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Association of DNA Mismatch Repair and Mutations in *BRAF* and *KRAS* with Survival after Recurrence in Stage III Colon Cancers from Phase III Adjuvant Chemotherapy Trials

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Abstract

Importance—The association of biomarkers with patient survival after recurrence (SAR) is poorly understood, yet may guide management and treatment.

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Objective—To determine the association of DNA mismatch repair (MMR) status and somatic mutations in *BRAF*^{V600E} or *KRAS* (exon 2) in the primary tumor with SAR in patients with stage III colon carcinomas treated with adjuvant FOLFOX-based chemotherapy.

Design—Tumor biomarkers were analyzed in relationship to SAR in participants in adjuvant chemotherapy trials.

Intervention—Patients with resected, stage III colon cancers who were randomized to adjuvant FOLFOX ± cetuximab (NCCTG N0147) or FOLFOX ± bevacizumab (NSABP C-08).

Main Outcome Measure(s)—Associations of biomarkers with SAR were analyzed using Cox proportional hazards models adjusted for clinicopathological features and time-to-recurrence. The interaction effect of primary tumor sidedness on the association of biomarkers with SAR was determined.

Results—Among patients with cancer recurrence [N0147 (N=871); C-08 (N= 524)], multivariable analysis revealed that those whose tumors had deficient (d) *vs* proficient (p) MMR had significantly better SAR (adjusted hazard ratio [$HR_{adj.}$], 0.70, 95% CI, 0.52 - 0.96, adjusted P [$P_{adj.}$] <.029). Patients whose tumors harbored mutant *BRAF*^{V600E} ($HR_{adj.}$, 2.45, 95% CI, 1.85 - 3.25, $P_{adj.}$<.0001) or mutant *KRAS* ($HR_{adj.}$, 1.21, 95% CI, 1.00 - 1.47, $P_{adj.}$=0.052) had worse SAR compared to tumors that had wild-type copies of both genes, although only results for *BRAF*^{V600E} achieved statistical significance. Significant interactions were found for MMR ($P_{adj.}$=.029) and *KRAS* ($P_{adj.}$=.025) by primary tumor site for SAR. Improved SAR was observed for patients with dMMR tumors of the proximal *vs* distal colon ($HR_{adj.}$, 0.57, 95% CI, 0.40 - 0.83, $P_{adj.}$ =.003), and worse SAR for mutant *KRAS* tumors of the distal colon (codon 12: $HR_{adj.}$, 1.76, 95% CI, 1.30 - 2.38, $P_{adj.}$ =.0003; codon 13: $HR_{adj.}$, 1.76, 95% CI, 1.08 - 2.86, $P_{adj.}$ =.022].

Conclusions and Relevance—In patients with recurrence, dMMR was significantly associated with better SAR and this benefit was limited to primary tumors of the proximal colon. Mutations in *BRAF*^{V600E} were significantly associated with worse SAR, and worse SAR for *BRAF*^{V600E} or *KRAS* mutant tumors was more strongly associated with distal cancers. These biomarkers have implications for patient management at recurrence.

Trial Registration—NCCTG NO147, NCT00079274; NSABP C-08, NCT00096278

Introduction

Prognostic biomarkers in patients with tumor recurrence have the potential to influence management and treatment decisions. Approximately 30% of patients with stage III colon carcinoma will experience recurrence of their disease despite adjuvant chemotherapy[1]. Studies have shown that DNA mismatch repair (MMR) status and mutations in *BRAF*^{V600E} or *KRAS* genes can provide prognostic information in patients with stage III disease[2]. However, the association of biomarkers with survival after recurrence (SAR) remains poorly understood, and studies have been underpowered given the relatively low frequency of these alterations and modest rates of tumor recurrence.

In stage III patients who participated in adjuvant chemotherapy trials, those whose tumors showed dMMR or microsatellite instability (MSI) have generally had better clinical outcomes compared to those with proficient (p) MMR or microsatellite stability[3]. However,

the association of dMMR/MSI with prognosis is less robust in stage III *vs* stage II disease[4], and limited data exist in patients treated with standard adjuvant FOLFOX in contrast to 5-fluorouracil alone[5-8]. As in patients with metastatic disease[9], *BRAF*^{V600E} mutations have been shown to be significantly associated with poorer survival[10-12] with a stronger impact seen for overall survival (OS) compared to disease-free (DFS) or progression-free survival[13] (PFS) for reasons that remain unclear. Since *BRAF*^{V600E} mutations are significantly enriched in sporadic colon cancers with dMMR/MSI (due to epigenetic inactivation of *MLH1*)[14,15], the combined MMR/*BRAF* variable may be more informative than either alone. In this regard, a new consensus guideline for the molecular testing of colorectal cancer (CRC) recommends that *BRAF* be analyzed in conjunction with MMR for prognostic stratification. Data for the association of *KRAS* mutation with clinical outcome have been less consistent than for *BRAF*^{V600E} [13,16-18]. In participants in the North Central Cancer Treatment Group (NCCTG) N0147 and the Pan European Trial Adjuvant Colon Cancer (PETACC)-8 adjuvant trials, stage III colon cancers with mutant *vs* wild-type (WT) *KRAS* had poorer DFS rates [16,19].

We studied the association of MMR and mutations in *BRAF*^{V600E} or *KRAS* in the primary tumor with SAR in participants in the NCCTG N0147 and the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-08 adjuvant chemotherapy trials. These trials evaluated FOLFOX chemotherapy alone or combined with cetuximab (N0147)[20] or bevacizumab (NSAPB C-08)[21] where neither antibody significantly improved patient outcome *vs* FOLFOX alone. We also determined whether the association of biomarkers with SAR depended on primary tumor site within the colon given recent data suggesting prognostic differences by tumor site[6,22].

Materials and Methods

The study population consists of patients with stage III colon adenocarcinoma who developed recurrence during participation in phase III adjuvant chemotherapy studies NCCTG N0147[N= 871][20] and NSABP C-08 [N=524][21]. We categorized primary tumor site as located proximal to, or at or distal to the splenic flexure. Each trial was approved by the respective Institutional Review Boards (IRB) and by the NCCTG (now part of Alliance for Clinical Trials in Oncology) or NSABP (now part of NRG Oncology). Each participant signed an IRB-approved, protocol-specific informed consent document. Data quality was ensured by review by the Statistics and Data Center of the Alliance or NRG, and by the study chairpersons per established policies.

Molecular Testing

DNA MMR proteins MLH1, MSH2, and MSH6 were analyzed in FFPE tumor tissues from the N0147 trial as previously described[10]; MLH1 and MSH2 expression were analyzed in tumors from C-08 as reported[23]. MMR protein loss was defined as the absence of nuclear staining in tumor cells in the presence of nuclear staining in normal colonic epithelium and lymphocytes. Tumors with loss of an MMR protein were categorized as having deficient (d) MMR, those with intact expression as having proficient (p) MMR. All biomarker assays were interpreted with investigators blinded to patient outcomes.

BRAF^{V600E} and *KRAS* mutation status were determined using genomic DNA extracted from macrodissected FFPE tumor tissue collected prospectively. In N0147, testing for the *BRAF* c.1799T>A (V600E) mutation in exon 15 was performed using a multiplex allele-specific PCR–based assay and an automated sequencing technique, as previously described[10]. *KRAS* exon 2 mutation status was analyzed using the DxS Mutation Test Kit KR-03/04 (DxS), assessing for seven different mutations in codons 12 and 13[16]. In N0147, molecular analyses was performed in a Clinical Laboratory Improvement Amendments (CLIA)-compliant laboratory. Mutation profiling of tumor specimens from C-08 was performed using OncoCarta and ColoCarta panel assays, with the running of samples on the MassSpec platform as described previously[23].

Statistical Analysis

SAR, defined as the time from recurrence to death due to any cause, was the primary study outcome. Due to the potential for significant confounding, all analyses were based on multivariable models that were adjusted for clinicopathological variables, time-to-recurrence, and biomarkers. The distribution of SAR between patient subgroups by biomarkers was estimated based on direct adjusted survival curves[24-26]. Since initial results showed significant differences in SAR among the four arms of the two adjuvant chemotherapy trials ($p = 0.026$), multivariable Cox models (stratified by the four treatment groups) were applied to assess the impact of biomarkers on SAR among patients with recurrence. Models were adjusted for age, sex, performance score, initial T/N stage, histologic grade, time from initial treatment to recurrence, primary tumor site, and biomarkers when applicable. The proportional hazard assumption was confirmed by examination of Schoenfeld residuals plot. Interaction effects of the primary tumor site on the impact of biomarkers on SAR were determined. Subgroup analyses were performed when there were statistically significant interaction effects. Association analyses were performed in patients from the mFOLFOX6 alone treatment arms from both studies due to clinical relevance. Two-sided P values are reported; values < 0.05 were considered statistically significant and were not adjusted for multiple comparisons. Analyses were performed using SAS version 9.4 (SAS Institute Inc., NC).

Results

Among the adjuvant trial participants, 3018 patients received mFOLFOX6 ± cetuximab (NCCTG N0147)[20] and 1961 patients received mFOLFOX6 ± bevacizumab (NSABP C-08)[21]. At a median follow-up of 6.0 years (N0147) and 6.3 years (C-08), 871 and 524 patients from each study, respectively, had a documented first recurrence and are included in this report. Among these patients, 848 had complete and available data on MMR and the mutational status of *BRAF* and *KRAS* genes (Fig. 1).

Molecular Markers and SAR

The multivariable associations of patient demographics and clinicopathological features, adjusting for biomarkers (MMR, *KRAS* and *BRAF*), with SAR are presented in Table 1. Patients with distal tumors had significantly better SAR than did patients with proximal tumors (adjusted hazard ratio [$HR_{adj.}$], 0.70, 95% confidence interval (CI), 0.58 - 0.84,

adjusted P value [$P_{adj.}$]=.0002). Longer time-to-recurrence (TTR) following primary resection was associated with significantly better SAR (for one year delay, $HR_{adj.}$ 0.79, 95% CI, 0.72 - 0.87, P_{adj}<.0001)[Table 1]. In addition, patient performance score, N stage, and histologic grade were significantly associated with SAR. Among patients who experienced recurrence, those whose tumors showed pMMR (*vs* dMMR) or had wild-type (WT) *KRAS* and *BRAF* (*vs* either mutated) had significantly longer median TTR (Supplemental Table 1).

Multivariable associations of molecular markers with SAR are shown in Table 2. After adjustment for covariates including TTR after primary treatment, patients with dMMR *vs* pMMR tumors had significantly better SAR ($HR_{adj.}$, 0.70, 95% CI, 0.52 - 0.96, P_{adj}=.028) [Fig. 2A, Table 2]. Patients with $BRAF^{V600E}$ mutant tumors had significantly worse SAR compared to those whose tumors had wild-type (WT) *BRAF* ($HR_{adj.}$, 2.45, 95% CI, 1.85 - 3.25, $P_{adj.}$<.0001) [Fig. 2B,C, Table 2]. Given that MMR status and $BRAF^{V600E}$ are strongly associated, we analyzed MMR/BRAF as a combined variable. Patients whose tumors had dMMR or pMMR plus mutant $BRAF^{V600E}$ had similarly poor adjusted median SAR times of 14.5 ($HR_{adj.}$, 1.52, 95% CI, 0.99- 2.34, P_{adj} =.058) and 15.4 months ($HR_{adj.}$, 2.64, 95% CI, 1.96 - 3.57, $P_{adj.}$<.0001), respectively, and shorter SAR compared to pMMR/WT BRAF (referent) [Table 2, Fig. 2D]. In contrast, patients whose tumors had dMMR or pMMR with WT *BRAF* showed better SAR with 30.3 and 28.4 month adjusted median SAR, respectively, and there was no statistical difference between these two groups (Table 2, Fig. 2D). Within the subset of dMMR tumors, we observed that those with *BRAF* mutations had significantly poorer SAR compared to those with WT *BRAF* ($HR_{adj.}$, 2.70, 95% CI, 1.23 – 5.93, P_{adj}= .0136)[Table 2]. Patients whose tumors harbored *KRAS* exon 2 mutations had shorter SAR compared to those whose tumors were WT for *KRAS* and *BRAF* (25.9 vs 32.1 months; p=.052)[Table 2]. When *KRAS* was analyzed by codon 12 or 13 mutations vs WT *KRAS*, the associations did not reach statistical significance (Table 2). Patients whose tumors had both WT *BRAF* and WT *KRAS* had the longest SAR (adjusted median of 32.1 months) of all groups that was significantly improved compared to patients whose tumors had $BRAF^{V600E}$ mutation (15 months; p<.0001) [Table 2].

Analysis by Primary Tumor Site

Based on statistically significant interactions between biomarkers and primary tumor site for SAR (Table 2), we separately examined the associations between biomarkers and SAR among patients with proximal or distal tumors (Table 3, Suppl. Fig. 1). After adjustment for covariates, patients with dMMR tumors of the proximal but not the distal colon had significantly better SAR [$HR_{adj.}$, 0.57, 95% CI, 0.40 - 0.83, $P_{adj.}$=.0028] (Table 3, Suppl. Fig. 1A), interaction p =.029 (Table 2). Patients with $BRAF^{V600E}$ mutated tumors had significantly shorter SAR for both proximal ($HR_{adj.}$, 1.90, 95% CI 1.37 – 2.64,, $P_{adj.}$ = 0.0001; Suppl. Fig. 1D) and distal cancers ($HR_{adj.}$, 5.84, 95% CI 3.27 – 10.43,$P_{adj.}$ < 0.0001) versus those whose tumors had WT *BRAF* (Suppl. Fig. 1B) or WT *BRAF*/WT *KRAS* (Suppl. Fig. 1C)[Table 3], although the interaction between *BRAF* and primary tumor site for SAR did not achieve significance ($P_{adj.}$ = 0.056) [Table 2]. A significant interaction was observed for *KRAS* mutations (codon 12, 13) [$P_{adj.}$ =.025] and the combined *KRAS*/*BRAF* variable (p=.0005) with primary tumor site for SAR (Table 2). Compared to tumors with WT *KRAS*, patients whose tumors harbored *KRAS* mutations at

codon 12 (HR $_{adj.}$, 1.76, 95% CI, 1.30 - 2.38, $P_{adj.}$ =0.0003) or codon 13 (HR $_{adj.}$, 1.76, 95% CI, 1.08 - 2.86, $P_{adj.}$ = 0.022) each had significantly worse SAR among distal, but not proximal cancers (Table 3, Suppl. Fig. 1C). For the combined MMR/*BRAF* variable, the adjusted median SAR was shorter for patients with dMMR and mutant $BRAF^{V600E}$ tumors of the distal *vs* proximal colon (5.7 vs 14.5 months). Furthermore, dMMR and mutant $BRAF^{V600E}$ tumors in the distal colon had significantly shorter SAR than did patients with pMMR and WT *BRAF* tumors ($HR_{adj.}$, 9.38, 95% CI, 3.23 - 27.28, $P_{adj.}$ < 0.0001; Table 3, Suppl. Fig. 1D).

Analysis by Study Treatment Arm

A statistically significant interaction was observed between the study treatment arm and MMR status ($P_{adj.}$ =.0026), and for the combined variable of MMR/*BRAF* ($P_{adj.}$ =.016) for SAR [Table 2]. The significantly favorable impact of dMMR on SAR shown in multivariable analysis was evident in the FOLFOX arms from both adjuvant trials (HR $_{adj.}$, 0.50, 95% CI, 0.31 - 0.81, $P_{adj.}$ = 0.0043, but was not observed in the FOLFOX + cetuximab arm of the N0147 trial (HR $_{adj.}$, 1.19, 95% CI, 0.78 - 1.82, $P_{adj.}$ = 0.43) [Suppl. Table 2]. An association of mutant $BRAF^{V600E}$ with significantly poorer SAR was observed in patients treated with FOLFOX alone or combined with cetuximab. However, patients whose cancers were dMMR and mutant $BRAF^{V600E}$ showed significantly poorer SAR when cetuximab was added to FOLFOX (HR $_{adj.}$, 2.95, 95% CI, 1.64 - 5.32, $P_{adj.}$ = 0.0003), but not in patients whose tumors were treated with FOLFOX alone (HR $_{adj.}$, 1.03, 95% CI, 0.52 - 2.01, $P_{adj.}$ = 0.94) [Suppl. Table 2]. A similar effect was observed for tumors with *KRAS* codon 12 mutations whereby their SAR was worse than in patients with WT *KRAS* tumors when treated with FOLFOX plus cetuximab, but not FOLFOX alone (Suppl. Table 2). Among patients with *KRAS* WT tumors, no differences in SAR were observed within proximal or distal primary tumors by treatment arm.

Discussion

We determined the impact of biomarkers on SAR in stage III colon cancer patients who participated in two large adjuvant chemotherapy trials of FOLFOX-containing therapy. In the overall cohort, patients whose tumors had mutant *BRAF* had significantly worse SAR with a 14.2 month decrease in adjusted median survival time compared to WT *BRAF* tumors. This result can explain, at least in part, prior data showing that mutant $BRAF^{V600E}$ was more strongly associated with OS compared to DFS or relapse-free survival in the N0147[6] and Pan European Trial Adjuvant Colon Cancer (PETACC)-3 adjuvant chemotherapy trials, respectively[13]. Furthermore, these findings suggest that the impact of $BRAF^{V600E}$ mutation on tumor aggressiveness is enhanced at the time of tumor recurrence since recurrence of these tumors led to accelerated patient mortality. In this regard, patients whose tumors harbored $BRAF^{V600E}$ mutations had a ~3-fold increase in early peritoneal metastases compared to those patients whose tumors showed WT *BRAF* in the stage III N0147 cohort[27]. These data are consistent with other reports showing adverse outcome [28] and significantly higher rates of peritoneal and distant lymph node metastases among $BRAF^{V600E}$ mutant metastatic CRCs[9].

Among patients with dMMR tumors, we found that their adjusted median SAR was 7 months longer than patients with pMMR tumors indicating a clinically significant survival advantage for this patient subset. This finding is consistent with the longer recurrence-free interval (i.e, TTR) observed for dMMR *vs* pMMR tumors in the overall study cohort. Importantly, the analysis was adjusted for covariates that included *BRAF* mutation status, TTR, and primary tumor site which were the variables whose inclusion in the multivariable model had the greatest impact on SAR in dMMR tumors. The longer SAR for patients with dMMR tumors may be explained, in part, by the increase in recurrence rates at regional *vs* distant sites, such as the liver, that was observed in the N0147 cohort[27]. Among patients whose tumors had mutant *KRAS*, a trend was seen toward poorer SAR that did not reach statistical significance for codon 12 or 13 mutations.

Sporadic colon cancers with dMMR are highly enriched with $BRAF^{V600E}$ mutations[5,14], and a forthcoming consensus guideline recommends that $BRAF^{V600E}$ mutation testing be done in conjunction with MMR analysis for prognostic stratification. A similarly poor SAR was observed for patients with $BRAF^{V600E}$ mutant dMMR or pMMR cancers with 14.5 and 15.4 month adjusted median SAR, respectively. In contrast, patients whose tumors had WT *BRAF* showed significantly better SAR with 30.3 (for dMMR) and 28.4 (for pMMR) month adjusted median SAR, respectively. Therefore, the mutational status of *BRAF* is an important determinant of SAR that confers adverse outcome in patients with both dMMR and pMMR cancers. In a pooled analysis of stage II and III patients from the NSABP C-07 and C-08 adjuvant studies where dMMR was associated with a lower rate of tumor recurrence, a trend toward worse SAR was seen for patients with dMMR colon cancers, although the analysis was not adjusted for *BRAF*[23]. The authors, however, postulated that the association of dMMR with shorter SAR was due to mutant $BRAF^{V600E}$ since patients with $BRAF^{V600E}$ mutant tumors had significantly shorter SAR[23]. In another study of patients with stage I-IV colorectal cancers, transcriptomic data were used to categorize tumors into four consensus molecular subtypes (CMS). The CMS1 subtype was enriched for tumors with MSI-H and $BRAF^{V600E}$ mutations, and patients with these tumors had a poorer SAR compared to the other three subtypes (CMS I-III) by univariate analysis[29]. However, the study data used to generate CMS were not adjusted for *BRAF (or KRAS*) status nor for TTR which was strongly associated with SAR as shown in our dataset.

We observed a statistically significant interaction between biomarkers (MMR, *KRAS*) and primary tumor site for SAR. The significant association of dMMR with better SAR was limited to cancers of the proximal *vs* distal colon. While not prognostic overall, analysis of *KRAS* mutations by primary tumor site revealed a significantly shorter SAR for patients with distal but not proximal cancers. This finding for SAR is consistent with TTR data from the N0147 cohort where the association of *KRAS* mutations with TTR and OS was stronger in patients with distal *c*ancers[6]. Conversely and relevant to anti-EGFR therapy, patient tumors with WT *KRAS* alleles had significantly better SAR for distal *vs* proximal cancers. However, stage III patients with WT *KRAS* tumors treated with FOLFOX + cetuximab *vs* FOLFOX had similar SAR irrespective of tumor site. In patients with metastatic CRC, a recent report suggests that distal cancers respond more favorably to cetuximab than do proximal tumors (CALGB 80405)[30]. Patients whose tumors harbored mutations in $BRAF^{V600E}$ had significantly poorer SAR independent of primary site, yet the association

was stronger for distal tumors. Of note, an association between primary tumor site and SAR was also been seen in stage III colon cancer patients treated with non oxaliplatin-containing chemotherapy in the PETACC-3 study[31]. Factors not studied in our report that may contribute to observed differences in prognosis by tumor site include epigenetic[16] and/or other genomic[31] alterations that may be embryologically influenced since the origin of the proximal colon is from the midgut and distal colon from the hindgut. In addition, microbial composition or metabolites may be relevant factors. Analysis of the associations between biomarkers and SAR by study treatment arm revealed that the better SAR for patients with dMMR tumors seen among FOLFOX-treated patients did not extend to those who also received cetuximab for reasons that are unclear. Due to the modest number of patients with complete biomarker data in C-08, results for SAR from the FOLFOX + bevacizumab study arm are not reported.

Strengths of our study include the two clinical trial cohorts receiving standard adjuvant FOLFOX-based chemotherapy with mature recurrence and survival data. All molecular analyses were performed on prospectively collected biospecimens. Our study findings are relevant to clinical practice in that National Comprehensive Cancer Network (NCCN) guidelines and a forthcoming consensus guideline recommend testing of all newly diagnosed CRCs for expanded *RAS* and $BRAF^{V600E}$ mutations in combination with MMR/MSI for prognostic stratification and identification of Lynch Syndrome patients. Study limitations include the fact that biomarkers were analyzed in only a subset of the C-08 cohort and that *KRAS* testing was limited to exon 2. However, a recent study found that clinicopathologic features, survival outcomes, and gene expression profiles were similar between patients whose CRC harbored *KRAS* codon 12/13 mutations and those with *KRAS* 61/146 or *NRAS* mutations[32]. Analysis of biomarkers by tumor site for SAR resulted in some small patient subsets for which cautious interpretation of the data is warranted. Lastly, no data were available on patient treatment after tumor recurrence for which we cannot exclude an impact on SAR.

In conclusion, the association of dMMR with more favorable SAR suggests that some of these patients may be candidates for an aggressive surgical approach at recurrence. Furthermore, therapy with an immune checkpoint inhibitor is a new therapeutic option in patients with metastatic dMMR/MSI CRCs where impressive tumor responses and extended PFS were observed[33]. In patients with both dMMR and pMMR tumors, $BRAF^{V600E}$ mutations were associated with significantly poorer SAR indicating the need for novel therapies in this subset[33,34]. The significant interactions of MMR and *KRAS* mutation status with SAR by primary tumor site indicates that these biomarkers should be interpreted in this context. Taken together, these data have important implications for stage III colon cancer patients at the time of tumor recurrence where they can be utilized to inform clinical decision-making.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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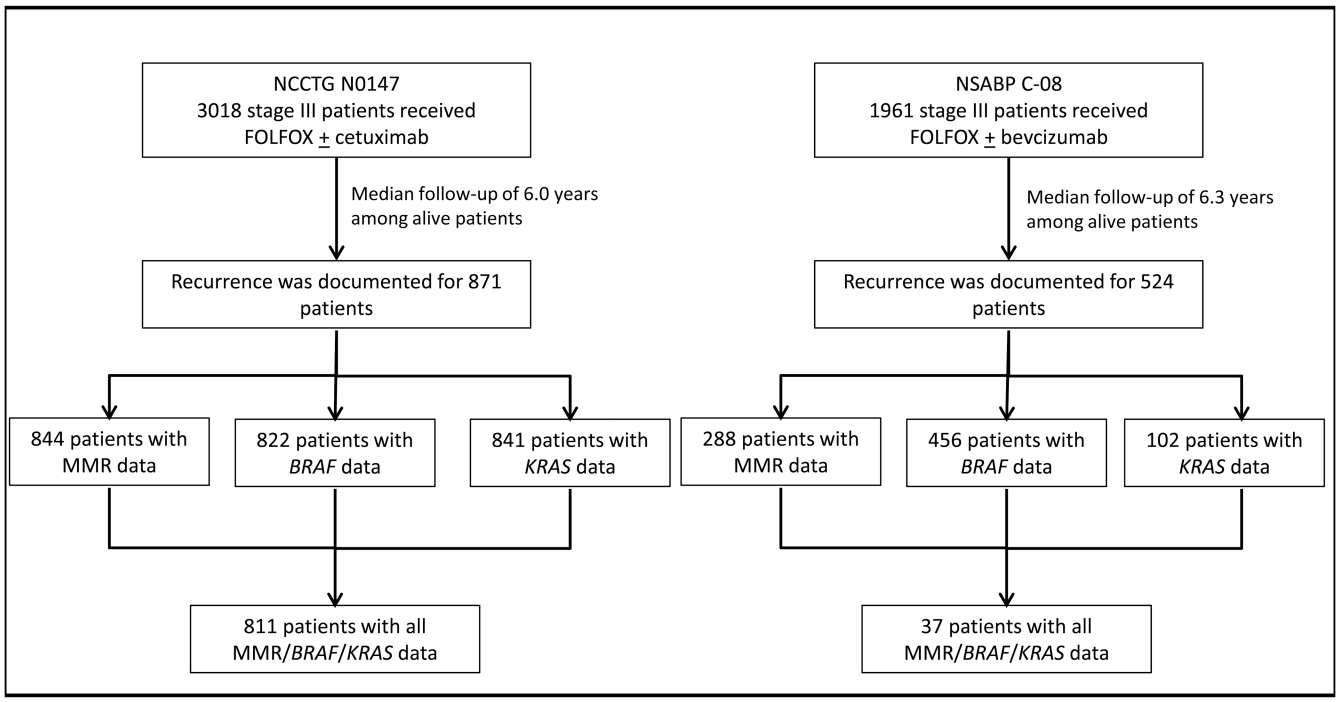

Figure 1.
Consort flow diagram of the study population.

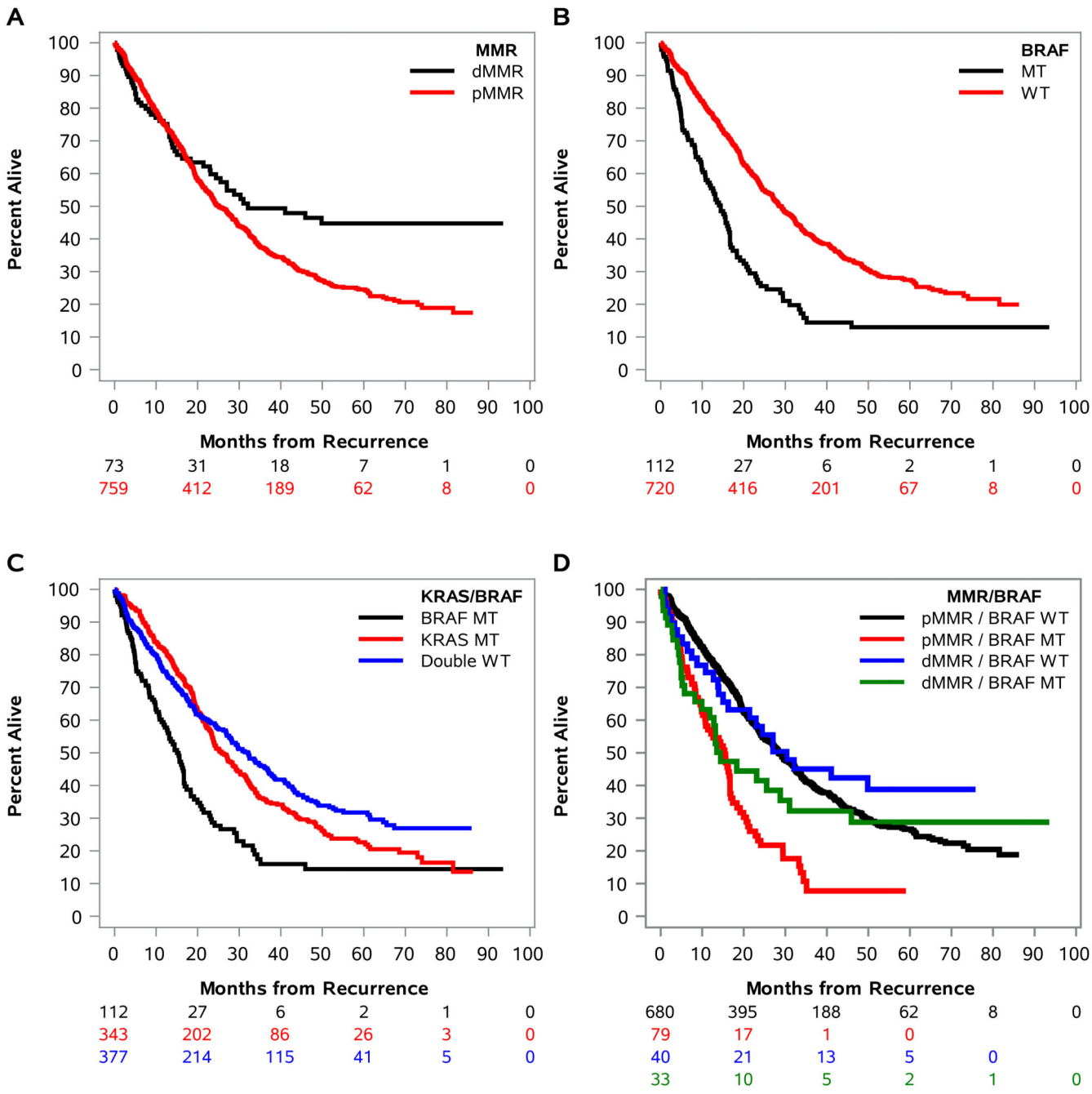

Figure 2.

In patients with stage III colon carcinoma treated with FOLFOX-containing adjuvant therapy, direct adjusted plots of survival after recurrence (SAR) are shown by DNA mismatch repair (MMR) status (*A*), mutated *vs* wild-type *BRAF* (*B*) or *KRAS* (*C*), and the combined variables of KRAS/BRAF (D), or MMR/*BRAF* (E). MUT: mutant; WT: wild-type. MMR status: deficient (d) or proficient (p).

Table 1

Multivariable [*$] associations between patient demographics and disease characteristics with survival after recurrence (SAR), adjusting for biomarkers (MMR, *KRAS*, and *BRAF*).

Biomarkers	N of patients (%)	HR	95% CI	P-value
Age, 10 year increase	832	1.06	0.98-1.15	0.17
Sex				
Female	387 (46.5%)	0.90	0.76-1.06	0.21
Male	445 (53.5%)	Ref		
Performance Score				
0	629 (75.6%)	Ref		
1	198 (22.7%)	1.23	1.01-1.49	0.037
2	5 (0.6%)	7.97	3.19-19.88	<.0001
T-stage				
T1/2	47 (5.6%)	Ref		
T3	631 (75.8%)	1.32	0.88-1.99	0.1794
T4	154 (18.5%)	1.41	0.91-2.19	0.1264
N-stage				
N1	339 (40.7%)	Ref		
N2	493 (59.3%)	1.39	1.17-1.66	0.0002
Primary tumor site				
Distal	384 (46.2%)	0.70	0.58-0.84	0.0002
Proximal	448 (53.8%)	Ref		
Histologic grade				
Low grade (1-2)	587 (70.6%)	Ref		
High grade (3/4/anaplastic)	245 (29.4%)	1.40	1.17-1.68	0.0003
Time-to-recurrence, 1 year increase	832	0.79	0.72-0.87	<.0001

Abbreviations: HR, hazard ratio; CI, confidence interval.

[*] Multivariable model in 832 patients includes complete data on all covariates (age, sex, performance score, T-stage, N-stage, primary tumor site, histologic grade, time-to-recurrence, MMR, KRAS, and BRAF. The HR, 95% CI and p-value associated with MMR, KRAS, BRAF are presented in Table 2.

[$] Stratified Cox models with four treatment arms as individual strata.

Table 2

Adjusted associations between biomarkers and survival after recurrence (SAR) [*$]

Biomarkers	Events/N	Adjusted median time in months (95% CI)[1,2]	HR	95% CI	P-value	Interaction P-value with tumor Site	Interaction P-value with treatment
MMR						0.029	0.0026
dMMR	54/73	32.1 (24.4-NR)	0.70	0.52-0.96	0.028		
pMMR	521/759	25.1 (23.3-28.3)	Ref				
KRAS						0.025	0.23
Codon 12 MT	201/275	23.8 (21.2-27.2)	1.20	0.98-1.47	0.076		
Codon 13 MT	55/68	27.2 (22.0-33.8)	1.24	0.91-1.67	0.17		
WT	319/489	28.1 (24.1-32.3)	Ref				
BRAF						0.056	0.57
MT	102/112	14.5 (10.8-16.7)	2.45	1.85-3.25	<.0001		
WT	473/720	28.7 (26.8-32.1)	Ref				
KRAS/BRAF						0.0005	0.67
BRAF MT	102/112	15.0 (12.1-16.9)	2.45	1.85-3.25	<.0001		
KRAS MT	256/343	25.9 (23.5-29.4)	1.21	1.00-1.47	0.052		
Both WT	217/377	32.1 (27.6-37.1)	Ref				
MMR/BRAF						0.076	0.016
MT BRAF dMMR	28/33	14.5 (11.8-45.9)	1.52	0.99-2.34	0.058		
WT BRAF dMMR	26/40	30.3 (21.4-NR)	0.85	0.56-1.28	0.43		
MT BRAF pMMR	74/79	15.4 (10.8-16.7)	2.64	1.96-3.57	<.0001		
WT BRAF pMMR	447/680	28.4 (26.2-31.9)	Ref				
BRAF in dMMR patients							
MT BRAF	28/33		2.70	1.23-5.93	0.0136		
WT BRAF	26/40		Ref				

Abbreviations: HR, hazard ratio; CI, confidence interval; MT, mutant; WT, wild-type; NR, not reached

[*] Adjusting for age, sex, performance score, T/N stage, primary tumor site, histologic grade, biomarkers (when applicable), and time-to-recurrence

[$] Stratified Cox models with four treatment arms as individual strata.

[1] Based on direct adjusted survival curves from Cox model

[2] Median not calculated for *BRAF* in dMMR patients due to small sample size (model convergence failed)

Table 3

Adjusted associations between biomarkers and survival after recurrence (SAR) among patients by primary tumor site [*$]

	Proximal tumor					Distal tumor				
Biomarkers	**Events/N**	**Adjusted median time in months (95% CI)[1]**	**HR**	**95% CI**	**P-value**	**Events/N**	**Adjusted median time in months (95% CI)[1]**	**HR**	**95% CI**	**P-value**
MMR										
dMMR	41/55	30.3 (16.3-NR)	0.57	0.40-0.83	0.0028	13/18	27.0 (24.4-NR)	1.26	0.69-2.28	0.45
pMMR	308/393	19.0 (17.2-20.6)	Ref			213/366	36.6 (33.3-43.3)	Ref		
KRAS										
Codon 12 MT	120/162	21.8 (19.7-25.3)	0.85	0.65-1.12	0.26	81/113	27.0 (23.2-34.4)	1.76	1.30-2.38	0.0003
Codon 13 MT	35/43	21.9 (16.6-30.2)	0.91	0.61-1.36	0.65	20/25	33.8 (24.4-48.1)	1.76	1.08-2.86	0.022
WT	194/243	16.7 (14.5-19.4)	Ref			125/246	43.3 (37.1-55.1)	Ref		
BRAF										
MT	85/94	13.2 (9.9-16.7)	1.90	1.37-2.64	0.0001	17/18	11.3 (6.5-23.4)	5.84	3.27-10.43	<.0001
WT	264/354	21.8 (19.3-23.8)	Ref			209/366	39.2 (33.9-44.2)	Ref		
KRAS/BRAF										
BRAF MT	85/94	12.8 (9.9-16.0)	1.90	1.37-2.64	0.0001	17/18	16.7 (6.6-25.5)	5.84	3.27-10.43	<.0001
KRAS MT	155/205	23.3 (21.1-27.2)	0.87	0.67-1.12	0.28	101/138	29.1 (24.5-36.3)	1.76	1.32-2.34	<.0001
Both WT	109/149	19.1 (14.7-26.8)	Ref			108/228	45.4 (38.5-61.4)	Ref		
MMR/BRAF										
MT BRAF dMMR	24/29	14.5 (11.8-NR)	1.08	0.66-1.75	0.76	4/4	5.7 (2.8-NR)	9.38	3.23-27.28	<.0001
WT BRAF dMMR	17/26	30.3 (16.3-NR)	0.59	0.35-0.99	0.047	9/14	27.0 (24.4-NR)	1.13	0.56-2.29	0.73
MT BRAF pMMR	61/65	15.0 (9.9-16.7)	1.92	1.35-2.73	0.0003	13/14	14.5 (6.6-NR)	5.38	2.81-10.31	<.0001
WT BRAF pMMR	247/328	20.7 (19.0-23.5)	Ref			200/352	39.2 (34.4-44.2)	Ref		

Abbreviations: HR, hazard ratio; CI, confidence interval; MT, mutant; WT, wild-type; NR, not reached

[*] Adjusting for age, sex, performance score, T/N stage, tumor site, histologic grade, biomarkers (when applicable), and time-to-recurrence

[$] Stratified Cox models with four arms as individual stratums.

[1] Based on direct adjusted survival curves from Cox model