Patient-specific meta-analysis of 12-gene colon cancer recurrence score validation studies for recurrence risk assessment after surgery with or without 5FU and oxaliplatin

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Background: Individualized estimates of the risk of recurrence in colon cancer patients are needed that reflect current medical practice and available treatment options.

Methods: Three validation studies of the 12-gene colon recurrence score assay were used with pre-specified patient-specific meta-analysis (PSMA) methods to integrate the 12-gene Oncotype DX Colon Recurrence Score result (RS) with the clinical and pathology risk factors stage, T-stage, mis-match repair (MMR) status, and number of nodes examined to calculate individualized recurrence risk estimates. Baseline risk estimation used the most recent studies, so the risk estimates reflect current medical practice. The effect of fluorouracil (5FU) was estimated with a meta-analysis of two studies. The effect of oxaliplatin was estimated using one of the RS assay validation studies, in which patients were randomized to 5FU with or without oxaliplatin.

Results: The RS result and each of the clinical-pathologic factors provided independent prognostic information for recurrence. Among stage II, T3, MMR-proficient patients with ≥12 nodes examined (the most common scenario), patients with RS ≤30 (approximately 48%) have estimated 5-year recurrence risk ≤10% with surgery alone. Among stage IIIA/B, T3, MMR-deficient patients with ≥12 nodes examined, patients with RS ≤19 (approximately 14%) have an estimated 5-year recurrence risk ≤10% with surgery alone. Among stage IIIA/B, T3, MMR-proficient patients with ≥12 nodes examined, those with RS ≤14 (approximately 6%) have estimated 5-year recurrence risk ≤10% with 5FU alone.

Discussion: The PSMA integrates the 12-gene colon RS result with clinical and pathology factors to provide individualized recurrence risk estimates that reflect current medical practice. The risk estimates are in a range that may help inform treatment decisions for a substantial number of stage II and stage III patients.

Keywords: Colon cancer; recurrence risk; 12-gene assay; meta-analysis

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Introduction

Colorectal cancer is the third most common cancer in the United States, with approximately 145,000 new cases diagnosed annually. Many of these patients present without evidence of metastatic disease where surgical resection of the primary is potentially curative. Most decisions regarding the indications for and specific adjuvant chemotherapy regimens in both stage II and stage III colon cancer are based on recurrence risk assessment using conventional clinical and pathologic risk factors. The 12-gene Oncotype DX Colon Recurrence Score® result (RS) test (Genomic Health, Inc., Redwood City, CA, USA, a wholly owned subsidiary of Exact Sciences) is a validated multigene assay that assesses recurrence risk in stage II and stage III colon cancer patients independent of conventional clinical and pathologic risk factors.

Since the late 1990's the overall rate of recurrence of colon cancer has declined (1). This reflects improvements in surgery, more accurate clinical and pathologic staging and the impact of chemotherapy in selected patients. However, there is no doubt that treatment decisions based on conventional clinical and pathologic lead to the unnecessary use of chemotherapy in some patients and its ineffective use in others. Prognostic tools are still needed to provide more precise, individualized estimates for the risk of recurrence of an individual patient. Theoretically, integrating information from the RS result and clinical-pathologic risk factors such as T-stage, number of lymph nodes examined, mis-match repair (MMR) status, and stage would add more precision, and it would be useful to provide recurrence risk estimates for specific treatment scenarios.

The clinical utility of the RS result is currently limited by reporting recurrence estimates from individual studies only. For stage II colon cancer patients, the report provides 3-year recurrence risk in patients managed by surgery alone using the QUASAR RS validation study (2). To estimate the effects of adjuvant chemotherapy, physicians must extrapolate by assuming a relative benefit from adjuvant fluorouracil (5FU)-based chemotherapy, approximately 20% based on the parent QUASAR study. To help physicians understand the effects of adding (or withholding) oxaliplatin in both stage II and III colon cancer patients, the Oncotype

DX® patient report provides 5-year risk of recurrence estimates in patients from the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07 validation study, in which patients were randomized to treatment with 5FU monotherapy or 5FU + oxaliplatin.

To improve the accuracy, precision and utility of test results, we calculated recurrence risk using patient-specific meta-analysis (PSMA) for stage II and stage III patients, combining recurrence risk estimates from three clinical validation studies for the RS assay. The meta-analysis integrated the RS result with other prognostic conventional clinical and pathologic risk factors, yielding refined recurrence risk estimates for treatment scenarios of surgery alone, surgery with adjuvant 5FU chemotherapy, and surgery with 5FU and oxaliplatin, that more accurately reflect expected outcomes from patients seen today.

We present the following article in accordance with the PRISMA reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-21-620/rc).

Methods

Three RS validation studies were available for the meta-analysis: CALGB 9581, NSABP C-07 and the SUNRISE study. As described in the original reports (3-5), the parent studies were approved by institutional review boards and all study participants provided informed consent. In each of three RS validation studies, tumor grade was centrally assessed according to the College of American Pathologists Consensus Statement.

The parent CALGB 9581 study (3) randomly assigned 1,713 patients with stage II colon cancer to treatment with edrecolomab or observation and found no survival difference. Venook *et al.* (6) reported a prospective-retrospective study of the association of the RS result with recurrence using a stratified cohort sample consisting of all 162 patients, pooling patients randomized to observation and edrecolomab, with available tissue and recurrence and a random selection of nonrecurring patients stratified on treatment for a total of 690 patients. CALGB 9581 enrolled from 1997 through 2002.

The NSABP C-07 study (4) (enrollment 2000–2002) randomly assigned patients with stage II and III colon

cancer to 5FU or 5FU plus oxaliplatin. Yothers *et al.* (7) reported a prospective-retrospective study of the association of the RS result with recurrence in 892 randomly selected patients from this study (half the patients with available tissue), 245 of whom experienced recurrence.

The SUNRISE Study in Japan reported by Yamanaka *et al.* (5) included a stratified cohort sample from 1,487 consecutive patients from 2000 through 2005 with stage II or III colon cancer who had surgery alone. The sample included 630 patients (1:2 ratio of recurrence to nonrecurrence). Sampling was stratified by stage (II *vs.* III), 597 of whom were evaluable for analysis with 202 experiencing recurrence.

Statistical analysis

The statistical methods were pre-specified and approved in advance by the investigators. Cox proportional hazards models were fit separately for each study with effects for RS result, number of nodes examined (<12 or ≥12), T-stage (T4 or T1–3), MMR status (proficient/unknown or deficient) and, where applicable, stage (II, IIIA/B or IIIC) and treatment (oxaliplatin + 5FU or 5FU). The endpoint was recurrence-free interval, defined as time to recurrence or death due to colon cancer. Patients who died without recurrence were censored at last follow-up. Since CALGB 9581 and the SUNRISE study used stratified cohort sampling (8), they were analyzed using the inverse stratum-specific sampling ratio as a weight with the robust regression parameter variance estimator of Lin and Wei (9). The significance of each factor in the multivariate models adjusting for the other factors was tested using a meta-analysis Wald test since likelihood ratio tests are not valid for cohort-sampling studies. The risk of recurrence at 1, 3 and 5 years after surgery was estimated using the "special population" PSMA method (10) to adjust for the effect of stage and oxaliplatin treatment. The log hazard ratio (HR) for the effect of 5FU was estimated separately for stage II and III using a fixed effects meta-analysis of log HR's extracted from reports of the QUASAR study (11) and the pooled analysis of NSABP trials reported by Wilkinson and colleagues (12) and integrated into the PSMA risk estimation. Since the overall recurrence risk has decreased over time, the events from the latest-enrolling two studies were used to estimate the baseline cumulative hazard. Potential interaction among the model factors was assessed using meta-analysis Wald tests weighting individual study interaction effect estimates by their inverse variance. Details

of the statistical methods are available online: https://cdn.amegroups.cn/static/public/jgo-21-620-1.pdf, including documentation of the originally planned methods. The only change from the planned methods was to estimate the effect of 5FU separately by stage (II *vs.* III) rather than overall. Analyses were conducted using SAS® Version 9.2 and SAS/Stat Version 14.1 (SAS Institute, Cary, NC, USA).

Results

Patient and tumor characteristics in the enrolled study populations are shown in *Table 1* and the distribution of the RS result integrated across the three studies is shown in Figure S1. Overall, 17% of patients had RS ≤20, 31% had RS ≤25, 48% had RS ≤30, and 22% had RS >40; the median RS result was 30 [interquartile range (IQR), 23–39] with mean 32 and standard deviation 12. There were relatively few T4 tumors in CALGB 9581 and C-07. The Cox model covariates were largely uncorrelated, with a maximum absolute correlation of only 0.13 (Table S1).

The Cox proportional hazards regression model fit to each of the three studies is shown in *Table 2*. The direction of association of each of the covariates with recurrence risk is consistent across studies. The HR's vary across studies, but meta-analysis Wald tests indicated that each covariate provides significant prognostic information independent of all the others. Meta-analysis Wald tests indicated no significant interaction among the common covariates RS result, number of nodes examined (<12 or ≥12), T-stage and MMR deficiency or between each of the common covariates and stage or treatment with oxaliplatin (Figures S2-S4). Therefore, the main effects model was judged appropriate. Exploratory meta-analyses examining tumor grade and right-sided tumor location showed no significant prognostic value for recurrence of either of these in either univariable or multivariable analysis together with the pre-planned covariates stage, RS result, number of nodes examined, T-stage and MMR deficiency (Tables S2,S3, Figure S5).

The HR's for 5FU for recurrence-free interval, based on a meta-analysis of two large studies (11,12), are shown in *Figure 1*. The estimated effect of 5FU in reducing the recurrence rate is larger in stage III [HR =0.63; 95% confidence interval (CI): 0.54–0.73] than in stage II (HR =0.77; 95% CI: 0.66–0.91). This difference was observed consistently across the two studies with a meta-analysis stage-by-treatment interaction test P value of 0.075.

A meta-analysis estimate of the absolute standardized

Table 1 Patient and tumor characteristics

Characteristic	CALGB 9581*# (n=690)	SUNRISE* (n=597)	NSABP C-07 (n=892)
Enrollment period	1997–2002	2000–2005	2000–2002
Number of patients (events)			
Surgery alone	690 [162]	597 [202]	–
Surgery + 5FU	–	–	449 [135]
Surgery + 5FU + oxaliplatin	–	–	443 [110]
Female gender	330 (48%)	278 (47%)	379 (42%)
Age			
Median (IQR)	66 (57–73)	66 (57–72)	60 (51–67)
Min–max	30–90	23–80	22–85
<12 nodes examined	327 (47%)	128 (20%)	344 (39%)
T4	41 (6%)	127 (20%)	57 (6%)
MMR deficient	137 (20%)	30 (5%)	84 (9%)
MMR proficient	519 (75%)	567 (95%)	626 (70%)
MMR status unknown	34 (5%)	0 (0%)	182 (20%)
RS result			
Median (IQR)	31 (25–39)	28 (20–37)	33 (25–41)
Min–max	2–78	0–68	4–80
Stage II	690 (100%)	247 (69%)	264 (30%)
Stage IIIA/B	–	278 (25%)	409 (46%)
Stage IIIC	–	72 (6%)	219 (25%)
Follow-up time (years)			
Median (IQR)	8.0 (5.5–9.5)	5.2 (4.9–7.5)	8.6 (8.0–9.6)
Tumor location			
Cecum	174 (26%)	48 (8%)	169 (19%)
Ascending colon	133 (20%)	109 (20%)	160 (18%)
Transverse colon+	159 (23%)	68 (10%)	202 (23%)
Descending colon	42 (6%)	36 (6%)	50 (6%)
Sigmoid colon/rectosigmoid	182 (26%)	336 (55%)	311 (35%)
Right-sided tumor location^	423 (62%)	225 (38%)	489 (55%)

*, percentage and summary statistics calculated using stratified cohort sampling weights; #, includes patients randomized to edrecolomab in CALGB 9581. Edrecolomab was ineffective; +, includes hepatic flexure, splenic colon and splenic flexure; ^, defined as the cecum, ascending colon, hepatic flexure and transverse colon. NSABP, National Surgical Adjuvant Breast and Bowel Project; 5FU, fluorouracil; IQR, interquartile range; MMR, mis-match repair; RS, 12-gene Oncotype DX Colon Recurrence Score result.

HR (13) for the joint contributions of all the covariates, including stage, to separation of risk in the population, controlling for treatment, is 2.19 (95% CI: 1.69–2.84), meta-analysis Wald test P<0.001. This meta-analysis estimate weighted the partial absolute standardized log HR from each study by its effective sample size, accounting

Table 2 Cox proportional hazards regression model HR with 95% CI and Wald test P values

Effect	CALGB 9581 (n=690)[a], 162 events	SUNRISE (n=597)[a], 202 events	NSABP C-07 (n=892), 245 events	Meta-analysis Wald test
RS, per 25 units	1.57 (1.14, 2.18); P=0.006	1.95 (1.35, 2.82); P<0.001	1.80 (1.39, 2.34); P<0.001	1.76 (1.47, 2.11); P<0.001
<12 vs. ≥12 nodes examined	1.12 (0.80, 1.57); P=0.51	1.84 (1.29, 2.64); P=0.001	1.47 (1.14, 1.91); P=0.003	1.44 (1.21, 1.72); P<0.001
T4 vs. T1-3	1.04 (0.51, 2.13); P=0.91	1.19 (0.81, 1.75); P=0.38	2.71 (1.81, 4.06); P<0.001	1.64 (1.27, 2.13); P<0.001
MMR deficient vs. proficient/ unknown	0.62 (0.39, 1.00); P=0.051	0.70 (0.30, 1.60); P=0.39	0.29 (0.13, 0.65); P=0.003	0.54 (0.38, 0.79); P=0.001
Stage IIIA/B vs. II	–	2.29 (1.62, 3.23); P<0.001	2.32 (1.55, 3.46); P<0.001	2.30 (1.77, 2.99); P<0.001
Stage IIIC vs. II	–	5.30 (3.41, 8.24); P<0.001	5.53 (3.69, 8.29); P<0.001	5.42 (4.02, 7.31); P<0.001
Oxali + 5FU vs. 5FU	–	–	0.80 (0.62, 1.03); P=0.089	–

[a], study used stratified cohort sampling. HR, hazard ratios; CI, confidence intervals; NSABP, National Surgical Adjuvant Breast and Bowel Project; RS, 12-gene Oncotype DX Colon Recurrence Score result; MMR, mis-match repair; Oxali, oxaliplatin; 5FU, fluorouracil.
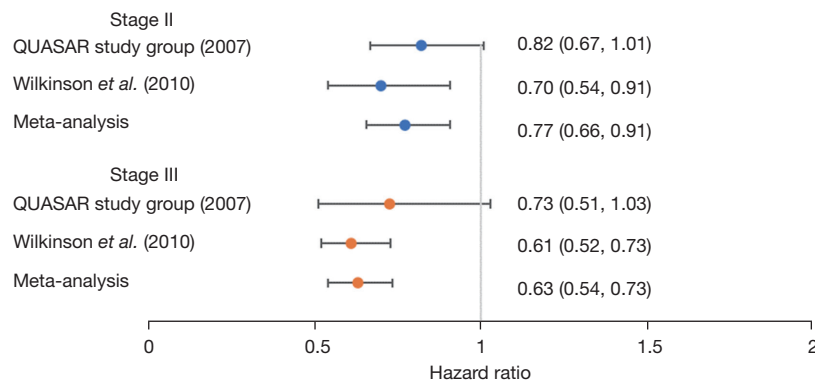

Figure 1 Meta-analysis estimate of 5FU treatment effect HR by stage. Meta-analysis test for interaction of stage with treatment P=0.075. 5FU, fluorouracil; HR, hazard ratio.

for stratified cohort sampling. A similar meta-analysis estimate of the partial absolute standardized HR (14) for the combination of the four covariates RS result, number of nodes examined (<12 vs. ≥12), MMR-deficiency and T-stage, controlling for stage (II, IIIA/B or IIIC) and treatment, is 1.55 (95% CI: 1.29–1.86), meta-analysis Wald test P<0.001. As shown in *Figure 2*, RS result contributed an estimated 48% [standard error (SE) 15%] of the risk score variance, MMR deficiency 30% (SE 14%), number of nodes examined 17% (SE 9%) and T-stage 5% (SE 3%). Percentage contribution is a measure of variable's prognostic importance for recurrence in the studied population.

In view of an analysis of pooled data from MMR-deficient patients in several randomized trials of 5FU by Sargent and colleagues (15), which indicated that 5FU alone is not effective, particularly in stage II patients, we decided

not to provide recurrence risk estimates with 5FU treatment for MMR-deficient patients. Cohen and colleagues (16) also concluded that fluoropyrimidine (FP) is ineffective compared to surgery alone in MMR-deficient patients.

Example estimates and 95% CI for the 5-year recurrence risk for scenarios based on number of nodes examined and MMR status are shown in *Figure 3* for stage II and IIIA/B, T3 patients. The complete set of 5-, 3- and 1-year recurrence risk estimates are available online: https://cdn.amegroups.cn/static/public/jgo-21-620-1.pdf. The various curves show the impact of each of the factors on the recurrence risk. As indicated by the lack of correlation among RS and the other covariates in the analysis, the distribution of RS is essentially unchanged across the different covariate levels. For stage II, T3, MMR-proficient patients with ≥12 nodes examined (the most common
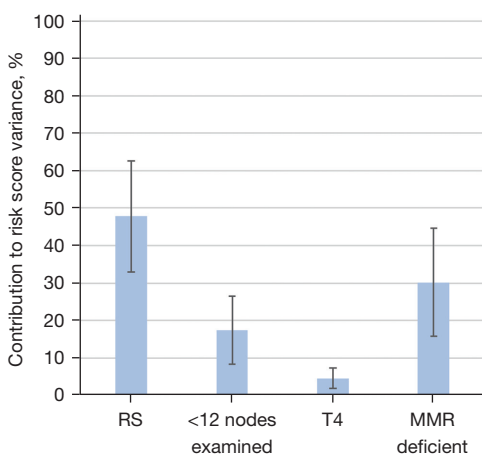
Figure 2 Meta-analysis estimate (with SE) of proportional contributions of each of the common covariates to the risk score variance, controlling for stage and treatment effects. RS, 12-gene Oncotype DX Colon Recurrence Score result; MMR, mis-match repair; SE, standard error.

scenario), patients with RS ≤30 (approximately 48% of patients) have an estimated 5-year recurrence risk ≤10% with surgery alone. Among stage IIIA/B, T3, MMR-deficient patients with ≥12 nodes examined, patients with RS ≤19 (approximately 14%) have an estimated 5-year recurrence risk ≤10% with surgery alone. Among stage IIIA/B, T3, MMR-proficient patients with ≥12 nodes examined, those with RS ≤14 (approximately 6%) will have estimated 5-year recurrence risk ≤10% with 5FU alone.

Comparisons of recurrence risk estimates from the PSMA with corresponding risk estimates from the existing Oncotype DX Colon Recurrence Score patient report are shown in *Figure 4*. The existing report provides 3-year risk estimates for stage II, MMR-proficient patients receiving surgery alone that depend on T-stage and RS result. For stage III patients, the existing report provides 5-year recurrence risk estimates for 5FU alone and 5FU with oxaliplatin, pooling across T-stages. The recurrence risk estimates provided by the PSMA are generally lower, although some for stage III patients are higher since the PSMA accounts for T-stage and number of nodes examined.

Discussion

In this analysis, the RS result and conventional clinical and pathologic risk factors were integrated using PSMA. These

known risk factors for recurrence have been reported previously (5-7,17). The meta-analysis showed that the RS result and the conventional clinical and pathologic risk factors T-stage, number of nodes examined, AJCC stage, and MMR status are largely independent of each other and furthermore are independently associated with the risk of recurrence. Treatment effects were estimated using a meta-analysis of two studies of 5FU and one of the three RS validation studies in which patients were randomized to 5FU alone or 5FU plus oxaliplatin. The integrated RS result and conventional risk factors provided refined recurrence risk estimates for untreated and 5FU- and oxaliplatin-treated stage II and stage III patients. These results more accurately reflect expected outcomes from patients seen today.

Cohen and colleagues (16) performed a pooled analysis of randomized studies of FP with oxaliplatin *vs.* FP alone in stage III colon cancer, which found an HR for disease-free survival of 0.47 (95% CI: 0.27–0.82) in microsatellite instability (MSI)/MMR-deficient patients and 0.82 (95% CI: 0.70–0.97) in microsatellite stable (MSS)/MMR-proficient patients. The HR of 0.47 in MMR-deficient patients is similar to the combined effects in stage III patients of 5FU and oxaliplatin ($HR_{5FU} \times HR_{oxali} = 0.63 \times 0.80 = 0.50$) from the present analysis, suggesting that the addition of oxaliplatin may allow MMR-deficient patients to also benefit from 5FU. The HR 0.82 in MMR-proficient patients is similar to the effect of oxaliplatin with 5FU *vs.* 5FU alone (0.80) in the present analysis, which would be expected if 5FU alone were effective in these patients (isolating the effect of oxaliplatin). These corroborating results suggest that the risk estimates for 5FU + oxaliplatin treatment in patients with stage III MMR-deficient tumors provided by the present analysis are appropriate.

The RS result is calculated from an analytically validated and standardized quantitative reverse transcriptase polymerase chain reaction assay that measures the expression of 12 genes (seven recurrence genes and five reference genes) in formalin-fixed paraffin-embedded (FFPE) primary colon tumor tissue (18). The applicability of the RS result is reinforced by the identity of the recurrence genes and the measured changes in tumor biology produced by their dysregulation. Six of the seven genes are in two key biologic pathways: cell cycle control (*MKI67*, *MYC*, *MYBL2*) and stromal response (*FAP*, *BGN*, *INHBA*). The seventh recurrence gene (*GADD45B*) is a marker of genotoxic stress and may regulate activity of stromal response genes, including *BGN* (18).

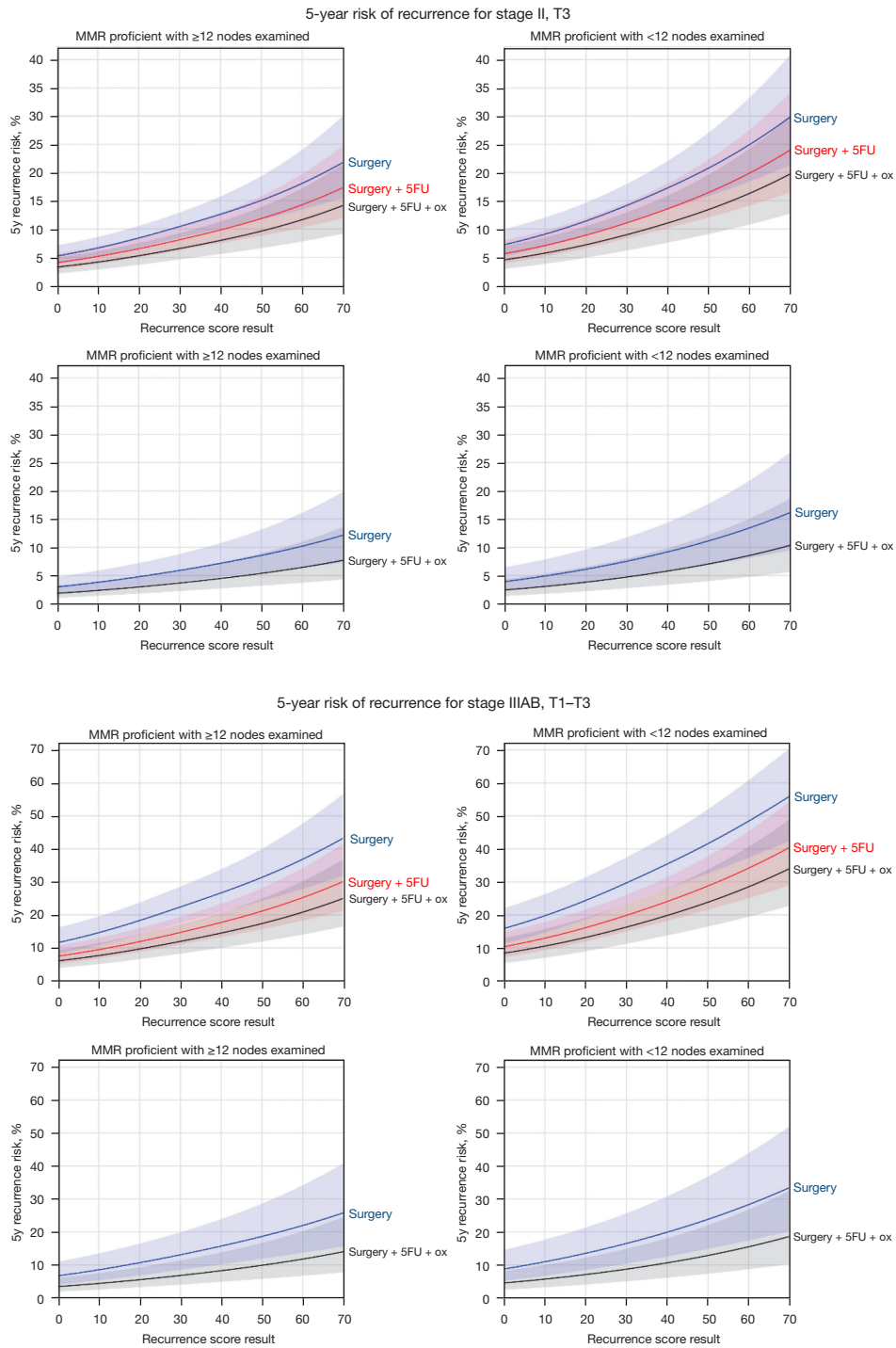Since the RS result contributes prognostic information

Figure 3 Example PSMA 5-year recurrence risk estimates and 95% CI. 5FU, fluorouracil; MMR, mis-match repair; PSMA, patient-specific meta-analysis; CI, confidence intervals.
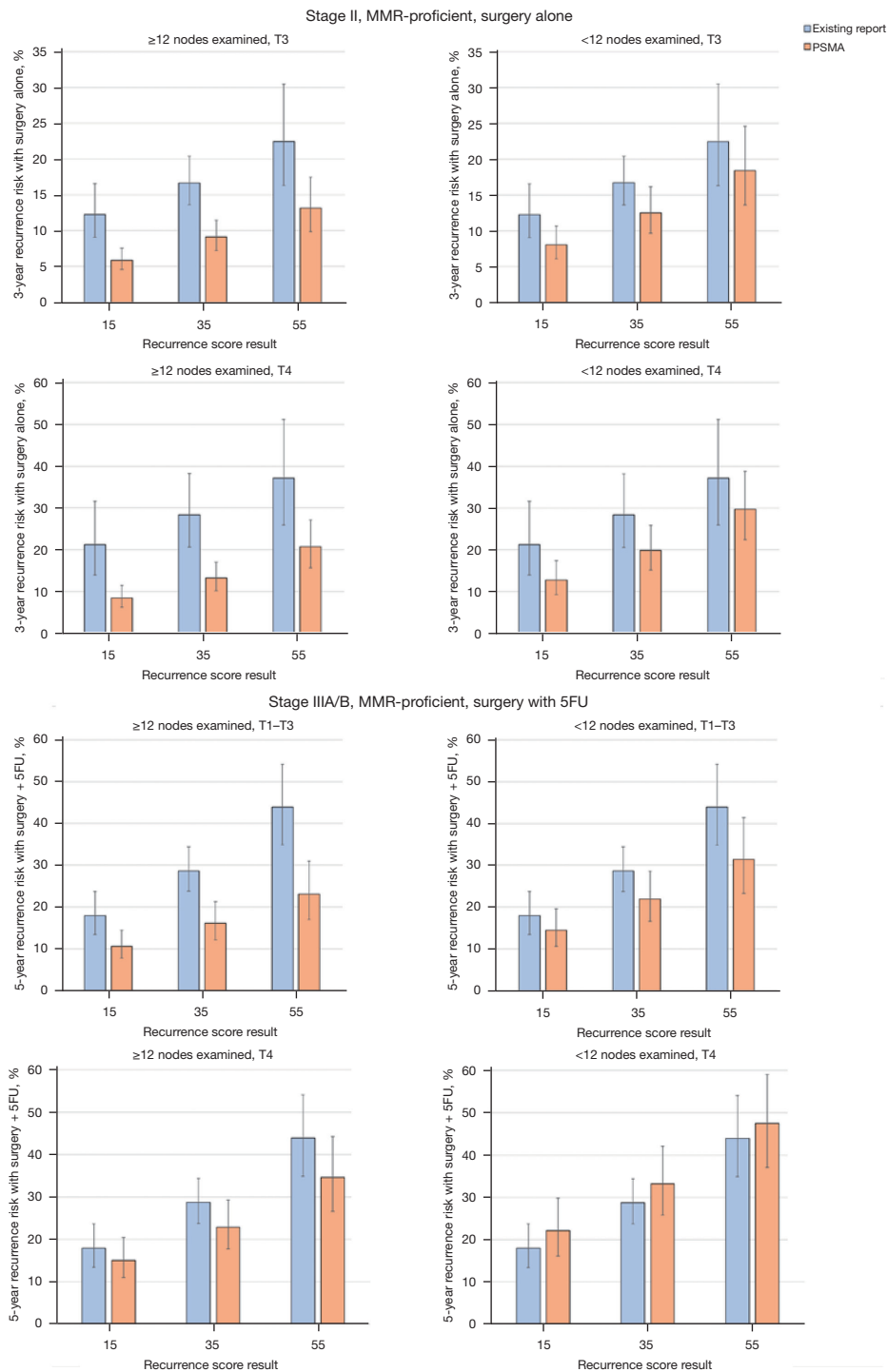
Figure 4 Comparison of recurrence risk estimates (95% CI) from the PSMA and the existing Oncotype DX Colon Recurrence Score patient report (Genomic Health, Inc., a wholly owned subsidiary of Exact Sciences Corporation). MMR, mis-match repair; PSMA, patient-specific meta-analysis; 5FU, fluorouracil; CI, confidence intervals.

independent of the conventional clinical and pathologic tumor features, the combination provides more prognostic information than the conventional clinical and pathologic features alone. RS result contributes about half of the total risk score variance, with the rest coming from the combined contributions of the conventional factors. The proportional contribution of each variable is influenced by its strength of association with the risk of recurrence and its distribution in the population. For example, the contribution of T-stage is small because relatively few tumors are T4 and since its association with recurrence is modest. Relatively few tumors are MMR-deficient but the association of MMR-deficiency with a reduction in recurrence risk is strong, so MMR contributes substantially more than T-stage to the risk score variance.

Strengths of this meta-analysis include a large data set, which allows more individualized risk estimates while maintaining reasonable precision (CI width), and the availability of risk estimates for various treatment scenarios. Limitations include that the estimated effect of 5FU is from a meta-analysis of a randomized study (11) and a non-randomized treatment comparison with covariate adjustment to reduce bias (12). However, the consistency of the results between these two studies suggests that the non-randomized comparison is providing reliable results. The SUNRISE study was a retrospective analysis that selected patients who had not received adjuvant chemotherapy after resection for stage II or III colon cancer and this may have led to selection of patients whom clinicians had considered to be at lower risk of recurrence, although patients in this study were treated during a time period when adjuvant chemotherapy for stage III disease was not used in Japan. Also, the PSMA risk assessment used a baseline risk assessment from the last two enrolling studies (NSABP C-07, enrolling from 2000–2002 and SUNRISE, enrolling from 2000–2005). If further improvements in patient outcomes have occurred since this time, they are not reflected in the present recurrence risk estimates. Finally, the RS result is not predictive, that is, it is not associated with the relative treatment effect of chemotherapy with 5FU or oxaliplatin. Therefore, the differences in absolute risk reduction due to chemotherapy with 5FU potentially with oxaliplatin reflect the recurrence risk together with a relative chemotherapy effect that is constant over the range of RS results. Nevertheless, having the risk estimates side-by-side for each treatment scenario should help facilitate discussions between physician and patient of the risks and benefits of each treatment option. The PSMA assesses

these risk and benefits in a patient-specific way, helping to personalize the decision-making process.

Part of the difference in recurrence rates between the existing patient report for stage II patients, which is based on the QUASAR RS validation study (2), and the current PSMA is due to a larger number of patients with <12 nodes examined (63%) in the QUASAR study. However, additional factors appear to be at play since risk estimates adjusted for number of nodes examined, T-stage, MMR-status and RS result still show decreases over time. Improved surgical techniques, better imaging, and other changes in the standard of medical care may have contributed to the decrease in risk since the 1990s. Regardless of the cause, it is important that recurrence risk estimates reflect the current standard of care.

The PSMA recurrence risks should be useful for treatment decisions for many patients. Five-year recurrence risk estimates with surgery alone for patients with stage II, T3, MMR-proficient tumors and ≥12 nodes examined are ≤10% for RS ≤30 (about 48% of patients). Similarly, for patients with stage IIIA/B, T3, MMR-deficient tumors with ≥12 nodes examined, patients with RS ≤19 (approximately 14%) have 5-year recurrence risk ≤10% with surgery alone. The low recurrence risk for these groups of patients could assist in considerations of whether to omit chemotherapy. Among stage IIIA/B, T3, MMR-proficient patients with ≥12 nodes examined, those with RS ≤14 (approximately 6%) have estimated 5-year recurrence risk ≤10% with 5FU alone, which could inform the considerations of omitting oxaliplatin. The risk estimates for patients with stage II MMR-deficient tumors are uniformly low, regardless of RS result and the clinical-pathology factors, so the RS result is not considered clinically useful for these patients. The one-year recurrence risk estimates may be useful in identifying patients at exceptionally high risk for an early recurrence who might benefit from additional adjuvant intervention.

In summary, the PSMA integrates the RS result with clinical and pathology factors to provide individualized recurrence risk estimates that reflect current medical practice. The risk estimates are in a range that may inform treatment decisions for a substantial number of stage II and stage III patients. Since even the most recent studies used in this meta-analysis enrolled about 15–20 years ago, it would be useful to validate the risk estimates in a current, independent cohort of patients to ensure that the observed association of recurrence risk with the gene expression and other the factors is still accurate. Future work could consider other gene sets identified using next generation

sequencing (NGS) and should evaluate the combination of genomic, clinical and pathology factors with ongoing assessments of circulating tumor DNA to further enhance recurrence rick assessment.

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This analysis was presented as a poster at the 2021 ASCO Annual Meeting.

Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-21-620/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-21-620/coif). MRC, FLB and CC are or were employees of and received remuneration and stock from Genomic Health, Inc., a wholly owned subsidiary of Exact Sciences Corporation, which provides test results based on the 12-gene assay. EO received lecture fees from Bayer Japan, Chugai Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., Eli Lilly, Ono Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd. NW reports support for the present manuscript via NCI grant #U10CA180868, directly to his institution only. TY reports grants from Taiho Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Ono Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Amgen K.K., Parexel International Inc., MSD K.K., Daiichi Sankyo Co., Ltd., and Sanofi K.K., outside the submitted work. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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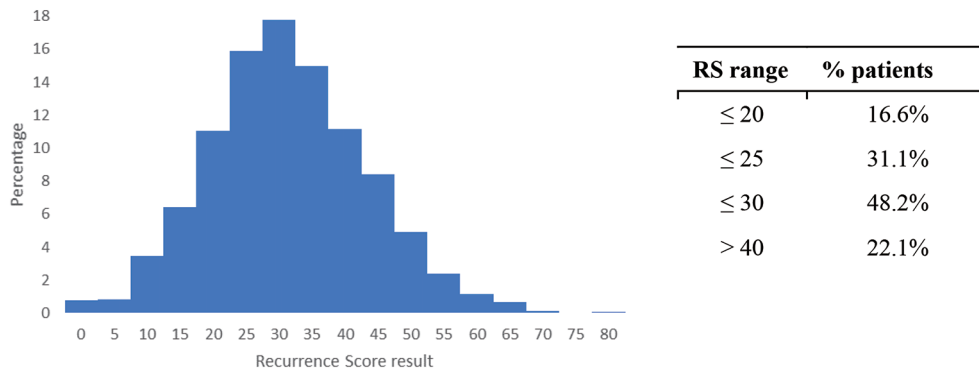
Figure S1 Distribution of recurrence score result in meta-analysis population. RS, 12-gene Oncotype DX Colon Recurrence Score result.

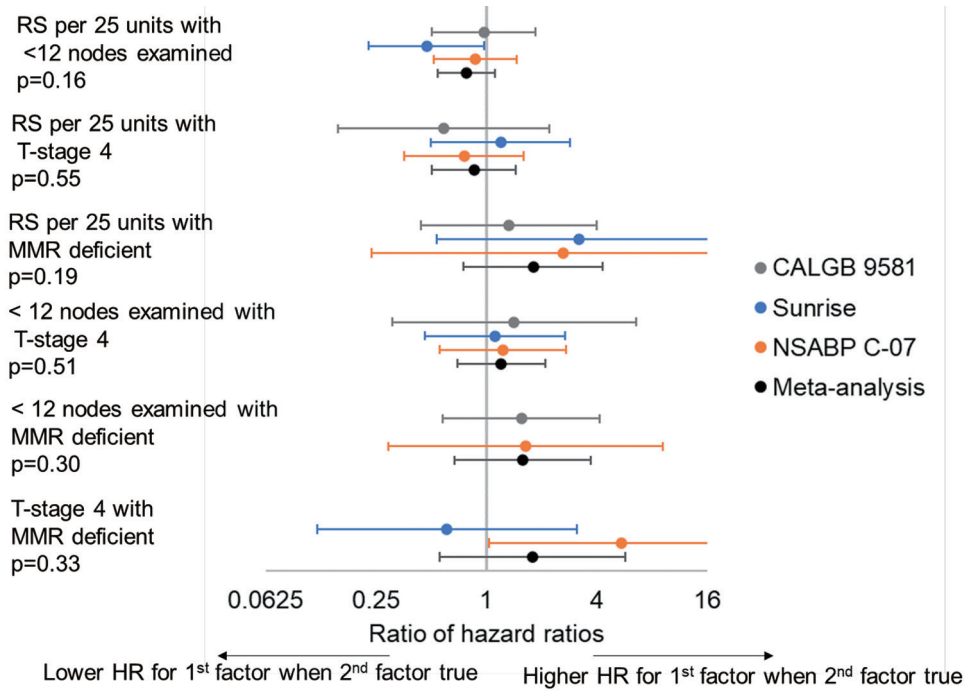| RS range	% patients
≤ 20	16.6%
≤ 25	31.1%
≤ 30	48.2%
> 40	22.1%

Figure S2 Meta-analysis tests of interaction among common covariates. Ratio of HR (estimates and 95% CI) with meta-analysis Wald test P values. Studies with no recurrence event in an interaction category excluded. RS, 12-gene Oncotype DX Colon Recurrence Score result; MMR, mis-match repair; HR, hazard ratios; CI, confidence intervals.
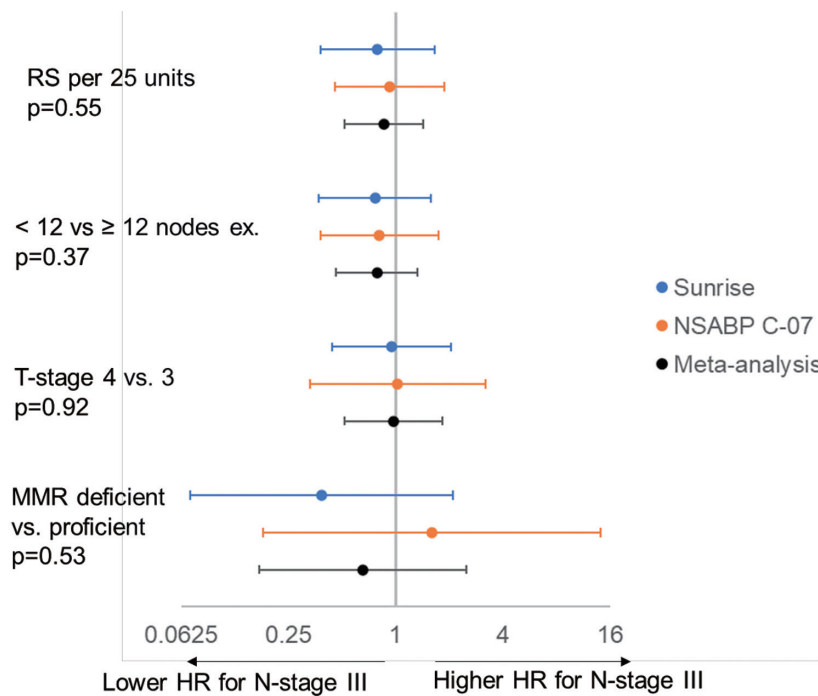
Figure S3 Meta-analysis tests of interaction of common covariates with stage III *vs.* II. Ratio of HR (estimates and 95% CI) with meta-analysis Wald test P values. RS, 12-gene Oncotype DX Colon Recurrence Score result; MMR, mis-match repair; HR, hazard ratios; CI, confidence intervals.
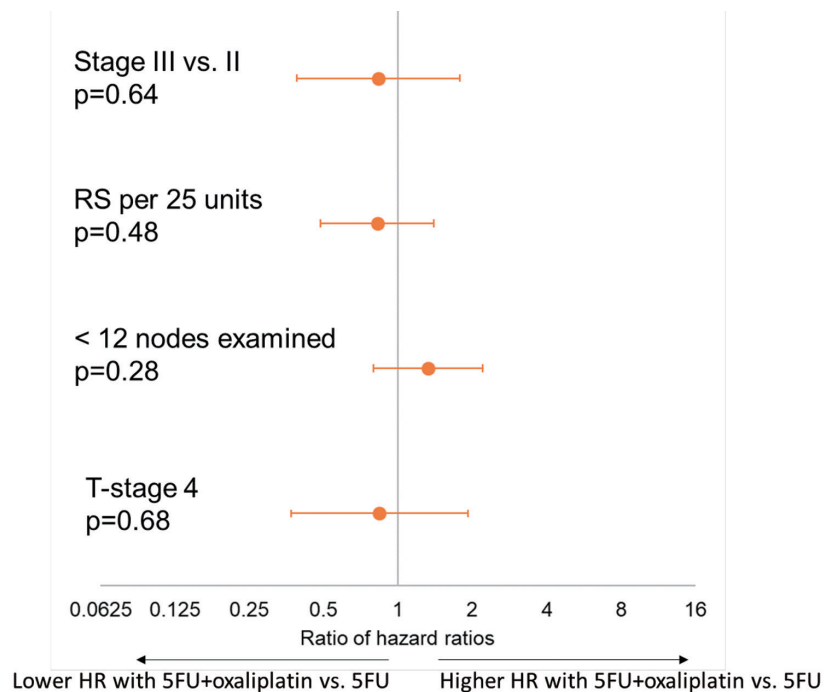

Figure S4 Tests of interaction of treatment with other covariates in NSABP C-07. Ratio of HR: estimates and 95% CI with Wald test P values. There were insufficient recurrence events among MMR-deficient patients to test for interaction. RS, 12-gene Oncotype DX Colon Recurrence Score result; HR, hazard ratios; 5FU, fluorouracil; CI, confidence intervals; MMR, mis-match repair.
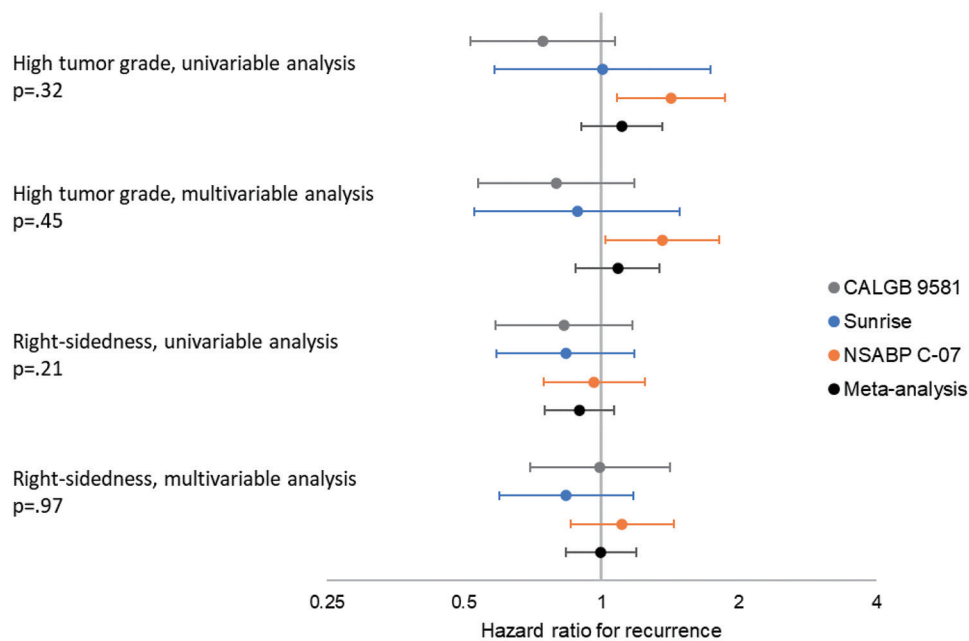
Figure S5 Meta-analysis estimates and 95% CI for HR of high tumor grade and right-sided tumor location with recurrence: univariable analysis and multivariable together with stage, RS, number of nodes examined, T-stage, and MMR status; meta-analysis Wald test P values. CI, confidence intervals; HR, hazard ratios; RS, 12-gene Oncotype DX Colon Recurrence Score result; MMR, mis-match repair.

Table S1 Correlations among model factors

Factor	<12 nodes examined	T-stage 4	MMR deficient	Stage IIIAB *vs.* II	Stage IIIC *vs.* II
RS	−0.02	0.13	0.03	0.02	0.03
<12 nodes examined		0.03	−0.12	0.03	−0.03
T-stage T4			0.08	0.00	0.01
MMR deficient				−0.02	−0.01

MMR, mis-match repair.

Table S2 Exploratory analysis assessing the incremental prognostic value of right-sidedness tumor location

Effect	CALGB 9581 (n=690)*, 162 events	SUNRISE (n=597)*, 202 events	NSABP C-07 (n=892), 245 events
RS per 25 units	1.57 (1.14, 2.18); P=0.006	1.99 (1.37, 2.88); P<0.001	1.78 (1.36, 2.32); P<0.001
<12 *vs.* ≥12 nodes examined	1.12 (0.79, 1.59); P=0.53	1.78 (1.23, 2.58); P=0.002	1.50 (1.16, 1.95); P=0.002
T-stage T4 *vs.* T3	1.04 (0.51, 2.13); P=0.92	1.18 (0.80, 1.73); P=0.41	2.70 (1.80, 4.04); P<0.001
MMR deficient *vs.* proficient/unknown	0.62 (0.39, 1.01); P=0.056	0.76 (0.32, 1.79); P=0.53	0.28 (0.12, 0.63); P=0.002
Stage IIIAB *vs.* II	–	2.31 (1.64, 3.25); P<0.001	2.32 (1.57, 3.52); P<0.001
Stage IIIC *vs.* II	–	5.29 (3.39, 8.27); P<0.001	5.64 (3.75, 8.47); P<0.001
Oxali + 5FU *vs.* 5FU	–	–	0.80 (0.62, 1.03); P=0.084
Right-sided tumor	0.99 (0.70, 1.14); P=0.97	0.84 (0.60, 1.17); P=0.30	1.12 (0.86, 1.45); P=0.40

Cox proportional hazards regression model HR with 95% CI and Wald test P values. RS, 12-gene Oncotype DX Colon Cancer Recurrence Score result; MMR, mis-match repair; Oxali, oxaliplatin; HR, hazard ratios; CI, confidence intervals.

Table S3 Exploratory analysis assessing the incremental prognostic value of tumor grade

Effect	CALGB 9581 (n=690)*, 162 events	SUNRISE (n=597)*, 202 events	NSABP C-07 (n=892), 245 events
RS per 25 units	1.56 (1.14, 2.15); P=0.006	1.97 (1.36, 2.84); P<0.001	1.67 (1.27, 2.19); P<0.001
<12 *vs.* ≥12 nodes examined	1.09 (0.80, 1.50); P=0.59	1.85 (1.29, 2.65); P=0.001	1.49 (1.15, 1.92); P<0.003
T-stage T4 *vs.* T3	1.07 (0.56, 2.03); P=0.85	1.19 (0.81, 1.75); P=0.38	2.72 (1.81, 4.07); P<0.001
MMR deficient *vs.* proficient/ unknown	0.71 (0.44, 1.16); P=0.17	0.73 (0.31, 1.72); P=0.47	0.27 (0.12, 0.61); P=0.002
Stage IIIAB *vs.* II	–	2.30 (1.63, 3.24); P<0.001	2.26 (1.52, 3.38); P<0.001
Stage IIIC *vs.* II	–	5.32 (3.42, 8.26); P<0.001	5.47 (3.65, 8.20); P<0.001
Oxali + 5FU *vs.* 5FU	–	–	0.82 (0.64, 1.06); P=0.14
High tumor grade	0.80 (0.54, 1.18); P=0.26	0.89 (0.53, 1.49); P=0.64	1.36 (1.09, 1.81); P=0.037

Cox proportional hazards regression model HR with 95% CI and Wald test P values. RS, 12-gene Oncotype DX Colon Cancer Recurrence Score result; MMR, mis-match repair; Oxali, oxaliplatin; HR, hazard ratios; CI, confidence intervals.