Impact of Molecular Alterations and Targeted Therapy in Appendiceal Adenocarcinomas

KANWAL P.S. RAGHAV,^a ADITYA V. SHETTY,^b SYED M.A. KAZMI,^b NIANXIANG ZHANG,^c JEFFREY MORRIS,^d MELISSA TAGGART,^e KEITH FOURNIER,^f RICHARD ROYAL,^f PAUL MANSFIELD,^f CATHY ENG,^a ROBERT A. WOLFF,^a MICHAEL J. OVERMAN^a

^aDepartment of Gastrointestinal Medical Oncology, ^bDivision of Cancer Medicine, ^cDepartment of Bioinformatics and Computational Biology, ^dDepartment of Biostatistics, ^eDepartment of Pathology, ^fDepartment of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston Texas, USA; ^gDepartment of Internal Medicine, The University of Texas Medical School at Houston, Houston, Texas, USA

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

Key Words. Appendix adenocarcinoma • Celecoxib • Cetuximab • COX-2 • KRAS • MSI

ABSTRACT

Background. Appendiceal adenocarcinomas (AAs) are rare and this has limited their molecular understanding. The purpose of our study was to characterize the molecular profile of AA and explore the role of targeted therapy against cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR).

Patients and Methods. We performed a retrospective review of 607 patients with AA at a single institution. A total of 149 patients underwent molecular testing for at least one of the following: activating mutations in *KRAS*, *BRAF*, *cKIT*, *EGFR*, or *PI3K*; protein expression of c-KIT or COX-2; or microsatellite instability (MSI) status by immunohistochemistry. Kaplan-Meier product limit method and log-rank test were used to estimate overall survival (OS) and to determine associations among OS, COX-2 expression, *KRAS* mutations, and other characteristics.

Results. Age, grade, stage, signet ring cells, mucinous histology, and completeness of cytoreduction score correlated with

survival outcomes. COX-2 expression, *KRAS*, *PI3K*, and *BRAF* mutations were seen in 61%, 55%, 17%, and 4% of patients, respectively. High MSI was seen in 6% of patients. *KRAS* mutation was strongly associated with well differentiated or moderately differentiated AA ($p < .01$). COX-2 expression ($p = .33$) and the presence of *KRAS* mutation ($p = .91$) had no impact on OS. The use of celecoxib in patients whose tumors expressed COX-2 ($p = .84$) and the use of cetuximab or panitumumab in patients with *KRAS* wild-type tumors ($p = .83$) also had no impact on OS.

Conclusion. In this cohort, we demonstrated that COX-2 expression and *KRAS* mutations were frequently seen in AA, although neither exhibited any prognostic significance. MSI was infrequent in AA. Targeted therapy against COX-2 and *EGFR* appeared to provide no clinical benefit. Well and moderately differentiated AA were molecularly distinct from poorly differentiated AA. *The Oncologist* 2013;18:1270–1277

Implications for Practice: Appendiceal adenocarcinomas (AAs) are rare, and current understanding of their molecular biology is poor. Surgical resection is the mainstay of therapy. Limited data exists to guide medical management, and the role of targeted therapy in AAs has not been studied. This study endeavors to define molecular alterations in AAs. Activating *KRAS* mutations represent the most common alteration, occurring in 55% cases. Interestingly, well and moderately differentiated tumors demonstrate similar high rates of *KRAS* mutation, contrary to the low rates seen in poorly differentiated tumors. These data link clinical behavior with molecular biology and suggest that moderately differentiated tumors resemble well-differentiated tumors and should be treated similarly. Further prospective trials are needed to evaluate the efficacy of targeted therapies such as anti-epidermal growth factor receptor therapy in AAs, prior to their implementation in clinical practice. Constructing a molecular sketch of AAs is a necessary first step toward recognizing molecular pathways involved in their carcinogenesis and advancing the role of targeted therapies in AAs.

INTRODUCTION

Appendix tumors are rare malignancies. Appendiceal neoplasms are incidentally found in about 0.9% of all appendectomy specimens [1]. The age-adjusted incidence of appendiceal malignancies appears to be increasing from 0.12 cases per 1,000,000 per year in 1973 to 5–6 cases per

1,000,000 per year in 2006–2007 [2, 3]. Primary appendiceal adenocarcinomas (AA) are the most common subtype of appendiceal tumors and constitute 50% to 70% of all appendiceal neoplasms and 0.5% of all neoplasms of gastrointestinal origin [3, 4].

Correspondence: Michael J. Overman, M.D., Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center; 1515 Holcombe Boulevard, Unit # 426, Houston, Texas 77030, USA. Telephone: 713-745-4317; Fax: 713-745-1163; E-Mail: moverman@mdanderson.org. Received May 28, 2013; accepted for publication August 28, 2013; first published online in *The Oncologist Express* on October 22, 2013. ©AlphaMed Press 1083-7159/2013/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2013-0186>

Classification of appendiceal epithelial neoplasms is controversial and is based on architectural and cytologic features [5, 6]. The clinical course is complicated and can vary from being relatively indolent to highly aggressive, depending on histologic subtype [2–4]. A review of the literature reveals scattered reports illustrating histologic subtype, age at diagnosis, grade, stage, presence of signet ring cell features, and extent of surgery as being significantly associated with survival outcomes [2–4, 7–9]. To date, only limited studies with small numbers of patients have evaluated the molecular profile of AA. Although there are anatomic associations between AA and colorectal cancer (CRC), AA are distinct entities with a unique biologic behavior. AA are commonly mucinous and tend to spread intraperitoneally, with limited incidence of nodal or distant metastases [9, 10].

Cyclooxygenase-2 (COX-2) expression and *KRAS* mutations have been implicated in colorectal carcinogenesis and have been shown to adversely affect the survival of patients with CRC [11–13]. Epidermal growth factor receptor (*EGFR*) inhibition using anti-*EGFR* antibodies has been demonstrated to improve survival in *KRAS* wild-type CRC [14–16]. Furthermore, COX-2 inhibition with celecoxib has been shown to reduce the occurrence of colorectal adenomas [17]. Selective COX-2 inhibition has also shown to inhibit tumor growth in nude mice implanted with COX-2-expressing CRC cell lines [18]. Extrapolating from these studies in CRC, COX-2 inhibition (celecoxib) and anti-*EGFR* therapy (cetuximab and panitumumab) have been used in the clinic, but at present no publications describe the results of such a therapeutic approach [19, 20].

As both the molecular profile and the role of molecularly targeted therapy remains uncharted in AAs, we sought to investigate the frequency of molecular alterations in these rare tumors and to ascertain the potential prognostic and therapeutic significance of targeting the COX-2 and *EGFR* pathways.

PATIENTS AND METHODS

Population

We performed a retrospective review of 607 patients with AA evaluated at The University of Texas MD Anderson Cancer Center (MDACC) between January 2002 and December 2010. Data were collected by reviewing electronic medical records under a protocol approved by the MDACC institutional review board. The inclusion criteria for the study required a histopathologic diagnosis of AA and the presence of a tested molecular alteration. Clinical and pathologic variables of interest reviewed included demographics (age at diagnosis, race, gender), tumor characteristics (grade; tumor, node, metastasis [TNM] stage; presence/absence of signet-ring cells) and treatment history (surgery, completeness of cytoreduction score [CCS]). A total of 149 (24%) patients were identified as having been tested for at least one of the following: activating DNA mutations in *KRAS*, *BRAF*, *PI3K*, *EGFR*, or c-KIT using polymerase chain reaction-based DNA sequencing; COX-2, cKIT, *EGFR*, or microsatellite instability (MSI) status (MLH-1, MSH-2, MSH-6, and PMS-2) expression analysis by immunohistochemistry. All testing was performed at a Clinical Laboratory Improvements Amendments-certified MDACC laboratory and reviewed by a referenced pathologist.

TNM staging was detailed according to the 2010 edition of the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) TNM staging system for appendiceal carcinomas [21]. The tumor grade was also classified according to 2010 AJCC/UICC TNM staging system as grade 1 (G1), grade 2 (G2) and grade 3 (G3) for well differentiated, moderately differentiated, and poorly differentiated histology, respectively [21]. The CCS was recorded as CCS 0 (no visible disease), CCS 1 (<2.5 mm visible disease), CCS 2 (between 2.5 and 25 mm visible disease), and CCS 3 (>25 mm visible disease) [9]. Mucinous adenocarcinomas were defined as tumors with >50% mucin present on pathologic review.

To assess the impact of molecularly targeted therapy for COX-2 and *EGFR*, patients treated with either anti-*EGFR* (cetuximab or panitumumab) or anti-COX-2 (celecoxib) therapy were reviewed for tumor markers (carcinoembryonic antigen, cancer antigen 125, cancer antigen 19–9, chemotherapy, radiologic restaging, and treating physician evaluation. Response to therapy was categorized as stable disease, progressive disease, or responding disease according to the treating physician's assessment.

Statistical Analysis

Kaplan-Meier product limit estimation was used to calculate the survival functions. The primary clinical endpoint was overall survival (OS) and was defined as the time from diagnosis to death. In the cohort of patients treated with targeted therapy, time to progression (TTP) was defined as the time from initiation of therapy to its discontinuation for toxicity, disease progression, or treating physician's discretion. Log-rank tests were used to test the survival differences for categorical variables. Fisher exact tests and chi-square tests were used to determine association between OS and other characteristics. For multivariate survival analysis, included covariates were age, grade, TNM stage, CCS, COX-2 expression, and *KRAS* mutation status. The multiple imputation technique was used to impute the missing data and then a proportional hazards Cox model was used to assess effects of the covariates on OS. The results were combined using Rubin methodology [22]. A *p* value < .05 was considered significant. All computations were carried out in SAS 9.2 and S-plus 8.0 or R 2.12.0 (SPSS software, IBM Corp., Armonk, NY, <http://www-01.ibm.com/software/analytics/spss/>).

RESULTS

Baseline Characteristics

A total of 149 patients underwent testing for at least one molecular alteration. The clinical and pathologic characteristics and treatment variables of the study population are summarized in Table 1. The median age at diagnosis was 50 years (range: 29.5–78.9 years). The majority of patients had stage IV disease (77%). The most common histologic grade was low grade or well differentiated histology (43%), and 61% of all cases were mucinous adenocarcinomas.

Molecular Profile

Results of the analysis for molecular alterations with their respective frequency of occurrence are summarized in Table 2. In AA, the mutation rates for *BRAF* and *PI3K* were 4% (2/50) and 17% (2/12), respectively. Both mutations in the *BRAF* gene involved codon 600 (GTG to GAG), resulting in a valine to glu-

Table 1. Summary of baseline characteristics for patients with appendiceal adenocarcinomas

Variable	Level	n	Percent (%)
Age	≤60 years	106	71.14
	>60 years	43	28.86
Gender	Female	79	53.02
	Male	70	46.98
Race	White	118	79.19
	Other	31	20.81
Grade ^a	1	64	42.95
	2	31	20.81
	3	54	36.24
Stage ^b	I/II/III	34	22.82
	IVA	56	37.58
	IVB/IVC	59	39.60
Nodal status	N0	100	67.12
	N1	16	10.74
	N2	13	8.72
	NX	20	13.42
Metastasis	M0	34	22.82
	M1	115	77.18
Mucinous adenocarcinoma	Yes	91	61.08
	No	58	38.92
Signet cells	Absent	109	73.15
	Present	40	26.85
Surgery	No	45	30.21
	Yes	104	69.79
CCS ^c	0/1	51	49.04
	2/3	53	50.96
COX-2 ^d	Negative	19	38.77
	Positive	30	61.23
KRAS ^e	Mutated	59	54.63
	Wild type	49	45.37
Celecoxib	No	139	93.29
	Yes	10	06.71
Cetuximab/panitumumab	No	120	80.54
	Yes	29	19.46

^aGrade: 1—well-differentiated; 2—moderately differentiated; 3—poorly differentiated.

^bStage: I/II/III—nonmetastatic disease; IVA—well-differentiated intraperitoneal metastatic disease; IVB/IVC—moderate to poorly differentiated metastatic disease.

^cCCS: 0/1—disease <2.5 mm; 2/3—disease >2.5 mm.

^dCOX-2: COX-2 expression was assessed using immunohistochemistry.

^eKRAS: KRAS mutation was assessed using polymerase chain reaction-based DNA sequencing.

Abbreviations: CCS, completeness of cytoreduction score; COX-2, cyclooxygenase-2 expression.

tamate substitution (V600E). Mutations in the *PI3K* gene were found in codon 545 and codon 1047, resulting in a charge change from negative to highly positive (E545K) and in a histidine to arginine substitution (H1047R), respectively. No *EGFR* mutations (0/7) or c-KIT mutations (0/5) were seen in the tested cohort. Furthermore, we also identified 35 patients

who were tested for MSI and 94% (33/35) of these were MSI stable. The two patients in whom MSI was high had loss of MLH1 (MLH1 gene promoter hypermethylation testing negative) and MSH2; however, the complete germline mutational testing on both of these patients was negative.

Survival Analysis

The median follow-up for the whole group was 60.3 months (95% confidence interval [CI]: 43.8–84.6). The median OS for all patients was 53.9 months (95% CI: 46.1–79.7). In subgroup analysis, median OS for the groups tested for COX-2 and *KRAS* were 55.7 months (95% CI: 39.2–not applicable [NA]) and 58.7 months (95% CI: 44.8–90.9), respectively. These results were not statistically different from those of patients without available COX-2 expression or *KRAS* mutation information (supplemental online Fig. 1).

Results of univariate survival estimates are shown in Table 3. Among the evaluated clinical and pathologic factors, age >60 years at diagnosis, poorly differentiated histology, advanced TNM stage, presence of signet cells, nonmucinous histology, and CCS ≥2 correlated with poor OS (Fig. 1). Grade was an important prognostic determinant of survival in metastatic AA. In patients presenting with metastatic AA, the median OS decreased significantly with poorer differentiation (G1: 87.3 months; G2: 33.1 months; and G3: 27.6 months; $p < .001$) (supplemental online Fig. 2).

Results of multivariate analysis are shown in supplemental online Table 1. In multivariate analysis, stage IV disease (hazard ratio [HR] = 2.76; 95% CI: 1.29–5.87; $p = .008$), CCS ≥2 (HR = 2.10; 95% CI: 1.10–3.99; $p = .023$), and age >60 years at diagnosis (HR = 2.84; 95% CI: 1.25–6.43; $p = .011$) were independently associated with worse OS. COX-2 expression (HR = 0.67; 95% CI: 0.19–2.39; $p = .524$) and *KRAS* (HR = 1.96; 95% CI: 0.84–4.57; $p = .116$) mutation had no significant impact on OS.

COX-2 Expression and COX-2 Inhibition

Among the 49 patients with tested COX-2 expression, immunohistochemistry showed COX-2 expression in 30 (61%) and revealed no expression of COX-2 in 19 (39%) patients. Results of COX-2 expression and association with clinical–pathologic variables are shown in supplemental online Table 2. COX-2 expression was significantly associated with age ($p = .025$) and gender ($p = .019$), being more commonly expressed in older individuals and in men. The difference in median OS of patients with tumors that expressed COX-2 (57.6 months; 95% CI: 42.3–NA) was not statistically significant ($p = .328$) compared with OS of patients with tumors that did not express COX-2 (42.2 months; 95% CI: 27.7–NA) (Fig. 2A).

Additionally, we identified 9/30 (30%) patients with COX-2-expressing tumors who received selective COX-2 inhibition, all with celecoxib. The median OS for patients with and without celecoxib treatment was 57.6 and 55.7 months, respectively ($p = .843$) (Fig. 2B).

In all but one case, celecoxib was used in conjunction with standard cytotoxic therapy at a dose of 200 mg twice daily (supplemental online Table 3). No radiographic responses were seen, and the best response of stable disease was seen in 44% of patients. The median TTP on celecoxib was 2.9 months (95% CI: 1.8–6.5 months) and there was no difference be-

Table 2. Molecular alterations in appendiceal adenocarcinomas

Molecular alteration	Type	Test	n (total)	Positive	Positive (term)	Percent
<i>KRAS</i>	Mutation	DNA-PCR	108	59	Mutated	54.6%
<i>COX-2</i>	Expression	IHC	49	30	Expressed	61.2%
<i>BRAF</i>	Mutation	DNA-PCR	50	2	Mutated	4.0%
MSI	Expression	IHC	35	33	MSI-Stable	94.2%
<i>PI3K</i>	Mutation	DNA-PCR	12	2	Mutated	16.7%
c-KIT	Mutation	DNA-PCR	5	0	Mutated	0%
c-KIT	Expression	IHC	6	1	Expressed	16.7%
<i>EGFR</i>	Mutation	DNA-PCR	7	0	Mutated	0%
<i>EGFR</i>	Expression	IHC	30	21	Expressed	70%

Abbreviations: COX-2, cyclooxygenase-2 expression; *EGFR*, epidermal growth factor receptor; IHC, immunohistochemistry; MSI, microsatellite instability; PCR, polymerase chain reaction.

Table 3. Univariate overall survival analysis for patients with appendiceal adenocarcinoma

Variable	Level	n	Events	Median OS (months)	95% CI	Log-rank p value
Age	≤60 year	106	50	62.7	49.4–97.5	.0352
	>60 year	43	26	42.3	31.3–NA	
Gender	Female	79	41	53.9	42.2–97.5	.9050
	Male	70	35	55.7	42.3–99	
Race	White	118	59	55.7	47.5–92	.4345
	Other	31	17	53.6	33–NA	
Grade ^a	1	64	27	87.4	55.7–NA	.0032
	2	31	17	51.7	33.1–NA	
	3	54	32	41.3	28.3–76	
Stage ^b	I/II/III	34	13	79.7	51.7–NA	.0002
	IVA	56	23	64.1	47.5–NA	
	IVB/IVC	59	40	33	26.2–53.9	
Nodal status	N0	100	47	64.0	47.0–94.0	.1183
	N1	16	7	87.0	34.0–NA	
	N2	13	8	41.0	24.0–NA	
Metastasis	M0	34	13	79.7	51.7–NA	.1060
	M1	115	63	47.5	41.3–64.1	
Mucinous adenocarcinoma	Yes	91	40	69.0	54.0–110.0	.0001
	No	58	36	49.0	28.0–63.0	
Signet cells	Absent	109	50	57.6	49.4–97.5	.0216
	Present	40	26	33	25.8–99	
Surgery	No	45	26	49.4	35.9–99	.0998
	Yes	104	50	57.6	47.5–97.5	
CCS ^c	0/1	51	22	87.4	53.9–NA	.0189
	2/3	53	28	42.3	31.3–99.8	
COX-2 ^d	Negative	19	13	42.2	27.8–NA	.3282
	Positive	30	12	57.6	42.3–NA	
<i>KRAS</i> ^e	Mutated	59	31	57.6	41–109.9	.9058
	Wild type	49	24	62.7	41.3–99.8	

^aGrade: 1–well differentiated; 2–moderately differentiated; 3–poorly differentiated.

^bStage: I/II/III–nonmetastatic disease; IVA–well-differentiated intraperitoneal metastatic disease; IVB/IVC–moderate to poorly differentiated metastatic disease.

^cCCS: 0/1 (disease <2.5 mm); 2/3 (disease >2.5 mm).

^dCOX-2: COX-2 expression was assessed using immunohistochemistry.

^e*KRAS*: *KRAS* mutation was assessed using polymerase chain reaction-based DNA sequencing.

Abbreviations: CI, confidence intervals; CCS, completeness of cytoreduction score; COX-2, cyclooxygenase-2 expression; NA, not applicable; OS, overall survival.

Figure 1. Kaplan-Meier estimates and plots of overall survival probabilities by age (A), CCS (B), grade (C), tumor, node, metastasis stage (D), signet ring cells (E), and mucinous histology (F). Tumor grade is classified as G1 (well differentiated), G2 (moderately differentiated), or G3 (poorly differentiated). TNM stage is grouped as I/II/III (nonmetastatic disease), IVA (well differentiated intraperitoneal metastatic disease), and IVB/IVC (moderate poorly differentiated metastatic disease). CCS is defined as CCS 0/1 (disease <2.5 mm) and CCS 2/3 (disease >2.5 mm).

Abbreviations: CCS, completeness of cytoreduction score; MAA, mucinous adenocarcinoma; NMAA, nonmucinous appendiceal adenocarcinoma.

tween mean TTP on celecoxib and TTP for prior systemic therapy ($p = .44$) (Fig. 3).

KRAS Mutations and EGFR Inhibition

Among the 108 patients with *KRAS* mutation analysis, 59 (55%) were found to have mutations; 49 (45%) were wild type. Results of *KRAS* mutation and association with clinical-pathologic variables are shown in supplemental online Table 4. Among the *KRAS* mutation subtypes, 86% (51/59) involved codon 12, and 14% (8/59) involved codon 13. The presence of *KRAS* mutations inversely correlated with the use of anti-*EGFR* antibody treatment ($p < .001$), presence of signet ring cells ($p < .001$), poorly differentiated histology ($p < .001$), and advanced TNM stage ($p = .003$). The difference in *KRAS* mutation rate by histologic grade was dramatic, with a rate of 21% in poorly differentiated cases but 74% in well or moderately differentiated cases ($p < .001$) (Fig. 4). In a subset of mucinous adenocarcinomas, *KRAS* mutations were seen in 18% of poorly differentiated tumors compared with 70% of well or moderately differentiated tumors ($p = .002$) (Fig. 4). The difference in median OS of patients with tumors that harbored *KRAS* mutations (57.6 months; 95% CI: 41.0–109.9) was

not statistically significant ($p = .906$) compared with median OS of patients with tumors that were *KRAS* wild type (62.7 months; 95% CI: 41.3–99.8) (Fig. 2C).

We identified 49 patients with *KRAS* wild-type tumors, 20 (40.8%) of whom received anti-*EGFR* antibody therapy with either cetuximab ($n = 16$) or panitumumab ($n = 4$) (supplemental online Table 5). The median OS for patients with and without cetuximab/panitumumab treatment was 68.4 and 51.7 months, respectively ($p = .832$) (Fig. 2D).

Cetuximab or panitumumab were used as monotherapy in 25% of cases (supplemental online Table 5). *EGFR* inhibition was most often used as either the second or third line of therapy. The best radiologic response with *EGFR* inhibition was responding disease and stable disease in 15% (3/20) and 35% (7/20) of patients, respectively. The median TTP on *EGFR* inhibition was 2.67 months (95% CI: 2.1 months–4.1 months), and the mean TTP on anti-*EGFR* therapy was significantly lower than TTP on immediate prior systemic therapy ($p = .01$) (Fig. 3).

In a further exploratory analysis, the entire cohort of patient who were treated with anti-*EGFR* antibody therapy was

Figure 2. Kaplan-Meier estimates and plots of overall survival probabilities by COX expression (A), celecoxib treatment in COX-2-expressing tumors (B), *KRAS* mutation status (C), and cetuximab/panitumumab treatment in *KRAS* wild-type tumors (D). COX-2 was assessed using immunohistochemistry. *KRAS* mutation was assessed using polymerase chain reaction–based DNA sequencing. *KRAS* negative and positive represent wild-type and mutant tumors, respectively.

Abbreviations: COX, cyclooxygenase; *EGFR*, epidermal growth factor receptor; Neg, negative; Pos, positive; Rx, prescription.

Figure 3. Scatter plots showing TTP on prior systemic therapies and study therapy. The study therapies are celecoxib (COX) (A) and *EGFR* inhibitors cetuximab and panitumumab (B). TTP was defined as duration from time of initiation of therapy to its discontinuation for any reason.

Abbreviations: *EGFR*, epidermal growth factor receptor; TTP, time to progression.

investigated. A total of 29 patients were treated with either cetuximab or panitumumab, 20 (69%) of whom had *KRAS* wild-type tumors and 9 (31%) of whom had tumors that were either mutants or did not undergo *KRAS* mutation testing. The median OS for patients with *KRAS*-mutated or unknown status tumors was significantly inferior to those with *KRAS* wild-type tumors ($p = .012$) (supplemental online Fig. 3).

DISCUSSION

Our study results suggest that COX-2 expression and *KRAS* mutations occur in about 61% and 55% of AA, respectively, but do not have any significant prognostic impact on survival. Furthermore, there is no evidence of benefit from the use of targeted therapies such as celecoxib, cetuximab, or panitumumab in AA. The molecular profile of AA reveals that mutations of c-KIT, *EGFR*, and *BRAF* pathways are seen un-

commonly in AA. Notably, *KRAS* mutations were seen more commonly in well or moderately differentiated tumors compared with those of poorly differentiated histology. Additionally, clinical–pathologic variables such as age, histologic grade, TNM stage, signet ring cells, and CCS are significant prognostic factors for AA.

This is the first study documenting a comprehensive molecular profile of AA in a large cohort of patients. Our analysis shows that the molecular profile of AA differs from that of CRC as assessed by The Cancer Genomic Atlas data [23]. Mutations involving *BRAF* (4% vs. 9.7%), *EGFR* (0% vs. 3.6%), and c-KIT (0% vs. 3.1%) are less frequent in AA. Whereas *PI3K* mutations occur at a rate similar to the rate in CRC (16% vs. 23%), *KRAS* mutations are seen at a higher rate in AA (55% vs. 41%). The rate of *KRAS* mutations found in our study was similar to that seen in a prior report in patients with pseudomyxoma peritonei [24]. This molecular profile lends supports to the assumption that, despite their anatomic congruity, AA and CRC are two molecularly distinct tumor types.

Of greater interest is the marked variation in the *KRAS* mutation rate depending on histology, with a threefold higher rate in well or moderately differentiated AA compared with poorly differentiated AA (74% vs. 21%; $p < .001$). This finding is consistent with previously published studies [24, 25]. *KRAS* mutation was strongly associated with moderately differentiated histology compared with poorly differentiated tumors (odds ratio [OR] 24.5, $p < .001$) (Fig. 4). Even within the subset of mucinous adenocarcinomas, *KRAS* mutations were strongly associated with well and moderately differentiated tumors (OR 10.69; $p = .002$). As has been shown in prior studies [2, 3], we demonstrated that histologic grade is the single most important prognostic factor affecting survival in AA; however, the biologic significance of the

Figure 4. Stacked bar graph showing distribution of *KRAS* mutations in well differentiated (grade 1), moderately differentiated (grade 2), and poorly differentiated (grade 3) appendiceal adenocarcinomas. (A): All tumors. (B): Mucinous adenocarcinomas.

Abbreviations: Mut, mutation; WT, wild type.

relative abundance of *KRAS* mutation in the well or moderately differentiated tumors requires further study. This finding indicates that well and moderately differentiated tumors may be related closely from a molecular perspective and are distinct from poorly differentiated AA, which argues reconsidering the combination of these two histologic grades in the recent seventh edition of the AJCC guidelines [21, 26, 27].

Our study also revealed that AA have a low prevalence of MSI (approximately 5%) compared with colon adenocarcinomas, where high levels of MSI are seen in approximately 15%–20% of cases [28]. MSI results from defective DNA mismatch repair. It is associated with increased mutations of several critical genes and plays a key role in carcinogenesis and progression of CRC [23]. MSI-high tumors in CRC are commonly hypermutated and have distinct rates of mutations compared with MSI-stable tumors [23]. Hypermutated tumors frequently have *BRAF* (V600E) mutations and the lack of MSI-high tumors could explain the lower incidence of *BRAF* mutations in AA. Similarly, MSI-high tumors have fewer *KRAS* mutations than MSI-stable tumors, which may explain the frequent *KRAS* mutations seen in this cohort of AA [28]. Furthermore, because nonhypermutated cancers have higher TP53 and APC mutations, it is likely that these mutations may play a more important role in AA [23]. Additional studies are needed to assess the biologic role of these checkpoints and molecular pathways in AA [29]. This difference of MSI status further strengthens the argument that AA and CRC are distinct tumor types at a molecular level and indicates that AA may progress through a different sequence of genetic events than CRC.

We also demonstrated that COX-2 expression and *KRAS* mutations are not prognostic. The subset analysis showed that COX-2 inhibition in COX-2-expressing tumors showed no clear benefit. Similarly, there was no clear benefit from *EGFR* inhibition in *KRAS* wild-type tumors. This is the largest case series investigating the role of these targeted therapies in AA. This exploratory finding, even within a small cohort, is of potential clinical significance for patients and should be investigated further.

Our study has limitations inherent to all retrospective analyses. Molecular testing was conducted at variable time points during the treatment course based on treating physician's discretion, and the reasons for ordering molecular tests could not be discerned. However, it should be noted that clinically the study does reflect standard appendix cancer in terms of classic clinical and pathologic prognostic factors such as age, tumor

grade, stage, signet ring cells, and extent of surgery, as shown in prior publications [2–5]. In addition, the demographic distribution (median age at diagnosis, race, and gender) of our cohort is similar to that of AA subgroups in prior studies [3, 4]. Despite these similarities, clinical heterogeneity of patient and treatment selection can confound survival results of the study. Moreover, although records were reviewed extensively and patient and treatment characteristics were recorded accurately, it is conceivable that some deficiencies and missing data may have biased the results. A variety of concurrent therapies were used with study drugs, and therefore the true independent treatment effect of celecoxib, cetuximab, and panitumumab could not be assessed. Interpretation of molecular analyses of *PI3K*, *EGFR*, and cKIT was limited by the small numbers. Therefore, our conclusions should be considered hypothesis-generating rather than definitive. Despite the modest size of this study, it is still the largest study of its kind in this rare tumor type.

CONCLUSION

This is the first study in a large cohort of patients with AA characterizing its molecular profile. AA demonstrates the frequent presence of *PI3K* and *KRAS* mutations along with a predominant microsatellite-stable phenotype. COX-2 expression and *KRAS* mutations, although seen frequently in AA, are not prognostic. Furthermore, although limited by small sample size, these data do not support a benefit from targeted therapies—namely, COX-2 inhibition and *EGFR* inhibition in AA—and suggests that further investigation is needed prior to routine clinical use. We also propose that moderately differentiated AA is molecularly distinct from poorly differentiated AA and should be regarded as such for staging and therapy.

Delineating the molecular landscape of AA is critical to understanding the true biology of this disease and is the necessary first step to developing effective therapeutic strategies that can translate into improved patient outcomes in this malignancy.

AUTHOR CONTRIBUTIONS

Conception/Design: Kanwal P.S. Raghav, Nianxiang Zhang, Keith Fournier, Richard Royal, Paul Mansfield, Cathy Eng, Michael J. Overman

Provision of study material or patients: Michael J. Overman

Collection and/or assembly of data: Kanwal P.S. Raghav, Aditya V. Shetty, Syed M.A. Kazmi, Michael J. Overman

Data analysis and interpretation: Kanwal P.S. Raghav, Aditya V. Shetty, Syed M.A. Kazmi, Nianxiang Zhang, Jeffrey Morris, Melissa Taggart, Keith Fournier, Richard Royal, Paul Mansfield, Cathy Eng, Michael J. Overman

Manuscript writing: Kanwal P.S. Raghav, Aditya V. Shetty, Syed M.A. Kazmi, Nianxiang Zhang, Jeffrey Morris, Melissa Taggart, Keith Fournier, Richard Royal, Paul Mansfield, Cathy Eng, Robert A. Wolff, Michael J. Overman
Final approval of manuscript: Kanwal P.S. Raghav, Aditya V. Shetty, Syed M.A. Kazmi, Nianxiang Zhang, Jeffrey Morris, Melissa Taggart, Keith Fournier, Richard Royal, Paul Mansfield, Cathy Eng, Robert A. Wolff, Michael J. Overman

DISCLOSURES

Cathy Eng: Eli Lilly (H); Genentech/Roche (H); Amgen (RF); Arqule (RF); Keryx (RF). The other authors indicated no financial relationships. (C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

REFERENCES

1. Connor SJ, Hanna GB, Frizelle FA. Appendiceal tumors: Retrospective clinicopathologic analysis of appendiceal tumors from 7,970 appendectomies. *Dis Colon Rectum* 1998;41:75–80.
2. McCusker ME, Cote TR, Clegg LX et al. Primary malignant neoplasms of the appendix: A population-based study from the surveillance, epidemiology and end-results program, 1973–1998. *Cancer* 2002;94:3307–3312.
3. Turaga KK, Pappas SG, Gamblin T. Importance of histologic subtype in the staging of appendiceal tumors. *Ann Surg Oncol* 2012;19:1379–1385.
4. Benedix F, Reimer A, Gastinger I et al. Primary appendiceal carcinoma—epidemiology, surgery and survival: Results of a German multi-center study. *Eur J Surg Oncol* 2010;36:763–771.
5. Misdraji J, Yantiss RK, Graeme-Cook FM et al. Appendiceal mucinous neoplasms: A clinicopathologic analysis of 107 cases. *Am J Surg Pathol* 2003;27:1089–1103.
6. Raghav KP, Taggart MW, Fournier KF et al. Is malignant dedifferentiation for mucinous appendiceal neoplasms a valid phenomenon or merely histopathologic ambiguity? *J Am Coll Surg* 2011;212:906.
7. Nitecki SS, Wolff BG, Schlinkert R et al. The natural history of surgically treated primary adenocarcinoma of the appendix. *Ann Surg* 1994;219:51–57.
8. Stewart JH 4th, Shen P, Russell GB et al. Appendiceal neoplasms with peritoneal dissemination: Outcomes after cytoreductive surgery and intraperitoneal hyperthermic chemotherapy. *Ann Surg Oncol* 2006;13:624–634.
9. Sugarbaker PH. New standard of care for appendiceal epithelial neoplasms and pseudomyxoma peritonei syndrome? *Lancet Oncol* 2006;7:69–76.
10. Compton C, Fenoglio-Preiser CM, Pettigrew N et al. American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Working Group. *Cancer* 2000;88:1739–1757.
11. Sheehan KM, Sheahan K, O'Donoghue DP et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. *JAMA* 1999;282:1254–1257.
12. Vogelstein B, Fearon ER, Hamilton SR et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988;319:525–532.
13. Andreyev HJ, Norman AR, Cunningham D et al. Kirsten ras mutations in patients with colorectal cancer: The multicenter “RASCAL” study. *J Natl Cancer Inst* 1998;90:675–684.
14. Van Cutsem E, Kohne CH, Lang I et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: Updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011;29:2011–2019.
15. Amado RG, Wolf M, Peeters M et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;26:1626–1634.
16. Allegra CJ, Jessup JM, Somerfield MR et al. American Society of Clinical Oncology provisional clinical opinion: Testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009;27:2091–2096.
17. Arber N, Eagle CJ, Spicak J et al. Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med* 2006;355:885–895.
18. Sheng H, Shao J, Kirkland SC et al. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 1997;99:2254–2259.
19. Andreopoulou E, Yee H, Warycha MA et al. Mucinous cancer of the appendix: Challenges in diagnosis and treatment. *J Chemother* 2007;19:451–454.
20. Andreopoulou E, Muggia F, Safa M et al. Phase II study of cetuximab in mucinous peritoneal carcinomatosis. *J Clin Oncol* 2006;24(18S):14020.
21. Edge S, Byrd DR, Compton CC. AJCC (American Joint Committee on Cancer) Cancer Staging Manual. 7th ed. New York, NY: Springer 2010.
22. Rubin DB. Multiple Imputation for Nonresponse in Surveys. New York, NY: John Wiley & Sons 1987.
23. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012;487:330–337.
24. Shetty S, Thomas P, Ramanan B et al. Kras mutations and p53 overexpression in pseudomyxoma peritonei: Association with phenotype and prognosis. *J Surg Res* 2013;180:97–103.
25. Zauber P, Berman E, Marotta S et al. Ki-ras gene mutations are invariably present in low-grade mucinous tumors of the vermiform appendix. *Scand J Gastroenterol* 2011;46:869–874.
26. Overman MJ, Fournier K, Hu CY et al. Improving the AJCC/TNM staging for adenocarcinomas of the appendix: The prognostic impact of histological grade. *Ann Surg* 2013;257:1072–1078.
27. Overman MJ, George B, Taggart MW et al. The natural history and role of surgical cytoreduction and systemic chemotherapy in high-grade mucinous adenocarcinomas of the appendix. *J Clin Oncol* 2013;31(4S):199.
28. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer—the stable evidence. *Nat Rev Clin Oncol* 2010;7:153–162.
29. Walther A, Johnstone E, Swanton C et al. Genetic prognostic and predictive markers in colorectal cancer. *Nat Rev Cancer* 2009;9:489–499.

See www.TheOncologist.com for supplemental material available online.