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Original Research

Prognostic variables in low and high risk stage III colon cancers treated in two adjuvant chemotherapy trials

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Abstract ***Background:*** Stratification of patients with stage III colon cancer into low ($T_{1-3}N_1$) and high (T_4 and/or N_2) risk groups is used to guide the duration of adjuvant chemotherapy. We determined the relative contribution of clinical and molecular features to survival by risk group.

Materials & methods: Stage III colon cancer (N = 5337) patients from two adjuvant trials of FOLFOX ± cetuximab [N0147 (Alliance), PETACC-8] were risk grouped, then subgrouped by clinical features and molecular variables [*KRAS* and *BRAF*/mismatch repair (MMR) combined variable]. Distributions of disease-free survival (DFS), overall survival (OS), and

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Deficient mismatch repair;
Prognosis;
Recurrence

survival after recurrence (SAR) were estimated. In multivariable Cox models, backward elimination was performed for analysis of candidate predictors of outcomes. Relative contributions of model-selected variables to outcomes by risk group were calculated using $\chi 2$.

Results: Among low risk tumours, mutant *KRAS* and male gender were significantly associated with poorer OS multivariately. In high risk tumours, significantly poorer OS was observed for right sidedness and for mutant *KRAS* and $BRAF^{V600E}$/pMMR, subgroups. Specifically, $BRAF^{V600E}$/pMMR (OS: HR = 1.75; 95% CI: 1.36–2.24; $P_{adj}<.0001$) and right- versus left-sidedness were associated with significantly poorer DFS, OS (HR = 1.56; 95% CI: 1.31–1.83; $P_{adj}<.0001$), and SAR (HR = 1.64; 95% CI: 1.37–1.95; $P_{adj}<.0001$). Poor prognosis of mutant *KRAS* for DFS and OS was similar among risk groups. *BRAF*/MMR and sidedness were associated with poorer SAR in both low and high risk tumours. Age, gender, and *KRAS* were the top three relative contributors to DFS and OS among low risk tumours; sidedness ranked first for DFS and OS, and second to *BRAF*/MMR for SAR among high risk tumours.

Conclusion: Sidedness and *BRAF*/MMR contributed the most to survival outcomes among high risk tumours and should be interpreted in the context of risk group.

1. Introduction

Despite adjuvant chemotherapy, nearly one-third of patients with stage III colon cancer will develop tumour recurrence [1–3] and most of these patients eventually die of their disease. An international adjuvant study known as IDEA (International Duration Evaluation of Adjuvant Therapy) evaluated the noninferiority of 3 months compared to the standard 6 months of adjuvant fluoropyrimidine plus oxaliplatin in patients with stage III colon cancer [4]. While noninferiority of 3 in comparison to 6 months of therapy was not confirmed in the overall population, a post hoc analysis of patients with low risk ($T_{1\text{-}3}N_1$) cancers revealed that 3 months of therapy was noninferior to 6 months and among those classified as high risk (T_4, N_2, or both), 6 months of therapy was superior to 3 months [4]. Based upon these data, T and N risk grouping is routinely used by clinicians and endorsed by ESMO [5] and NCCN [6] guidelines, to determine the recommended duration of adjuvant chemotherapy for stage III patients. To date, however, the relative contribution of clinical and molecular features to patient survival in low and high risk groups of stage III patients has not been studied. Improving patient outcomes in the adjuvant setting will require development of prediction models that incorporate clinical and biological data into T and N staging to guide precision oncology approaches.

Using pooled data from two phase III trials of adjuvant fluoropyrimidine plus oxaliplatin chemotherapy (NCCTG N0147, PETACC-8) where patient outcomes were similar by treatment arm [1,3], we categorized stage III patients into low risk ($T_{1\text{-}3}$ and N_1) and high risk (T_4, N_2, or both) groups [4]. Stratified by risk groups, we examined the relationship of clinical and molecular variables to survival outcomes, and then utilized Cox model selection procedures to identify candidate predictors of disease-free survival (DFS), overall survival (OS), and survival after recurrence (SAR). Importantly, insight into tumour biology and metastatic potential can be gained by evaluation of SAR. Data indicate that the impact of the $BRAF^{V600E}$ point mutation on patient prognosis in colon cancer is dependent on the status of the DNA mismatch repair (MMR) system [7]. Accordingly, we examined *BRAF* [wild type (WT) or mutant V600E) and MMR as a combined variable (*BRAF*/MMR) with four possible combinations (*BRAF* WT/pMMR, *BRAF* WT/dMMR, $BRAF^{V600E}$/pMMR, $BRAF^{V600E}$/dMMR). Right- versus left-sided colon cancers are enriched in $BRAF^{V600E}$ and microsatellite instability (MSI) [due to deficient MMR (dMMR)] [8,9] that can contribute to differences in prognosis by primary tumour sidedness. Information gained from this study may inform patient management including decision-making in the adjuvant setting.

2. Patients and methods

2.1. Patient population

Patients with resected stage III colon cancers (N = 5337) had participated in two trials of adjuvant FOLFOX ± cetuximab [North Central Cancer Treatment Group (NCCTG) N0147 [1] (Alliance) and PETACC-8 [3]] where outcomes were similar by study arm that enabled data pooling. The study population includes patients with available data for all biomarkers

that were analysed in prospectively collected tissues. Trial identification numbers: NCT00079274; NCT00265811.

2.2. Molecular analysis

DNA mismatch repair (MMR) status was determined in tumour tissue by analysis of MMR protein (MLH1, MSH2, and MSH6) by immunohistochemistry (IHC). If IHC was indeterminate [10], microsatellite instability (MSI) testing was performed. Tumours were classified as dMMR if there was loss of one or more MMR proteins or if tumours exhibited high-level MSI.

Tumour tissue was analysed for mutations in *KRAS* (codon 12 or 13 in exon 2) or the $BRAF^{V600E}$ point mutation (exon 15) genes, as described previously [11,12]. Written informed consents were obtained from patients at study entry, and the study was approved by the Mayo Clinic Institutional Review Board.

2.3. Statistical analyses

Patients were risk-grouped using T and N stage data and the associations of the study variables with patient DFS, OS, and SAR were analysed univariately and in multivariable Cox models. DFS was defined as the time from the date of random assignment to recurrence or death due to all causes, whichever occurred first. SAR was defined as the time from recurrence to death from any cause. Five-year survival rates were determined based on Kaplan-Meier estimates. Cox models were used for evaluating associations between outcomes and clinical/molecular factors, stratifying by treatment arm. Study variables included in the initial model were age, sidedness, histologic grade, gender, lymph nodes, performance status, and *KRAS* and *BRAF*/MMR. After determination of the optimal functional form (continuous or categorical) of clinical and molecular variables, a backward elimination selection was performed to identify the important independent prognostic factors. The proportional hazards assumption was confirmed by examination of the Schoenfeld residual plot [13]. The relative contributions of model-selected variables to outcomes by risk group were calculated using the $\chi 2$ from Harrell's rms R package (version 3.2.3; http://biostat.mc.vanderbilt.edu/rms) based on multivariable models. In an exploratory analysis, Cox models for survival outcomes were utilized to examine interaction between sidedness and treatment arm among WT *KRAS* tumours. The relative contribution of each factor represents the percentage of chi-square made up of the total for the model. Two-sided P values are reported; $P < .05$ was considered statistically significant and was not adjusted for multiple comparisons. Analyses were performed using SAS software (version 9.4; SAS Institute Inc.). Data were frozen as of 8/5/15. Data collection and statistical analyses were performed by the Alliance Statistics and Data Center.

3. Results

3.1. Study population stratified by risk group

Patients with stage III colon cancer (N = 5337) from two phase III adjuvant trials were divided into low risk ($T_{1\text{-}3}N_1$) [N = 2770 (51.9%)] and high risk (any T_4 and/or N_2) [N = 2565 (48.1%)] groups. Among low risk patients, 726 (31.3%) tumours harboured mutant *KRAS*, 224 (9.6%) had $BRAF^{V600E}$, and 253 (9.1%) showed dMMR. Corresponding numbers in the high

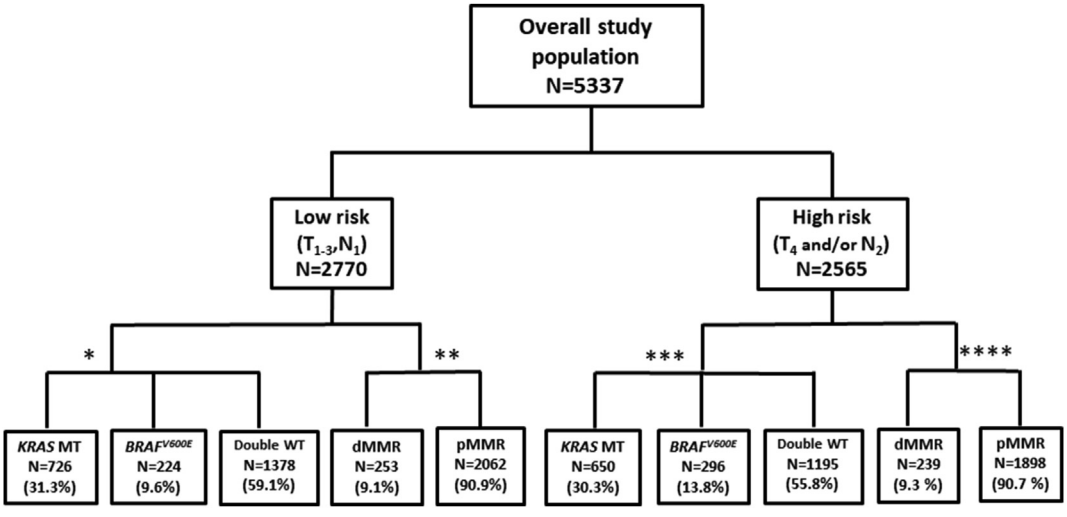

Fig. 1. Flow chart of the study population of stage III colon cancer patients from two adjuvant chemotherapy trials by T (tumour), N (lymph node) risk group. Tumour molecular characteristics within each risk group are shown. dMMR, deficient mismatch repair; pMMR, proficient mismatch repair. *Missing data, unable to subgroup (n = 442); **Missing MMR status (n = 422); ***Missing data, unable to subgroup (n = 424); ****Missing MMR data (n = 428).

risk group were 650 (30.3%) for *KRAS*, 296 (13.8%) for $BRAF^{V600E}$, and 239 (9.3%) for dMMR. A flow chart of the study population is shown in Fig. 1. Given that the association of *BRAF* with prognosis is ideally interpreted in the context of MMR status, we evaluated a *BRAF*/MMR combined variable. For *BRAF*/MMR, numbers for low and high risk subgroups are shown in Table 1. Median patient follow-up was 83.4 months.

In both risk groups, patients with $BRAF^{V600E}$/dMMR tumours were signficantly older and more likely female consistent with a sporadic origin [9], and tumours showed high grade histology and a predilection for the right colon (Table 1). In comparison, patients with WT *BRAF*/dMMR tumours were younger and more likely to be male (Table 1), as were patients with mutant *KRAS*/dMMR tumours (Supplemental Table 1) independent of risk group. $BRAF^{V600E}$/pMMR tumours were significantly associated with high grade histology and right versus left sidedness compared to WT *BRAF*/pMMR tumours in both risk groups (Table 1). *KRAS* mutant/pMMR tumours were more likely to have low grade histology and were evenly distributed by primary site (Supplemental Table 1). Among patients with high risk tumours, an increased number of positive regional lymph nodes was seen in patients with $BRAF^{V600E}$/pMMR tumours compared to the other subgroups (Table 1).

3.2. Association of molecular and clinical variables with clinical outcome by risk group

To enhance the interpretation of clinical and molecular features in stage III tumours, we analysed their relationship to clinical outcome variables by risk group. In a univariate analysis, primary tumour sidedness was prognostic for OS and SAR among patients with low risk tumours and for DFS, OS, and SAR among those with high risk tumours (Table 2). Specifically, patients with right- and left-sided tumours had significantly

Table 1
Association of *BRAF*/MMR and clinicopathological features in low and high risk groups of stage III colon cancer patients.

	Low Risk (T_{1-3} N1)				
	BRAF WT/pMMR (n = 1845)	*BRAF* WT/dMMR (n = 133)	*BRAF* MT/pMMR (n = 105)	*BRAF* MT/dMMR (n = 108)	*P* value
Age, median (range)	59 (19–82)	55 (28–80)	62 (28–80)	66 (43–86)	<.0001
Sidedness					
Left (%)	1153 (63%)	26 (19%)	32 (31%)	9 (8%)	<.0001
Right (%)	682 (37%)	106 (80%)	72 (69%)	99 (92%)	
Grade					
Low (%)	1603 (87%)	79 (60%)	78 (75%)	53 (49%)	<.0001
High (%)	240 (13%)	53 (40%)	27 (25%)	55 (51%)	
Gender					
Male (%)	1037 (56%)	73 (55%)	55 (52%)	29 (27%)	<.0001
Female (%)	808 (44%)	60 (45%)	50 (48%)	79 (73%)	
LNs, median (range)	2 (1–3)	1 (1–3)	1 (1–3)	2 (1–3)	.5705
Performance Status					
0	1454 (80%)	107 (82%)	81 (80%)	79 (74%)	.3878
1	359 (20%)	23 (18%)	20 (20%)	28 (26%)	
	High Risk (T_4 and/or N_2)				
	BRAF WT/pMMR (n = 1596)	*BRAF* WT/dMMR (n = 128)	*BRAF* MT/pMMR (n = 183)	*BRAF* MT/dMMR (n = 103)	*P* value
Age, median (range)	58 (19–85)	51 (23–80)	61 (27–81)	67 (45–84)	<.0001
Sidedness					
Left (%)	972 (61%)	35 (28%)	49 (27%)	6 (6%)	<.0001
Right (%)	612 (39%)	90 (72%)	134 (73%)	97 (94%)	
Grade					
Low (%)	1212 (77%)	57 (45%)	96 (53%)	43 (42%)	<.0001
High (%)	371 (23%)	69 (55%)	86 (47%)	59 (58%)	
Gender					
Male (%)	911 (57%)	75 (59%)	83 (45%)	22 (21%)	<.0001
Female (%)	685 (43%)	53 (41%)	100 (55%)	81 (79%)	
LNs, median (range)	6 (1–33)	5 (1–31)	7 (1–51)	5 (1–22)	<.0001
Performance Status					
0	1202 (77%)	99 (80%)	132 (74%)	69 (68%)	.1326
1	357 (23%)	25 (20%)	47 (26%)	32 (32%)	

WT: wild-type; MT: mutant. LN: lymph nodes.

Table 2
Univariate analysis of clinical and molecular features with patient survival in low and high risk stage III colon cancers.

Variable	DFS			OS			SAR		
	Total	Hazard Ratio (95% CI)	*P* value	Total	Hazard Ratio (95% CI)	*P* value	Total	Hazard Ratio > (95% CI)	*P* value
Low Risk									
Age	2770	1.02 (1.01–1.03)	<.0001[1]	2770	1.04 (1.03–1.05)	<.0001[1]	473	1.02 (1.00–1.03)	.0070[1]
Sidedness	2754		.7893[2]	2754		.0248[2]	472		.0002[2]
Left	1584	Ref		1584	Ref		272	Ref	
Right	1170	1.02 (0.87–1.20)	.7893[1]	1170	1.25 (1.03–1.52)	.0248[1]	200	1.57 (1.24–2.00)	.0002[1]
BRAF/MMR	2191		.0674[2]	2191		.0427[2]	370		.0019[2]
WT/pMMR	1845	Ref		1845	Ref		322	Ref	
MT/pMMR	105	1.24 (0.85–1.82)	.2662[1]	105	1.61 (1.06–2.44)	.0259[1]	23	2.17 (1.35–3.48)	.0014[1]
WT/dMMR	133	0.72 (0.47–1.11)	.1345[1]	133	0.68 (0.40–1.17)	.1615[1]	15	0.64 (0.30–1.37)	.2503[1]
MT/dMMR	108	0.62 (0.38–1.02)	.0617[1]	108	0.79 (0.45–1.37)	.3993[1]	10	2.05 (0.96–4.36)	.0635[1]
KRAS	2770		.0001[2]	2770		.0005[2]	473		.1861[2]
Wild-type	2044	Ref		2044	Ref		308	Ref	
Mutated	726	1.40 (1.18–1.66)	.0001[1]	726	1.44 (1.17–1.76)	.0005[1]	165	1.18 (0.92–1.50)	.1861[1]
PS	2714		.0002[2]	2714		<.0001[2]	464		.0029[2]
0	2186	Ref		2186	Ref		361	Ref	
1	515	1.36 (1.12–1.65)	.0016[1]	515	1.63 (1.30–2.04)	<.0001[1]	99	1.51 (1.14–2.00)	.0036[1]
Gender	2770		<.0001[2]	2770		<.0001[2]	473		.6734[2]
Male	1527	Ref		1527	Ref		282	Ref	
Female	1243	0.71 (0.60–0.83)	<.0001[1]	1243	0.64 (0.53–0.79)	<.0001[1]	191	0.95 (0.74–1.21)	.6734[1]
Histologic Grade	2758		.7869[2]	2758		.3201[2]	469		.0047[2]
Low	2299	Ref		2299	Ref		384	Ref	
High	459	1.03 (0.83–1.28)	.7869[1]	459	1.14 (0.88–1.47)	.3201[1]	85	1.52 (1.14–2.04)	.0047[1]
LNs	1455	0.99 (0.98–1.00)	.0256[1]	1455	0.99 (0.97–1.00)	.0595[1]	266	1.00 (0.98–1.01)	.5615[1]
High Risk									
Age	2565	1.01 (1.00–1.01)	.0040[1]	2565	1.02 (1.01–1.02)	<.0001[1]	988	1.01 (1.01–1.02)	0.0006[1]
Sidedness	2547		<.0001[2]	2547		<.0001[2]	984		<.0001[2]
Left	1376	Ref		1376	Ref		497	Ref	
Right	1171	1.32 (1.18–1.49)	<.0001[1]	1171	1.69 (1.47–1.94)	<.0001[1]	487	1.74 (1.50–2.02)	<.0001[1]
BRAF/MMR	2010		.2052[2]	2010		<.0001[2]	803		<.0001[2]
WT/pMMR	1596	Ref		1596	Ref		635	Ref	
MT/pMMR	183	1.23 (0.99–1.54)	.0618[1]	183	1.73 (1.38–2.18)	<.0001[1]	85	2.86 (2.24–3.64)	<.0001[1]
WT/dMMR	128	1.08 (0.82–1.41)	.5897[1]	128	1.10 (0.80–1.51)	.5647[1]	47	1.16 (0.81–1.67)	0.4079[1]
MT/dMMR	103	1.19 (0.89–1.60)	.2486[1]	103	1.54 (1.12–2.11)	.0073[1]	36	2.23 (1.55–3.21)	<.0001[1]
KRAS	2565		.0005[2]	2565		.0003[2]	988		.4288[2]
Wild-type	1915	Ref		1915	Ref		690	Ref	
Mutated	650	1.26 (1.10–1.43)	.0005[1]	650	1.31 (1.13–1.51)	.0003[1]	298	1.06 (0.91–1.24)	.4288[1]
PS	2500		<.0001[2]	2500		<.0001[2]	965		.0242[2]
0	1942	Ref		1942	Ref		735	Ref	
1	545	1.21 (1.05–1.39)	.0083[1]	545	1.35 (1.15–1.58)	.0002[1]	221	1.23 (1.03–1.45)	.0199[1]
Gender	2565		.2976[2]	2565		.1804[2]	988		.4310[2]
Male	1388	Ref		1388	Ref		538	Ref	
Female	1177	0.94 (0.83–1.06)	.2976[1]	1177	0.91 (0.79–1.04)	.1804[1]	450	0.94 (0.81–1.09)	.4310[1]
Histologic Grade	2545		.0002[2]	2545		<.0001[2]	981		<.0001[2]
Low	1823	Ref		1823	Ref		678	Ref	
High	722	1.27 (1.12–1.45)	.0002[1]	722	1.50 (1.30–1.73)	<.0001[1]	303	1.56 (1.34–1.82)	<.0001[1]
LNs	1323	1.00 (0.99–1.01)	.9633[1]	1323	1.00 (0.99–1.01)	.8460[1]	529	1.00 (0.99–1.01)	.9623[1]

Age and LNs are continuous variables (1 unit increase).
[1] Covariate Wald *P* value.
[2] Type 3 Wald *P* value. LNs: lymph nodes.

poorer survival. Interestingly, women had significantly better DFS and OS than did men that was exclusive to low risk tumours (Table 2). Data for age and performance status (PS) are shown in Table 2. Univariately and in both low and high risk groups, patients with $BRAF^{V600E}$/pMMR tumours had significantly shorter OS and SAR compared to those with WT *BRAF*/pMMR tumours (versus reference) (Table 2). Among high risk tumours, those that were $BRAF^{V600E}$/dMMR also showed significantly poorer OS (HR = 1.54; 95% CI: 1.12–2.11; $P = .0073$) and SAR (HR = 2.23; 95% CI: 1.55–3.21; $P < .0001$) versus reference (Table 2). These findings for *BRAF*/MMR in high risk patients are shown in Kaplan-Meier plots (Fig. 2A and B). Mutant

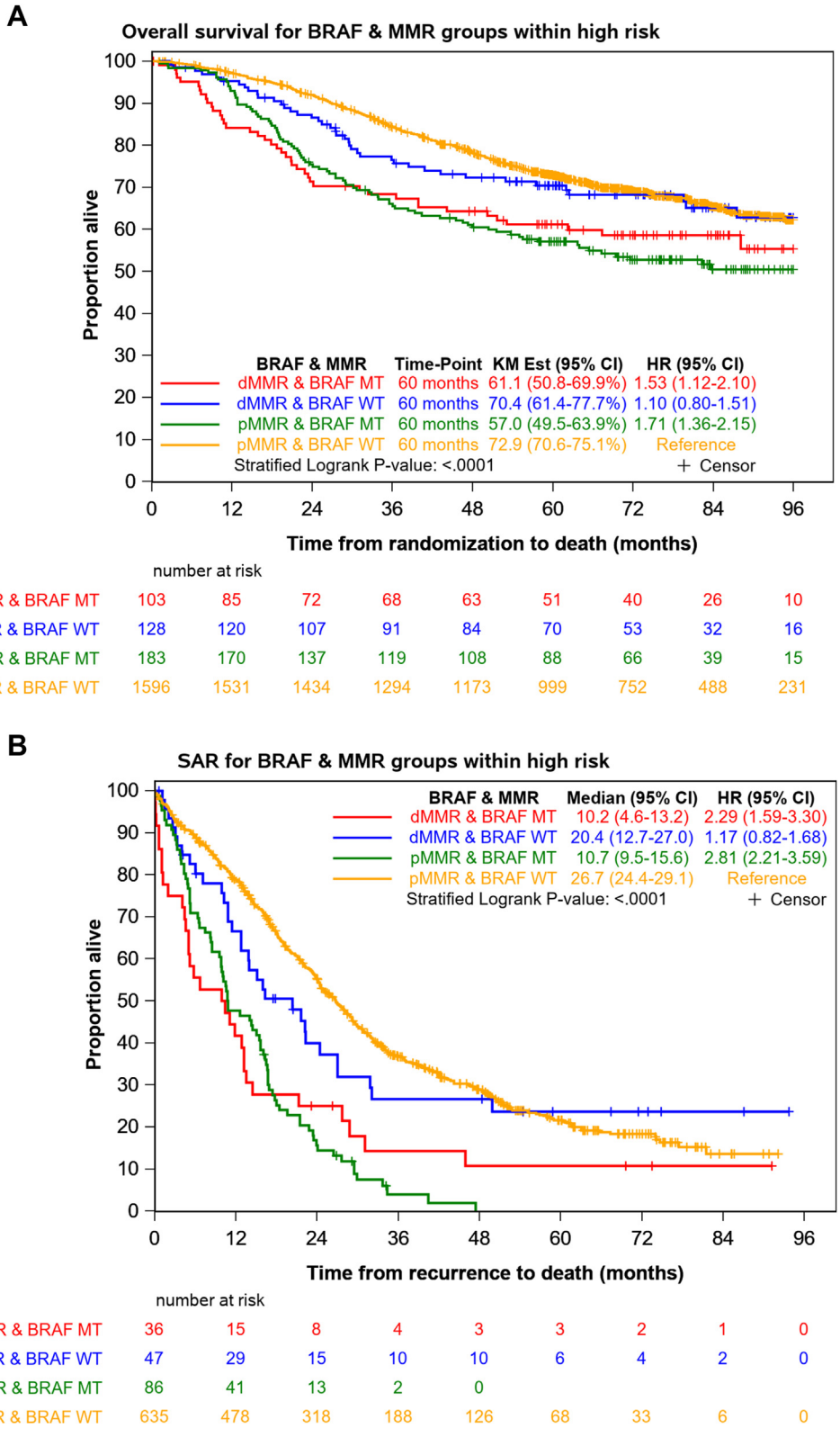

Fig. 2. Univariate association of *BRAF*/MMR with overall survival (OS) and survival after recurrence (SAR) in patients with stage III colon cancer. All patients were treated with FOLFOX-based adjuvant chemotherapy. (A), Kaplan-Meier (KM) plot of OS for the *BRAF*/MMR combined variable in patients with high (T_4 and/or N_2) risk tumours. (B) K-M plot of SAR is shown for *BRAF*/MMR among patients with high risk tumours. *P* values are derived from the stratified logrank test. HR, hazard ratio; CI, confidence interval; deficient (d) MMR; proficient (p) pMMR; WT, wild type.

versus WT *KRAS* was associated with significantly shorter DFS and OS, but not SAR in both risk groups (Table 2).

By multivariable analysis, high versus low risk patients had significantly poorer DFS (HR = 2.42; 95% CI: 2.16–2.71; P_{adj}<.0001), OS [HR = 2.61; 95% CI: 2.29–2.98; P_{adj}<.0001], and SAR [HR = 1.54; 95% CI: 1.32–1.81; P_{adj}<.0001] independent of covariates. At 5 years of follow-up, only 57.2% (95% CI: 55.1–59.5) of high risk patients were alive and disease-free compared to 79.2% (95% CI: 77.5–81.0) of low risk patients. Analysis of individual variables by risk group was then performed. Significantly poorer OS was found for patients with high versus low risk tumours of the right colon (HR = 2.84; 95% CI: 2.36–3.41; $P_{adj} < .0001$) and left-colon (HR = 2.35; 95% CI: 1.94–2.85; $P_{adj} < .0001$), and those harbouring $BRAF^{V600E}$ (HR = 3.08; 95% CI: 2.12–4.48; $P_{adj} < .0001$), mutant *KRAS* (HR = 2.43; 95% CI: 1.97–3.00; $P_{adj} < .0001$), proficient MMR (HR = 2.46; 95% CI: 2.14–2.83; $P_{adj} < .0001$), or deficient MMR (HR = 4.18; 95% CI: 2.69–6.49; $P_{adj} < .0001$ [Fig. 3A–F].

The multivariable association of clinical and molecular features with patient survival by risk group is shown in Table 3. We performed model selection procedures in low and high risk groups, separately, where initial models included variables of age, gender, primary tumour sidedness, PS, *BRAF*/MMR, and *KRAS*. Since all associations with outcome variables (DFS, OS, SAR) by risk group showed $P < .10$, we sought to determine which variables independently contributed the most to prognosis by performing backward elimination utilizing Cox models. This procedure was repeated for each of the three survival outcomes. The multivariable association of primary tumour sidedness with DFS and OS was limited to high risk patients. Specifically, high risk patients with right- versus left-sided tumours had significantly worse

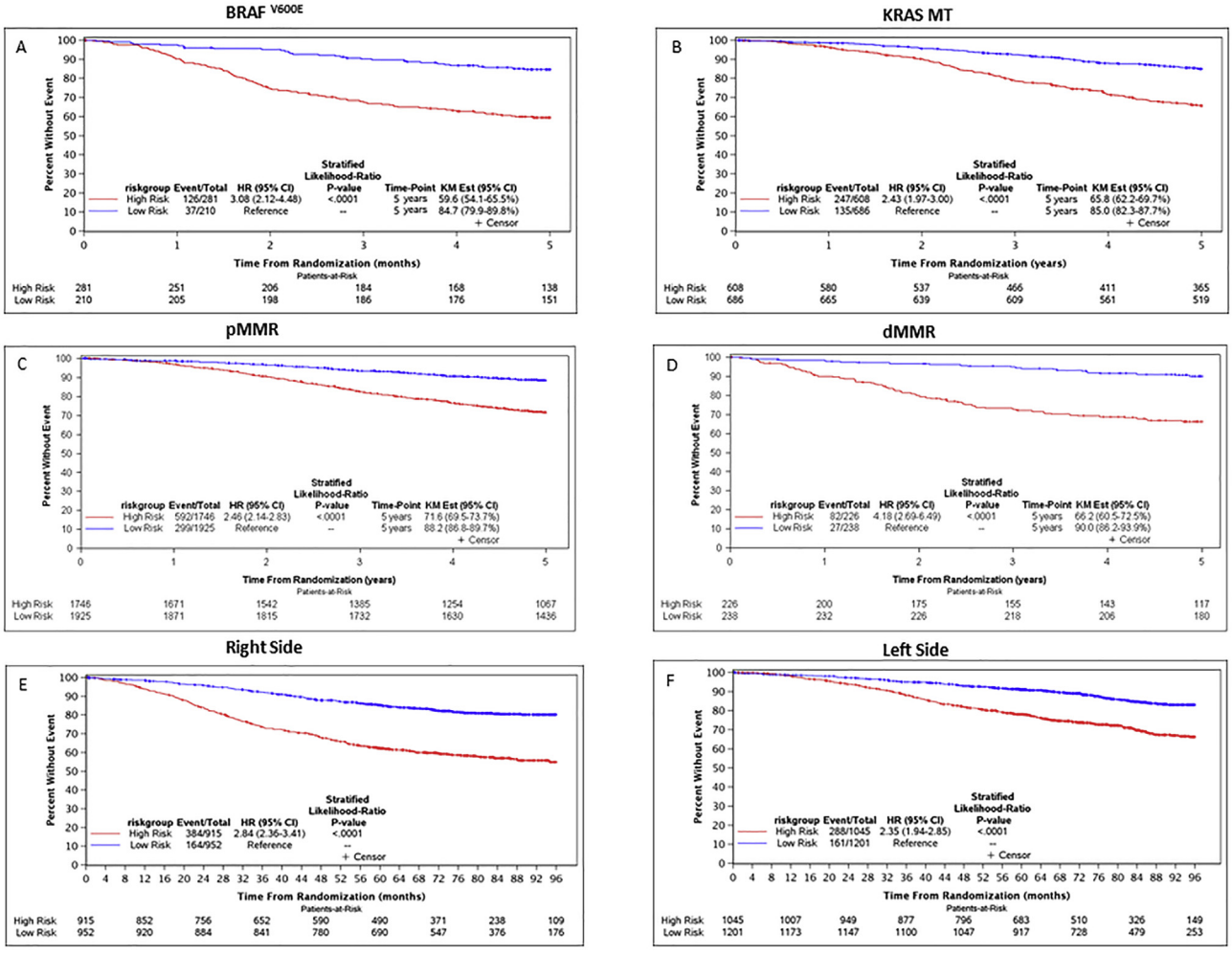

Fig. 3. Overall survival (OS) by $BRAF^{V600E}$ (A), *mutant (MT) KRAS (B)* pMMR (C), dMMR (D) and right (E) or left (F) primary tumour sidedness in low ($T_{1\text{-}3}N_1$) versus high (T_4 and/or N_2) risk stage III colon cancer patients. pMMR: proficient mismatch repair; dMMR: deficient mismatch repair.

Table 3
Multivariable Cox models with backward elimination in low and high risk stage III colon cancers.

Variable	Low Risk (T_{1-3}, N_1)					
	DFS		OS		SAR	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.02 (1.01, 1.03)	<.0001	1.03 (1.02, 1.04)	<.0001	1.02 (1.00, 1.03)	.0462
Gender						
Male	REF		REF			
Female	0.71 (0.60, 0.84)	<.0001	0.65 (0.53, 0.80)	<.0001		
KRAS						
Wild-type (WT)	REF		REF			
Mutated (MT)	1.38 (1.16, 1.64)	.0003	1.41 (1.14, 1.73)	.0012		
PS						
0	REF				REF	
1	1.31 (1.08, 1.59)	.0059	1.53 (1.22, 1.92)	.0002	1.66 (1.22, 2.28)	.0014
BRAF & MMR						
pMMR & WT					REF	
pMMR & MT					1.82 (1.11, 2.98)	.0173
dMMR & WT					0.60 (0.28,1.28)	.1869
dMMR & MT					1.91 (0.89, 4.09)	.0964
Sidedness						
Left					REF	
Right					1.76 (1.33, 2.32)	<.0001
	High Risk (T_4 and/or N_2)					
	DFS		OS		SAR	
Variable	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
KRAS						
Wild type (WT)	REF		REF		REF	
Mutated (MT)	1.25 (1.10 1.43)	.0009	1.43 (1.20, 1.70)	<.0001	1.23 (1.02, 1.48)	.0268
PS						
0	REF		REF		REF	
1	1.22 (1.06, 1.402	.0063	1.34 (1.12, 1.59)	.0010	1.17 (0.98, 1.42)	.0913
BRAF & MMR						
pMMR & WT			REF		REF	
pMMR & MT			1.75 (1.36, 2.24)	<.0001	2.62 (2.04, 3.42)	<.0001
dMMR & WT			1.09 (0.78, 1.51)	.6233	1.21 (0.84, 1.74)	.3020
dMMR & MT			1.30 (0.92, 1.83)	.1325	1.80 (1.21, 2.67)	.0035
Sidedness						
Left	REF		REF		REF	
Right	1.29 (1.15, 1.46)	<.0001	1.56 (1.31, 1.83)	<.0001	1.64 (1.37, 1.95)	<.0001

DFS (HR = 1.29; 95% CI: 1.15–1.46, $P_{adj} < .0001$) and OS (HR = 1.56; 95% CI: 1.31–1.83, $P_{adj} < .0001$) independent of covariates (Table 3). For SAR, significantly shorter SAR was observed for right- versus left-sided tumours in both risk groups (low risk: HR = 1.76, 95% CI: 1.33–2.32, $P_{adj} < .0001$; high risk: HR = 1.64, 95% CI: 1.37–1.95, $P_{adj} < .0001$). Female versus male gender was associated with significantly better DFS and OS (HR = 0.65, 95% CI: 0.53–0.80; $P_{adj} < .0001$) in low risk but not high risk patients; gender was eliminated from the model for SAR (Table 3). PS was significantly associated with all outcome variables independent of risk group. Age was significantly prognostic only among low risk patients.

Among patients with low risk tumours, *BRAF*/MMR was eliminated from the models for DFS and for OS. Patients with low risk tumours that were $BRAF^{V600E}$/pMMR had significantly shorter SAR (HR = 1.82; 95% CI: 1.11–2.97, $P_{adj} = .017$) (Table 3). Similarly, low risk $BRAF^{V600E}$/dMMR tumours had shorter SAR (HR = 1.91; 95% CI: 0.89–4.08, $P_{adj} = .096$) that did not achieve statistical significance likely due to small patient numbers. Mutant *KRAS* was associated with significantly shorter DFS and OS (HR = 1.41; 95% CI: 1.14–1.73, $P_{adj} = .0012$), but not SAR among low risk tumours (Table 3). Among high risk tumours, *BRAF*/MMR was prognostic for OS and SAR. Compared to those with WT *BRAF*, $BRAF^{V600E}$/pMMR tumours had significantly shorter OS (HR = 1.75; 95% CI: 1.36–2.24, $P_{adj} < .0001$) which did not achieve statistical significance for $BRAF^{V600E}$/dMMR tumours (Table 3). For the endpoint of SAR, however, high risk $BRAF^{V600E}$/pMMR tumours (HR = 2.62; 95% CI: 2.04–3.42, $P_{adj} < 0.0001$) and $BRAF^{V600E}$/dMMR (HR = 1.80; 95% CI: 1.21–2.67, $P_{adj} = .0035$) tumours each had significantly shorter SAR (Table 3). Mutant

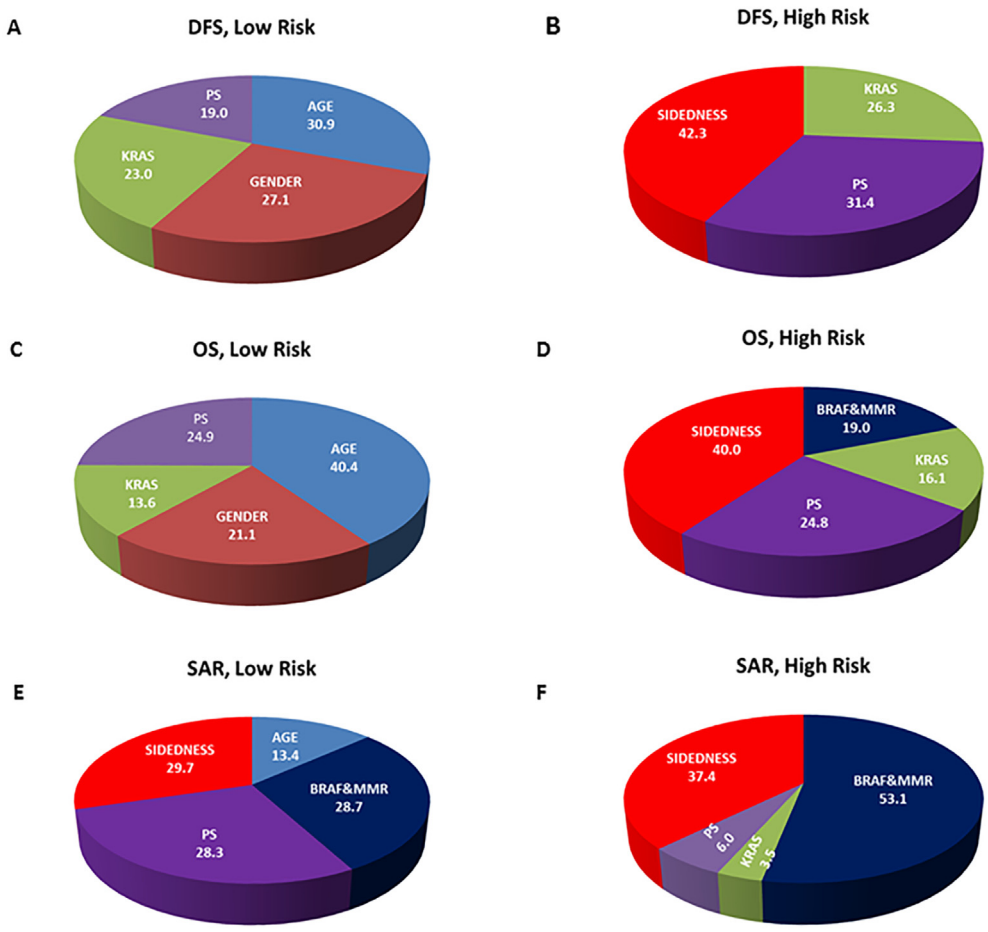

Fig. 4. Relative contribution (in percentage) of clinical and molecular tumour features to the prediction of disease-free survival (DFS)[*top*], overall survival (OS)[*middle*], and survival after recurrence (SAR) [*bottom*] among all stage III patients with low risk ($T_{1\text{-}3}N_1$)[*left panel*] or high risk (T_4 and/or N_2) [*right panel*] cancers. MMR, mismatch repair; PS, performance status; primary tumour site, left vs. right.

KRAS was associated with significantly shorter DFS, OS (OS: HR = 1.43; 95% CI: 1.20–1.70, $P_{adj} < .0001$), and SAR.

3.3. Relative contribution to patient survival

We determined the relative contribution (in percent) of each clinical and molecular feature to patient survival in low and high risk groups. Among low risk tumours, sidedness was one of the top three contributors only to SAR (Fig. 4). However, sidedness was the highest contributor to DFS (42.3%) and OS (40%) among high risk tumours, and was second (37.4%) only to *BRAF*/MMR (53.1%) as a contributor to SAR. Therefore, sidedness ranked first as a contributor to DFS and OS among high risk patients and ranked second for SAR (Fig. 4B, D, and F). The contribution of *BRAF*/MMR to SAR increased nearly twofold in high versus low (53.1% vs. 28.7%) risk patients (Fig. 4E and F). Age and gender were the top two contributors to DFS and OS among low, but not high, risk tumours. Mutant *KRAS* had a similar relative contribution to DFS and OS in low and high risk patients whereby it ranked third for DFS and was fourth for OS among both low and high risk tumours, respectively (Fig. 4). *KRAS* did not contribute appreciably to SAR.

3.4. Primary tumour sidedness and risk group

Patients with right- versus left-sided tumours in both risk groups were older and their tumours were significantly more likely to be high grade and to harbour mutant *KRAS* and any *BRAF*/dMMR (*data not shown*). Those with right-sided tumours were also more likely to be female among low, but not high, risk patients. None of the other variables differed signficantly by tumour site. Significantly poorer DFS, OS, and SAR were found for patients with high versus low risk tumours of both the right colon (DFS: HR = 2.63; 95% CI: 2.23–3.10; $P_{adj} < .0001$); *SAR:* HR = 1.48; 95% CI: 1.19–1.84; $P_{adj} = .0003$) and the left colon (DFS: HR = 2.23; 95% CI: 1.91–2.61; $P_{adj} < .0001$); SAR: HR = 1.59; 95% CI: 1.26–2.00; $P_{adj} < .0001$).

We performed an exploratory analysis of patient survival by tumour sidedness and study treatment arm

in stage III colon cancers. Of note, the addition of cetuximab to FOLFOX failed to improve the primary endpoint of patient DFS in the two adjuvant trials included in this report [1,3]. A multivariate test of interaction between sidedness and treatment arm for survival outcomes did not achieve statistical significance. Furthermore, similar DFS and OS were observed by treatment arm in both right- and left-sided cancers with WT *KRAS* (*data not shown*).

4. Discussion

After publication of the IDEA study [4], T, N risk grouping has been widely adopted by oncologists for guiding the recommended duration of adjuvant chemotherapy in stage III colon cancer and has been endorsed by current guidelines. In this report, we sought to identify clinical and/or molecular feature(s) that can best predict patient survival by risk group [low risk ($T_{1\text{-}3}N_1$); high risk (T_4, N_2, or both)] using data from two phase 3 adjuvant trials that evaluated a fluoropyrimidine plus oxaliplatin. Risk grouping was shown to provide robust prognostic stratification in our cohort. We then compared the association of variables with survival between risk groups. By multivariable analysis, mutant *KRAS* was significantly associated with poorer DFS and OS independent of risk group, and showed a similar relative contribution to these survival outcomes. In contrast, $BRAF^{V600E}$/pMMR tumours had significantly poorer OS that was limited to high risk tumours; yet the association with SAR was significant in both risk groups. The association of primary tumour sidedness with DFS and OS was limited to high risk tumours, although significantly shorter SAR was observed for right versus left-sided tumours in both low and high risk groups. Importantly, the relative contribution of sidedness was highest among variables examined for DFS and OS among high but not low risk tumours, and its contribution to SAR was only exceeded by $BRAF^{V600E}$/pMMR tumours among high risk patients. Differences in the biology of colon cancer based on sidedness has been shown by multi-omics including differentially expressed genes (*TP53, KRAS,* $BRAF^{V600E}$*, PIK3CA, SMAD4, CTNNB1,* and *PTEN* [14]), miRNAs, and DNA methylation profiles [15,16]. In addition, transcriptomically determined consensus molecular subtypes (CMS) were shown to vary by tumour sidedness with a decrease in CMS1 and CMS3 and an increase in CMS2 prevalence moving from the right to left colon [14]. CMS2 colon cancers are characterized by WNT and Myc signaling activation. The gut microbiome may also be a factor in that CRCs with a high abundance of *Fusobacterium nucleatum* DNA were found to increase in prevalence from rectum to cecum [17]. DNA abundance of this anaerobic bacterium was inversely related to intratumoral $CD3^+$ T-cell density [18] and was associated with significantly poorer prognosis in patients with CRC [19].

Prior studies have not adequately examined *BRAF* in the context of MMR status which can lead to misinterpretation of its prognostic impact and inconsistencies between studies [11,20]. *BRAF*/MMR was prognostic among high risk patients for OS and was the primary driver of SAR as shown by its relative contribution, which increased nearly twofold in high versus low risk tumours (28.7%–53.1%). Accordingly, the adverse impact of oncogenic $BRAF^{V600E}$ on patient survival is strongest following tumour recurrence. An important observation was that high risk tumours harbouring $BRAF^{V600E}$/pMMR or $BRAF^{V600E}$/dMMR each had significantly poorer SAR (versus reference). These data indicate that any 'protective' effect of dMMR seen in $BRAF^{V600E}$ tumours is lost after tumour recurrence. These results for SAR support data in metastatic CRCs where $BRAF^{V600E}$ is associated with poor prognosis independent of MMR status [21,22]. Further analysis revealed that poorer SAR seen for $BRAF^{V600E}$/dMMR tumours was limited to patients who received cetuximab (Supplemental Table 2). For patients with metastatic CRC harbouring $BRAF^{V600E}$, targeted therapy with encorafenib and cetuximab has been shown to improve outcomes and is now an FDA-approved treatment option [23]. Furthermore, use of an immune checkpoint inhibitor is an FDA-approved treatment for patients with metastatic CRCs with dMMR irrespective of *BRAF* status both as first-line [24] and as salvage therapy [25]. Furthermore, a phase 3 adjuvant trial is ongoing to evaluate an anti-PD-L1 antibody in patients with resected stage III colon cancers with dMMR [26].

We observed that women had significantly better DFS and OS than did men that was confined to low risk tumours. Furthermore, gender and age were the top two contributors to DFS and OS among low, but not high, risk tumours. In support of these findings, consistent results were obtained from analysis of time-to-recurrence for these variables (*data not shown*). Population-based data have shown a survival advantage for female compared to male CRC patients whose mechanism has been speculated to be related to the effect of sex hormones, either endogenous or through hormonal replacement therapy [27–29]. Not unexpectedly, PS was an important contributor to patient outcomes. In an exploratory analysis, we examined the potential predictive role of sidedness for benefit from cetuximab given data in metastatic CRC whereby patients with right versus left-sided primary tumours have poorer survival when treated with an anti-EGFR antibody plus chemotherapy [30,31]. However, an interaction test was not significant and similar patient survival was found for WT *KRAS* tumours of the right and left colon by treatment arm.

Strengths of our study include prospective collection of tumour tissues with analysis of molecular features, the randomized phase III trial designs, and the uniform treatment and rigorous patient follow-up. Study limitations include the retrospective pooled analysis and the absence of expanded *RAS* mutation assessment, although mutations in *NRAS* and *HRAS* occur in fewer than 5% of CRCs [32] such that their inclusion would be unlikely to have substantially altered our results. While similar patient outcomes were seen by study arm in both the N0147 and PETACC-8 adjuvant trials [1,3], we acknowledge the potential for cetuximab to influence biomarker-related outcomes. However, analysis by study arm was performed and the main treatment-related finding was that $BRAF^{V600E}$/dMMR tumours showed poorer SAR only among those who received FOLFOX and cetuximab versus FOLFOX alone (Supplemental Table 2), yet cautious interpretation is warranted given small patient numbers. The fact that all patients received adjuvant therapy precludes a predictive analysis. We emphasize that with the exception of MMR status, molecular profiling of patients with stage III colon cancer is not standard of care although such data have prognostic value, but have not been shown to be predictive for adjuvant treatment.

In conclusion, the prognostic impact and relative contribution of clinical and molecular features show important differences by IDEA study-defined risk grouping in patients with stage III colon cancer. Whereas the relative contribution of *KRAS* to survival was largely independent of risk group, the impact of *BRAF*/MMR on outcome was limited to high risk tumours for OS, and was the primary driver of SAR especially among high risk patients. Importantly, worse SAR observed for $BRAF^{V600E}$ tumours was independent of MMR status which indicates loss of a 'protective' effect of dMMR at tumour recurrence. As with *BRAF*/MMR, the prognostic impact of primary tumour sidedness was primarily seen in high risk tumours where it was a top contributor to all survival outcomes in high risk, but not low risk, patients. Taken together, these data indicate that the prognostic impact of *BRAF*/MMR and tumour sidedness depend on risk group and should be interpreted in this context. Our data serve to refine prognostication by risk group in stage III patients, and demonstrate the clinical utility of integrating molecular analysis with anatomic tumour staging.

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Author contribution

Frank Sinicrope: Study conceptualization, formal data analysis and interpretation, manuscript writing, review and editing, supervision and funding acquisition; **Sakti Chakrabarti**: Formal data analysis, manuscript writing and review; **Pierre Laurent-Puig**: Data acquisition, Manuscript review; **Luke Huebner**: Statistical analysis, quality control of data and algorithms, manuscript review; **Thomas C. Smyrk**: Data acquisition, manuscript review; **Josep Tabernero**: Data acquisition, manuscript review; **Enrico Mini**: Data acquisition, manuscript review; **Richard M. Goldberg**: Data acquisition, manuscript review; **Aziz Zaanan**: Data acquisition, manuscript review; **Gunnar Folprecht**: Data acquisition, manuscript review; **Jean Luc Van Laethem**: Data acquisition, manuscript review; **Karine Le Malicot**: Data acquisition, manuscript review, quality control of data and algorithms, Statistical analysis; **Qian Shi**: Statistical analysis, quality control of data and algorithms, Data acquisition, manuscript review; **Steven R. Alberts**: Data acquisition, manuscript review; **Julien Taieb**: Data acquisition, manuscript review.

Conflict of interest statement

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2020.11.016.

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