surgery-alone studies and at least one study of surgery plus FU/LV. HRs were generally similar with overlapping CIs, with a few exceptions that could be chance findings due to multiplicity of testing across multiple genes and studies. These results support pooling data across stages for gene discovery and algorithm development.

Recurrence risk genes were expected to have a similar relationship with RFI when measured in patients treated with surgery alone or surgery followed by FU/LV. A total of 48 (13%) of the 375 genes studied in all four development studies were significantly ($P < .05$) associated with RFI in both surgery alone studies and at least one study of surgery plus FU/LV. Fewer than one of these 48 genes is expected to be a false discovery. Cluster analysis identified two relatively distinct gene groups: a stromal gene group (containing several subgroups such as early response) and a cell cycle gene group (Fig 2). Higher expression of stromal genes (eg, *BGN, FAP, GADD45B*, and *PAI*) was associated with higher risk of recurrence, whereas higher expression of cell cycle genes (eg, *Ki-67, MYBL2*, and *MCM2*) was associated with lower risk of recurrence.

In contrast to recurrence risk genes, the genes predictive of differential FU/LV benefit are required to exhibit a different relationship with outcome (ie, different HRs) in patients treated with surgery alone compared with patients treated with surgery plus FU/LV. A total of 66 (18%) of 375 genes studied in all four development studies had interactions of gene expression and treatment that were significant at the less than .10 level if the data across the four studies were pooled (13 genes with $P < .01$; 45 genes with $P < .05$); four of these genes were also associated with risk of recurrence at the $P < .05$ level. Approximately 37 of these 66 genes are expected to be false discoveries; this was expected, given the lower statistical power associated with the analysis of interaction.


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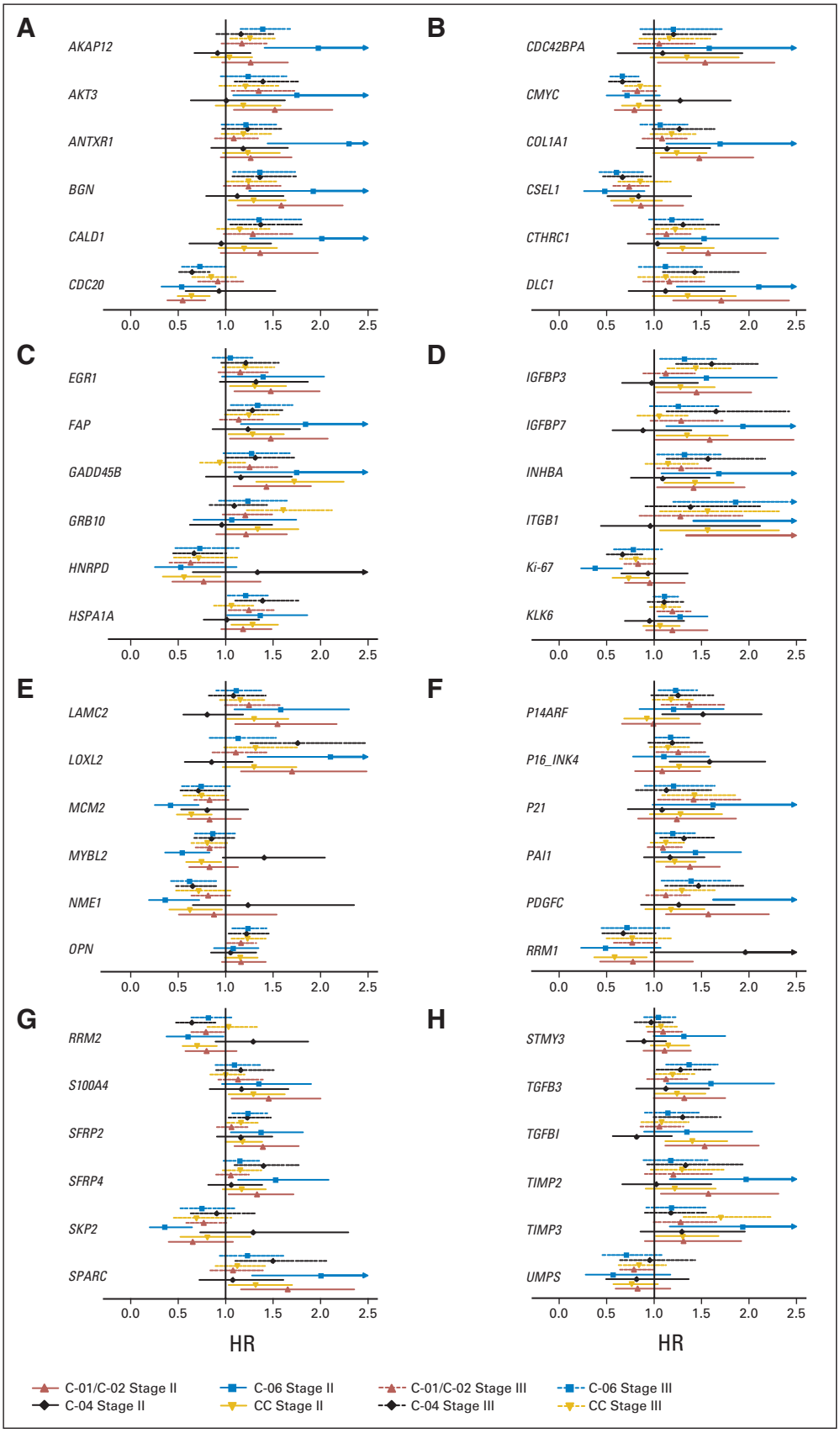


**Fig 1.** Hazard ratio estimates and 95% CIs for gene expression from univariate Cox PH regression models of recurrence-free interval in patients on studies C-01/C-02, CC, C-04, and C-06 by tumor stage for the 48 genes that were significantly related to recurrence-free interval (A) in both of the surgery-only studies as well as (B) in at least one study of surgery + fluorouracil and leucovorin.

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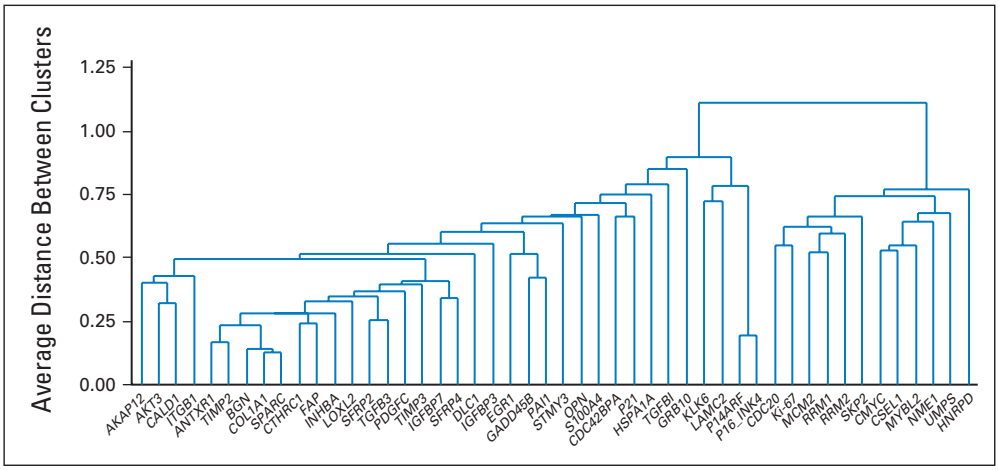


**Fig 2.** Unsupervised hierarchical clustering of the 48 genes significantly related to recurrence-free interval in both surgery-only studies and at least one study of surgery + fluorouracil and leucovorin using data from all four studies.

Among 66 potentially predictive genes, there were a large number of genes involved in multiple stages of the cell cycle and apoptosis (ie, *MAD2L1, AURKB, BIK*, *BUB1, CDC2*), and higher expression was associated with greater differential benefit from FU/LV (Appendix Fig A5, online only). There were also a prominent stress response/hypoxia signature (ie, *HSPE1, NR4A1, RhoB, HIF1A*); multifunctional transcription factors (*RUNX1, CREBBP, KLF5*); and genes associated with wnt signaling (*AXIN2* and *LEF*), MMR (*MSH2* and *MSH3*), and angiogenesis (*EFNB2*). Higher expression of some of these genes (eg, *RUNX1, CREBBP, KLF5*, and *EFNB2*) was associated with lower benefit from FU/LV.

Seven of the 48 recurrence risk genes and six of the 66 chemotherapy benefit genes were selected to create the final recurrence score and treatment score algorithms (Appendix Figs A1 and A5; described in the Appendix). The results of bootstrap analyses to assess the predictive ability of the recurrence score are listed in Table 1 for patients with stage II disease treated with surgery alone (C-01/C-02 and CC cohorts). Patients were divided into three recurrence risk groups on the basis of the calculated recurrence score (ie, $< 30$, 30-40, and $\geq 41$). The recurrence score separated the 632 patients with stage II disease into groups that had a sizable difference in estimates of risk of recurrence between the high- and the low-risk groups.

The results of bootstrap analyses to assess the predictive ability of the treatment score are listed in Table 2 for the 870 patients with stage II disease treated with surgery alone or surgery plus FU/LV. Patients were divided into three benefit groups (Appendix). For comparison, the overall 3-year risk of recurrence of patients with stage II disease was 14% for the surgery-alone group and was 10% for patients treated with surgery plus FU/LV. The correlation between the recurrence score and treatment score was relatively low ($r = -0.4$) in these studies, which suggests that the determinants of recurrence risk and differential FU/LV benefit may be distinct.

The performance of these algorithms was evaluated on the data set used for algorithm development; hence, the results in Tables 1 and 2 are likely to be optimistic. These algorithms will be validated on an independent data set of patients with stage II colon cancer from the QUASAR study.[11]

## DISCUSSION

Our strategy for discovering genes related to recurrence risk and differential benefit with adjuvant FU/LV chemotherapy has been to perform multiple, large, independent studies to identify those genes most consistently and strongly related to clinical outcome (Appendix Fig A1).[40,41] The results reported here are based on data from 1,851 patients, using standardized assay technology in a single laboratory. This approach is in contrast to algorithms developed from much larger numbers of genes and significantly smaller sample sizes.[17-21]

We have identified 48 genes that have significant and similar relationships with RFI and 66 genes that have different relationships with RFI in patients treated with surgery alone compared with patients treated with surgery plus FU/LV: the former genes are likely to predict recurrence, whereas the latter genes are likely to predict differential benefit with adjuvant FU/LV therapy. A large proportion of these genes remain significantly associated with RFI after analysis is controlled for the effects of numerous clinical/pathologic covariates, including nodal status, which also contributed significantly to prediction of recurrence risk.

This report highlights several challenges to biomarker development in colon cancer. First, our ability to identify genes predictive of differential treatment benefit was limited by the lack of large, randomized clinical trials with tumor specimens (beyond the QUASAR validation trial). Second, the lower power of the test of interaction leads to a high FDR among the candidate predictive genes, of greater than 50% in our studies. Finally, the question of whether stage II and III disease are biologically similar or dissimilar is unresolved. However, for the vast majority of genes, we saw no strong difference between stages in the relationship of gene expression and RFI, and an additional analysis that compared patients who had stage II disease and $\geq 12$ nodes examined with patients who had stage III disease confirmed these findings (data not shown).

Our strategy has led to the discovery of recurrence risk genes that can be confidently associated with clinical outcome and are generally different from the genes identified in our breast cancer studies (with the exceptions of *Ki-67* and *MYBL2*)[42] or the genes previously reported in colon cancer.[17-21]


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Higher expression of cell cycle genes was associated with an increased RFI; this observation is similar to that reported for *Ki-67* in colon cancer[43,44] but is opposite to the relationship observed in breast cancer studies.[42] The association of stromal gene expression with colon cancer recurrence provides an elegant molecular explanation for Dukes' original observation that invasion is the critical characteristic that should be used in staging colorectal cancer.[45,46]

Some of the genes that were identified as predictive of chemotherapy benefit are not unexpected. Sensitivity to FU should be affected by factors related to the level of proliferation (ie, cell-cycle related genes), the induction of apoptosis and hypoxia,[47] FU metabolism, and MMR.[48] However, it is not clear why the expression of other genes, such as *GJB2* or *HES6*, which are associated with gap junction communication and notch signaling respectively, would predict FU benefit. The quantitative expression of the genes related to FU activation/metabolism (*TS, DPD, TP*)[15,49-54] or to the markers (*hMLH1, hMSH2*) associated with MMR[55-57] were not associated with differential FU/LV benefit in our studies; current guidelines by the American Society of Clinical Oncology conclude that there is insufficient evidence to recommend the use of these markers as predictors of response to therapy.[23]

This report describes our process for identifying genes that can be used to estimate recurrence risk and differential FU/LV chemotherapy benefit on the basis of the relationship of quantitative gene expression at the time of diagnosis and clinical outcome in patients with stage II/III colon cancer treated with surgery or surgery plus FU/LV. The results of these four studies have been used to develop a multigene assay (Figs 3 and 4) for prediction of recurrence risk and differential benefit of adjuvant FU/LV chemotherapy that can be used to divide patients with stage II colon cancer into groups with different likelihoods of recurrence and treatment benefit. The requirement for external validation is being addressed in QUASAR,[11] a large, independent study of patients with stage II colon cancer randomly assigned to surgery alone or to surgery followed by adjuvant FU/LV chemotherapy.

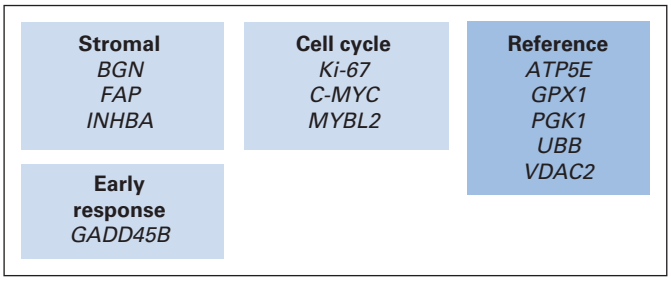


**Fig 3.** Recurrence score gene panel and algorithm. The recurrence score that is based on 12 genes (seven cancer-related genes and five reference genes) is derived from the reference-normalized expression measurements in four steps and is scaled from 0 to 100. First, expression of each gene is normalized relative to the expression of the five reference genes (ie, *ATP5E, GPX1, PGK1, UBB*, and *VDAC2*). Reference-normalized expression measurements range from two to 15, with a 1-unit increase reflecting approximately twice as much input RNA. Expression of individual genes is given a threshold as follows: if *FAP, Ki-67* or *MYBL2* measurement is less than 6.5 $C_T$, it is considered to be 6.5; if GADD45B measurement is less than 5 $C_T$, then it is considered to be 5. Genes are grouped on the basis of function and/or correlated expression. Second, the stromal and cell cycle group scores are calculated as averages of the reference-normalized, individual gene expression measurements as follows: stromal group score = (*BGN* + *FAP* + *INHBA*) ÷ 3; cell cycle group score = (*Ki-67* + *C-MYC* + *MYBL2*) ÷ 3. Third, the unscaled recurrence score ($RS_u$) is calculated with the use of coefficients that are defined on the basis of regression analysis of gene expression and recurrence in four development studies: $RS_u = +0.15 \times$ stromal group score $- 0.30 \times$ cell cycle group score $+ 0.15 \times$ *GADD45B*. A plus sign indicates that increased expression is associated with increased risk of recurrence, and a minus sign indicates that increased expression is associated with decreased risk of recurrence. Fourth, the recurrence score (RS) is rescaled as follows: $RS = 44 \times (RS_u + 0.82)$; if RS is less than 0, then RS = 0, and if RS is greater than 100, then RS = 100.

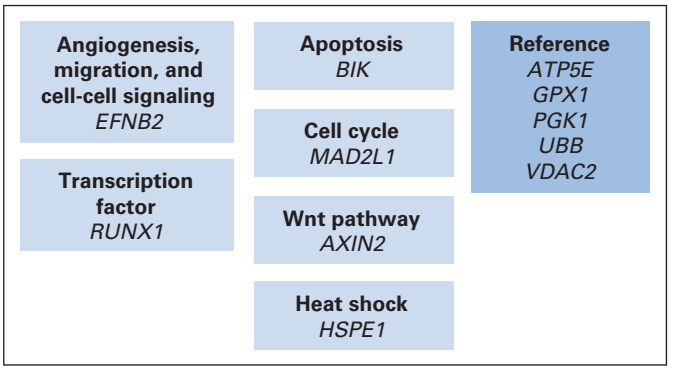


**Fig 4.** Treatment score gene panel and algorithm. The treatment score that is based on 11 genes (six cancer-related genes and the same five reference genes; Fig 3) is derived from the reference-normalized expression measurements in three steps and is scaled from 0 to 100. First, expression of each gene is normalized relative to the expression of the five reference genes (*ATP5E, GPX1, PGK1, UBB*, and *VDAC2*). Expression of individual genes is given a threshold as follows: if *MAD2L1* or *RUNX1* measurement is less than 5.5 $C_T$, it is considered to be 5.5; if *BIK* measurement is less than 6 $C_T$, then it is considered to be 6; and if *EFNB2* measurement is less than 5 $C_T$, then it is considered to be 5. Second, the unscaled treatment score ($TS_u$) is calculated with the use of coefficients that are defined on the basis of regression analysis of gene expression and chemotherapy benefit in four development studies: $TS_u = -0.3 \times$ *EFNB2* $- 0.04 \times$ *RUNX1* $+ 0.1 \times$ *MAD2L1* $+ 0.3 \times$ *BIK* $+ 0.1 \times$ *AXIN2* $+ 0.1 \times$ *HSPE1*. A plus sign indicates that increased expression is associated with increased chemotherapy benefit, and a minus sign indicates that increased expression is associated with decreased chemotherapy benefit. Third, the treatment score (TS) is rescaled as follows: $TS = 37 \times (TS_u - 1)$; if TS is less than 0, then TS = 0, and if TS is greater than 100, then TS = 100.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

*Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.*

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