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Gastrointestinal Cancer

Wild-type *APC* Is Associated with Poor Survival in Metastatic Microsatellite Stable Colorectal Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. *APC* • Metastatic colorectal cancer • Microsatellite stable • prognostic • Wnt signaling

Abstract

Background. The prognostic implication of wild-type *APC* (*APC*-WT) in microsatellite stable (MSS) metastatic colorectal cancer (mCRC) is not well defined.

Materials and Methods. *APC* prognostic value was evaluated retrospectively in two independent cohorts of patient with MSS mCRC with a confirmatory analysis from a public data set from Memorial Sloan Kettering Cancer Center (MSKCC).

Results. In comparison with the *APC*-mutant (*APC*-MT) population (n = 255), *APC*-WT patients (n = 86) tended to be younger (59% of age < 40 vs. 26% of age > 50), right-sided (41.7% vs. 27%), $BRAF^{V600E}$ mutated (23.3% vs. 0.8%), and *KRAS* wild type (65.1% vs. 49.8%). Alternative WNT pathway alterations, *RNF43* and *CTNNB1*, were over-represented in the *APC*-WT versus *APC*-MT population (7% vs. 0.4% and 4.7% vs. 0.4%, respectively). *APC*-WT patients had a worse overall survival (OS) than *APC*-MT patients (22.6 vs. 45.6 months, p < .0001). Using a multivariate model correcting for primary tumor location, *RAS* and *BRAF* status, *APC-WT* was predictive of poor survival (*APC*-MT vs. *APC*-WT, hazard ratio [HR], 0.62; 95% confidence interval [CI], 0.44–0.86, p = .0037). The prognostic implication of *APC*-WT on OS was confirmed further in a similar multivariate model of 934 stage IV patients from MSKCC public database (*APC*-MT vs. *APC*-WT, HR, 0.63, 95% CI, 0.49–0.81, p < .0001).

Conclusion. *APC*-WT is associated with poor OS in MSS mCRC regardless of *RAS* and *BRAF* status. Compared with *APC*-MT mCRC tumors, *APC*-WT tumors were associated with other Wnt activating alterations, including *RNF43* and *CTNBB1*. Our data suggest alternative therapy needs to be investigated in *APC*-WT patients. ***The Oncologist*** 2021;26:208–214

Implications for Practice: Patients with microsatellite stable metastatic colorectal cancer with wild-type *APC* had a worse overall survival than patients with mutated *APC* regardless of *RAS/RAF* status. *APC* status should be considered as a stratification factor in prospective trials, and novel therapeutic strategies need to be developed for this subgroup of patients.

Introduction

With an estimated 51,020 deaths in 2019, colorectal cancer is still a leading cause of cancer-related death in the U.S. [1]. Despite the advancements in cytotoxic, biological, and targeted therapy over the last 2 decades, the overall survival of metastatic colorectal cancer at 5 years is 14% [2, 3]. It is increasingly acknowledged that colorectal cancer is a highly heterogeneous disease with diverse molecular and clinical features that affect therapeutic outcomes [4]. A comprehensive genomic disease classification-beyond *RAS*, *BRAF*, and microsatellite stable (MSS) and microsatellite instable (MSI) is needed to better guide the management of this disease [5, 6].

Adenomatous polyposis coli (*APC*) is a tumor-suppressor gene that acts as a gatekeeper of the Wnt/β-catenin pathway by regulating β-catenin phosphorylation [4]. Somatic

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Table 1. Characteristics of patients with microsatellite stable metastatic colorectal cancer

Characteristics	COH/UCD				MSKCC			
	Total (%)(n = 341)	*APC*-WT (n = 86)	*APC*-MT (n = 255)	p value	Total (%)(n = 934)	*APC*-WT (n = 216)	*APC*-MT (n = 718)	p value
Age at diagnosis, median (range), yr	56 (16–88)	55 (16–85)	56 (20–88)	.16	53 (13–93)	53 (13–83)	53 (22–93)	.25
Gender, n (%)								
Male	192 (56.3)	44 (51.2)	148 (58.0)		494 (52.9)	113 (52.3)	381 (53.1)	
Female	149 (43.7)	42 (48.8)	107 (42.0)	.32	440 (47.1)	103 (47.7)	337 (46.9)	.88
Site,[a] n (%)								
Right	103 (30.7)	35 (41.7)	68 (27.0)		241 (26.1)	75 (35.5)	166 (23.3)	
Left	233 (69.3)	49 (58.3)	184 (73.0)	.014	681 (73.9)	136 (64.5)	545 (76.7)	6.60×10^{-04}
$BRAF^{V600E}$, n (%)								
Mutated	22 (6.5)	20 (23.3)	2 (0.8)		49 (5.2)	31 (14.4)	18 (2.5)	
Nonmutated	319 (93.5)	66 (76.7)	253 (99.2)	2.60×10^{-11}	885 (94.8)	185 (85.6)	700 (97.5)	6.98×10^{-10}
KRAS, n (%)								
Mutated	158 (46.3)	30 (34.9)	128 (50.2)		424 (45.4)	92 (42.6)	332 (46.2)	
Nonmutated	183 (53.7)	56 (65.1)	127 (49.8)	.017	510 (54.6)	124 (57.4)	386 (53.8)	.35
NRAS, n (%)								
Mutated	15 (4.4)	3 (3.5)	12 (4.7)		38 (4.1)	1 (.5)	37 (5.2)	
Nonmutated	326 (95.6)	83 (96.5)	243 (95.3)	.77	896 (95.9)	215 (99.5)	681 (94.8)	6.70×10^{-04}
TP53, n (%)								
Mutated	257 (75.4)	69 (80.2)	188 (73.7)		727 (77.8)	169 (78.2)	558 (77.7)	
Nonmutated	84 (24.6)	17 (19.8)	67 (26.3)	.25	207 (22.2)	47 (21.8)	160 (22.3)	.93

[a]Data not available: 5 cases in the COH/UCD and 12 cases in theMSKCC cohort.

Abbreviations: *APC*-MT, *APC* mutation; *APC*-WT, wild-type *APC*; COH, City of Hope National Medical Center; UCD, University of California, Davis.

Table 2. Alterations in genes associated with Wnt signaling pathway

Characteristics	COH/UCD				MSKCC			
	Total (%) (*n* = 341)	*APC*-WT (*n* = 86)	*APC*-MT (*n* = 255)	*p* value	Total (%) (*n* = 934)	*APC*-WT (*n* = 216)	*APC*-MT (*n* = 718)	*p* value
ARID1A								
Mutated	13 (3.8)	3 (3.5)	10 (3.9)	0.999	34 (3.6)	3 (1.4)	31 (4.3)	.059
Nonmutated	328 (96.2)	83 (96.5)	245 (96.1)		900 (96.4)	213 (98.6)	687 (95.7)	
CTNNB1								
Mutated	5 (1.5)	4 (4.7)	1 (0.4)	.015	11 (1.2)	8 (3.7)	3 (0.4)	.00063
Nonmutated	336 (98.5)	82 (95.3)	254 (99.6)		923 (98.8)	208 (96.3)	715 (99.6)	
FBXW7								
Mutated	24 (7.0)	7 (8.1)	17 (6.7)	.82	71 (7.6)	7 (3.2)	64 (8.9)	.005
Nonmutated	317 (93.0)	79 (91.9)	238 (93.3)		863 (92.4)	209 (96.8)	654 (91.1)	
RNF43								
Mutated	7 (2.1)	6 (7.0)	1 (0.4)	.0013	23 (2.5)	21 (9.7)	2 (0.3)	3.26×10^{-12}
Nonmutated	334 (97.9)	80 (93.0)	254 (99.6)		911 (97.5)	195 (90.3)	716 (99.7)	
SOX9								
Mutated	27 (7.9)	2 (2.3)	25 (9.8)	.035	78 (8.4)	10 (4.6)	68 (9.5)	.024
Nonmutated	314 (92.1)	84 (97.7)	230 (90.2)		856 (91.6)	206 (95.4)	650 (90.5)	

Abbreviations: COH, City of Hope National Medical Center; MSKCC, Memorial Sloan Kettering Cancer Center; MT, mutant; UCD, University of California, Davis; WT, wild type.

Table 3. Univariate survival model

Clinicopathologic variable	COH/UCD (*n* = 341)		MSKCC (*n* = 934)	
	HR (95%CI)	*p* value	HR (95%CI)	*p* value
Age at diagnosis, continuous, yr	1.00 (0.98–1.01)	.553	1.01 (1.00–1.02)	.294
Gender, female vs. male	1.00 (0.76–1.32)	.986	0.96 (0.78–1.19)	.713
Primary tumor location, right vs. left	1.61 (1.20–2.16)	.0017	1.64 (1.30–2.07)	3.66×10^{-05}
APC, mutated vs. nonmutated	0.54 (0.40–0.73)	5.95×10^{-05}	0.55 (0.44–0.70)	8.36×10^{-07}
KRAS, mutated vs. nonmutated	1.11 (0.85–1.46)	.447	1.37 (1.10–1.70)	.0044
NRAS, mutated vs. nonmutated	1.20 (0.59–2.45)	.622	1.75 (0.11–2.76)	.0153
BRAF, mutated vs. nonmutated	2.00 (1.30–3.08)	.0016	3.10 (2.27–4.24)	9.67×10^{-13}
$BRAF^{V600E}$, mutated vs. nonmutated	2.56 (1.50–4.35)	5.47×10^{-04}	4.61 (3.22–6.61)	8.17×10^{-17}
TP53, mutated vs. nonmutated	1.33 (0.93–1.88)	.114	1.00 (0.77–1.30)	.989
ARID1A, mutated vs. nonmutated	1.11 (0.56–2.17)	.769	1.03 (0.56–1.87)	.934
CTNNB1, mutated vs. nonmutated	0.95 (0.35–2.55)	.913	1.67 (0.86–3.26)	.133
FBXW7, mutated vs. nonmutated	0.48 (0.25–0.91)	.025	1.20 (0.79–1.84)	.394
RNF43, mutated vs. nonmutated	4.18 (1.84–9.48)	6.38×10^{-04}	3.75 (2.23–6.32)	6.8×10^{-07}
SOX9, mutated vs. nonmutated	0.51 (0.28–0.93)	.029	0.75 (0.49–1.15)	.187

Abbreviations: CI, confidence interval; COH, City of Hope National Medical Center; HR, hazard ratio; MSKCC, Memorial Sloan Kettering Cancer Center, UCD, University of California, Davis.

APC mutations occur in approximately 75% of sporadic colorectal cancer and play a crucial role in the initiation of the adenoma-carcinoma pathway [7–9]. A comparison of genomic alterations between early and late-onset colorectal cancer showed a decreased incidence of *APC* mutations in younger patients [10]. Colorectal cancers with mutated *APC* conferred better overall survival (OS) than wild-type tumors when treated with an epidermal growth factor receptor (EGFR) inhibitor [11]. These data suggest that *APC* mutation may account for EGFR inhibitor sensitivity and provide rationale for refining treatment guidelines in addition to extended *RAS/RAF* testing [11, 12].

Although several studies suggest that patients with colorectal cancer lacking *APC* mutation carry a worse outcome than *APC*-mutated patients [13–15], no prior studies have focused on the impact of *APC*-WT status on the survival of a large cohort of MSS metastatic CRC, especially when factoring in other established prognostic variables such *RAS*, *BRAF*, and sidedness. In this study, we analyzed the genomic profiles of 341 clinically characterized, sporadic, stage IV MSS colorectal tumors. A multivariate model including clinical covariates and *RAS/BRAF* status was deployed to further define the prognostic potential of *APC*. Our observations were further validated in a cohort of 934 patients with

stage IV MSS colorectal cancer from Memorial Sloan Kettering Cancer Center (MSKCC) public data base.

Materials and Methods

Patients

We analyzed 287 patients with MSS stage IV colorectal cancer who had undergone treatment at the City of Hope National Medical Center (COH; Duarte, CA) between 2013 and 2019 and 54 patients with MSS stage IV colorectal cancer who had undergone treatment at University of California, Davis (UCD; Davis, CA) between 2012–2019. Patients' demographics including gender, age, primary tumor location, and survival status were obtained from chart abstraction of each patient's electronic medical record. This study was approved by the Institutional Review Board IRB 14361 (COH) and IRB 1484151-1 (UCD). Memorial Sloan Kettering Cancer Center public data set of 934 patients with metastatic colorectal cancer was used for the validation of our results. [15]

Genomic Analysis

Comprehensive genomic profiling of COH and UCD cohorts were conducted through next-generation sequencing via FoundationOne (Foundation Medicine Inc., Cambridge, MA). The genomic analysis was conducted on DNA extracted form formalin-fixed paraffin-embedded tumor samples retrieved from surgical resection, or biopsies [16]. Samples from MSKCC were sequenced using the MSK-IMPACT assay in the clinical laboratories of the Molecular Diagnostics Service at MSKCC [15].

Mutation Annotation

Somatic mutations were annotated based on OncoKB, a precision oncology knowledge base, for their mutation type and clinical implication (supplementary online Table 1) [17]. Variants implicated as oncogenic, likely oncogenic, and predicted oncogenic were recruited to this analysis. Variants with unknown significance, including likely neutral, inconclusive, and unknown, were filtered out.

Statistical Analysis

For baseline clinical and genomic characteristics, Fisher's exact test was used to compare proportions between patient groups based on *APC* mutation status. OS was examined from the date of documentation of metastatic disease to the date of death. Differences in OS between groups with or without clinically relevant mutations at a given gene of interest were compared using Kaplan-Meier curves, with *p* values calculated via log-rank test. Both univariate and multivariate Cox regression models were applied to estimate the hazard ratios and confidence intervals of survival based on mutation status and other clinical factors.

Results

Study Population

We analyzed a total of 341 patients with stage IV MSS colorectal adenocarcinomas (287 from COH, 54 from UCD).

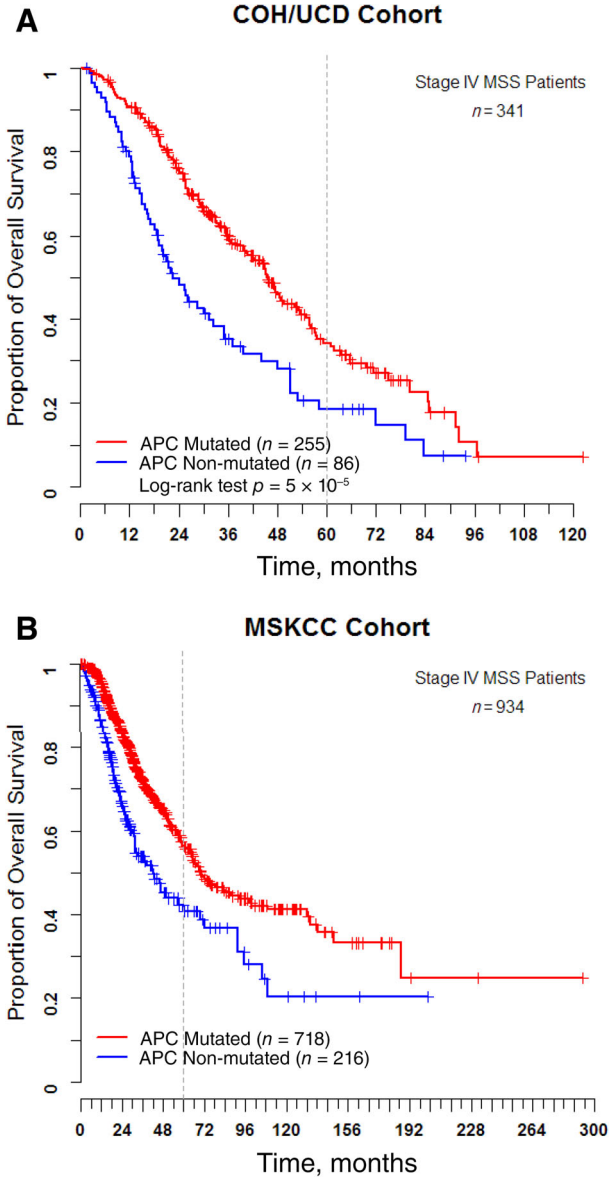

Figure 1. Kaplan-Meier curves for overall survival of patients with MSS mCRC by *APC* status. **(A):** Kaplan-Meier curves for overall survival of the COH/UCD cohort. **(B):** Kaplan-Meier curves for overall survival of the MSCKK cohort.
Abbreviations: COH, City of Hope National Medical Center; MSKCC, Memorial Sloan Kettering Cancer Center; MSS, microsatellite stable; UCD, University of California, Davis.

Median age was 56 years, 56.3% were male, and 43.7% were female. A total of 69.3% (233 of 336) of patients had a left-sided primary tumor. The genomic features of our cohort were, in general, similar to those reported in the MSKCC database (Table 1). *APC* mutations were found in 74.8% (255 of 341) of tumors in the pooled COH/UCD cohort. The frequencies of mutations in *KRAS*, *NRAS*, *BRAF*, and *TP53* were 46.3% (158 of 341), 4.4% (25 of 341), 6.5% (22 of 341), and 75.4% (257 of 341), respectively.

Wild-type-*APC* Was Associated with Right-Sided Primary, *BRAF*^{V600E} Mutation, and Younger Age

Wild-type *APC* (*APC*-WT) was present in 25.2% (86 of 341) of the COH/UCD cohort (Table 1). Right-sided primary tumor

Table 4. Multivariate survival model

	COH/UCD (*n* = 341)		MSKCC (*n* = 934)	
Clinicopathologic variable	**HR (95% CI)**	***p* value**	**HR (95% CI)**	***p* value**
Age at diagnosis, continuous, yr	0.99 (0.98–1.01)	.245	1.00 (0.99–1.01)	.633
Gender, female vs. male	0.95 (0.71–1.27)	.735	0.96 (0.77–1.19)	.721
Primary tumor location, right vs. left	1.45 (1.05–2.02)	.026	1.16 (0.90–1.50)	.258
APC, mutated vs. nonmutated	0.62 (0.44–0.86)	.0037	0.63 (0.49–0.81)	3.87×10^{-04}
KRAS, mutated vs. nonmutated	1.16 (0.86–1.58)	.330	1.72 (1.35–2.20)	1.42×10^{-05}
NRAS, mutated vs. nonmutated	1.62 (0.77–3.40)	.203	2.70 (1.68–4.34)	4.32×10^{-05}
$BRAF^{V600E}$, mutated vs. nonmutated	1.73 (0.89–3.36)	.105	4.79 (3.10–7.39)	1.55×10^{-12}

Abbreviations: CI, confidence interval; COH, City of Hope National Medical Center; HR, hazard ratio; MSKCC, Memorial Sloan Kettering Cancer Center, UCD, University of California, Davis.

was found in 41% of patients with *APC*-WT versus 27% of patients with *APC* mutation (*APC*-MT; $p = .014$). Right-sided tumors were also more frequent in *APC*-WT versus *APC*-MT tumors within the MSKCC cohort (35.5% vs. 23.3%, $p = .00066$). $BRAF^{V600E}$ and *APC* mutations rarely co-occurred. In the COH/UCD cohort, 23.3% of the *APC*-WT tumors harbored $BRAF^{V600E}$ mutation, whereas only 0.8% of the *APC*-MT tumors coexisted with $BRAF^{V600E}$ mutations ($p < .0001$). Similar findings were observed in the MSKCC cohort (14.4% *BRAF* mutations in *APC*-WT vs. 2.5% in *APC*-MT, $p < .0001$). Wild-type *KRAS* was found in 65.1% of *APC*-WT tumors and 49.8% of *APC*-MT tumors ($p = .017$), whereas no significant difference was observed in the MSKCC cohort. No significant difference in *NRAS* mutations was observed between *APC*-WT and *APC*-MT tumors in the COH/UCD cohort, whereas a significant increase in *NRAS* mutations was noted in the *APC*-MT population in MSKCC cohort (5.2% in *APC*-MT vs. 0.5% in *APC*-WT, $p = .00067$).The frequency of mutations in *TP53* were similar between *APC*-WT and *APC*-MT population in both cohorts (Table 1). Interestingly, 59.3% of early onset patients (age < 40) from COH/UCD cohort were *APC*-WT, whereas 25.4% of later onset patients (age > 50) were *APC*-WT ($p = .000563$). MSKCC cohort analysis confirmed a similar trend, 30.3% of early onset patients were *APC*-WT, whereas 22.2% of late onset patients were *APC*-WT ($p = .064$; supplemental online Table 2).

Wild-type-*APC* Was Associated with Alternative WNT Pathway Activating Mutations

Given that WNT pathway is activated in the majority of colorectal cancers, we analyzed mutation frequencies of WNT signaling-related molecules other than *APC* in the COH/UCD cohort and MSKCC cohort. We identified five recurrently mutated genes (mutations present more than once in the COH/UCD and MSKCC cohort) involved in the WNT signaling pathway, which include *ARID1A*, *CTNNB1*, *FBXW7*, *RNF43*, and *SOX9* (Table 2). No significant difference was observed in the frequencies of mutations in *ARID1A* and *FBXW7* in the *APC*-WT versus the *APC*-MT tumors in the COH/UCD cohort, whereas the frequencies of mutations in *FBXW7* was higher in the *APC*-mutated tumors in the MSKCC cohort. The active mutation of *CTNBB1*, a key molecule of WNT pathway downstream of *APC*, was significantly more prevalent in the *APC*-WT tumors compared with the *APC*-MT tumors (COH/UCD cohort, 4.7% vs. 0.4%, $p = .015$; MSKCC cohort, 3.7% vs. 0.4%, $p = .00063$). Mutations in *RNF43*, a WNT signaling inhibitor, were significantly more frequent in the *APC*-WT tumors compared with the *APC*-MT tumors (COH/UCD cohort, 7% vs. 0.4%, $p = .0013$; MSKCC cohort, 9.7% vs. 0.3%, $p < .0001$). In contrast, *SOX9*, a positive regulator of WNT signaling, was significantly more mutated in *APC*-MT tumors compared with *APC*-WT tumors (COH/UCD cohort, 9.8% vs. 2.3%, $p = .035$; MSKCC cohort, 9.5% vs. 4.6%, $p = .024$). Within the *APC*-WT population, $BRAF^{V600E}$ mutation was associated with higher *RNF43* mutations, whereas non-$BRAF^{V600E}$ was associated with higher *RAS* mutation frequency and a trend of higher alternative WNT pathway mutations, such as ARID1A, CTNNB1, and FBXW7 (supplemental online Table 3). In summary, alternative WNT pathway activating mutations were significantly more prevalent in the *APC*-WT tumors compared with the *APC*-MT tumors.

Wild-type-*APC* Was Associated with Poor Prognosis in Metastatic MSS Colorectal Cancer

A systematic univariate survival analysis including patient age, gender, primary tumor location, and genetic alterations in our study showed significant differences in survival associated with tumor location, *APC*, *BRAF* and *RNF43* mutation (Table 3). Similar to numerous prior studies, $BRAF^{V600E}$ mutation was associated with poor prognosis in the COH/UCD cohort (hazard ratio [HR], 2.56; 95% confidence interval [CI], 1.50–4.35; $p < .0001$) and MSKCC cohort (HR, 4.61; 95% CI, 3.22–6.61; $p < .0001$). Right-sided tumors were associated with poor prognosis in both the COH/UCD and MSKCC cohort (COH/UCD cohort: HR, 1.61; 95% CI, 1.20–2.16; $p = .0017$; MSKCC cohort: HR, 1.64; 95% CI, 1.30–2.07; $p < .0001$). Although rare, *RNF43* mutations were associated with poor prognosis in both cohorts (COH/UCD cohort: HR, 4.18; 95% CI, 1.84–9.18; $p < .0001$; MSKCC cohort: HR, 3.75; 95% CI, 2.23–6.32; $p < .0001$). *APC*-WT was associated with inferior outcome in both cohorts (*APC*-MT vs. *APC*-WT, COH/UCD cohort, HR, 0.54; 95% CI, 0.40–0.73; $p < .0001$; MSKCC cohort, HR, 0.55; 95% CI, 0.44–0.70, $p < .0001$). The median overall survival of *APC*-WT patients from the COH/UCD cohort was 22.6 months compared with 45.6 months in *APC*-MT patients ($p < .0001$; Fig. 1A). In the MSKCC cohort, the median overall survival of *APC*-WT patients was 42.6 months, whereas the median overall

survival of *APC*-MT patients is 70.0 months ($p < .0001$; Fig. 1B). In addition, we observed similar results when the COH cohort and UCD cohort were analyzed separately (supplemental online Fig. 1). The longer OS values of the MSKCC cohort are likely due to the high metastasectomy rate in the MSKCC cohort (>50%), whereas most of the patients seeking care at our clinic were not eligible for metastasectomy. On multivariate analysis that includes primary tumor location, *RAS*, and *BRAF* status and *APC* status, APC-WT continued to be predictive of poor survival in the COH/UCD cohort (*APC*-MT vs. *APC*-WT: HR, 0.62; 95% CI, 0.44–0.86; $p = .0037$) and the MSKCC cohort (*APC*-MT vs. *APC*-WT: HR, 0.63; 95% CI, 0.49–0.81; $p < .0001$). Our multivariate model also confirmed the inferior outcome of right-sided primary and *BRAF* mutant tumors (Table 4). Taken together, our analysis indicates that wild-type *APC* is an independent poor prognostic marker for MSS metastatic colorectal cancer.

Discussion

Somatic mutations in *APC* are present in approximately 70%–80% of colorectal cancers [9, 18]. Compared with MSS colorectal cancers, tumors with MSI have a lower frequency of *APC* mutations [19]. Although several studies have suggested worse outcomes for unselected patients with colorectal cancer with *APC*-WT tumors, the prognostic implication of this genomic alteration in MSS metastatic colorectal cancer is not well defined [14, 15]. In this retrospective study, we focused our analysis on patients with MSS metastatic colorectal cancer from two independent institutions. We found *APC*-WT is associated with right-sided primary, *BRAF* mutation, and younger age. Using a multivariate model correcting for tumor location and *RAS/RAF* status, *APC*-WT was an independent poor prognostic marker in MSS metastatic colorectal cancer. The poor prognostic value of *APC*-WT was also significant when the COH cohorts and UCD cohorts were analyzed separately. In addition, our observations were validated using an independent cohort of 934 patients with metastatic colorectal cancer from the MSKCC data set.

There is no clear explanation for the worse outcome of *APC*-WT colorectal cancers. The copresence of $BRAF^{V600E}$ mutation and the increased incidence of right colonic tumors in *APC*-WT tumors may explain some of the poor outcomes associated with this group. However, *APC*-WT maintains a poor prognostic value after correcting for these variables [8, 20–24]. This indicates that additional biological variables associated with *APC*-WT status may contribute to the poor prognosis of this group. Consistent with prior reports, we showed that *APC*-WT colorectal cancer occurs more often in younger patients than older patients [10]. A study of 24 first-line clinical trials with metastatic colorectal cancer demonstrated that younger age was associated with worse progression-free survival (PFS) and OS irrespective of type of therapy received and molecular status [25]. Therefore, additional biological variables that are yet to be defined and that contribute to a more aggressive histology in younger individuals may partly explain the poor prognosis of this population. In addition, *APC*-WT tumors have been associated with an epithelial–mesenchymal transition (EMT) signature [13]. EMT has been suggested as one of the mechanisms leading to anti-EGFR resistance and a worse overall colorectal cancer outcome [26–28]. Finally, *APC*-WT status, as a standalone finding, has been recently associated with a diminished benefit from anti-EGFR therapy in a retrospective analysis from the CALGB 80405 study. Because our data reflect a modern era of treatment that includes anti-EGFR therapy, a differential outcome that is in favor of the *APC*-MT group may reflect a benefit from anti-EGFR driven within its *RAS*-WT subpopulation [11]. Taken together, these results suggest that *APC*-WT colorectal cancer may drive a distinct mechanism of tumorigenesis which then confers relative resistance to chemotherapy or targeted therapy, therefore leading to a worse overall outcome.

CTNNB1 and *RNF43* were enriched within the *APC*-WT colorectal cancer population, suggesting an alternative mechanism of WNT pathway activation in this subgroup. Prior studies and our own findings indicate that *CTNNB1* and *APC* mutations are mutually exclusive, suggesting that *CTNNB1* mutation could substitute for *APC* mutation as the initiator genomic alteration in colorectal tumor development [29]. As a tumor-suppressor gene, *RNF43* encodes a transmembrane ubiquitin ligase that downregulates the expression of membrane WNT receptor. Its mutation augments the activity of WNT ligands upon binding to WNT receptors [30, 31]. *RNF43* mutations were mutually exclusive with *APC* mutation, and have correlated with $BRAF^{V600E}$ mutation and MSI [32, 33]. *RNF43*-mutated tumors have conferred a worse OS in patients with *RAS*-WT colorectal cancer when treated with cetuximab [11]. Indeed, in our data analysis, patients with *RNF43*-mutated tumors exhibited earlier death within the *APC*-WT population (supplemental online Fig. 2). It is possible that *RNF43* led to more robust WNT activation than *APC* mutations, leading to a more sustained progrowth signaling and worse overall outcome.

We acknowledge the limitations of the retrospective analysis of our study. However, the large sample size and the reproducibility of our data within each institute (COH and UCD) as well as in an independent public data set lends strong support to our findings. Despite the potential patient heterogeneity across three institutes and the likely variable rate of metastasectomies that would explain the different OS among institutes, *APC* status was maintained as a negative prognostic marker across all centers. Few discrepancies in the occurrence rate of low frequency gene mutations were noted between the COH/UCD cohort and MSKCC cohort. These may be explained by sample power and, less likely, by variations in sequencing platform. Given the nature of retrospective study, and the heterogeneity of treatment in different lines of treatment, we did not analyze the impact of *APC* status on PFS or response rate. This study suggests *APC* status should be considered as a stratification biomarker in future prospective trials.

Conclusion

Our data demonstrated that *APC*-WT is an independent poor prognostic marker for metastatic colorectal cancer. Our findings suggest that incorporating *APC* mutation assessment along with other known classifiers such as *RAS* and *BRAF* may improve colorectal cancer risk stratification.

Our data suggest that alternative therapeutic interventions may be needed for patients with *APC*-WT metastatic colorectal cancer.

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Disclosures

May Cho: AstraZeneca, QED Biopharma, Bristol-Myers Squibb, Taiho, HelioDx, I-Mab Biopharma, Eisai, Incyte, Ipsen, Tempus (SAB), Immunocore Limited, Bristol-Myers Squibb, Seattle Genetics, Exelexis, AstraZeneca, Incyte (RF); **Marwan Fakih:** Amgen (H), AstraZeneca, Amgen, Novartis (RF), Amgen, Array, Bayer, Pfizer (C/A), Amgen, Guardant 360 (Other-Speaker' bureau). The other authors indicated no financial relationships.

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