

# Potential actionable targets in appendiceal cancer detected by immunohistochemistry, fluorescent in situ hybridization, and mutational analysis

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**Background:** Appendiceal cancers are rare and consist of carcinoid, mucocoele, pseudomyxoma peritonei (PMP), goblet cell carcinoma, lymphoma, and adenocarcinoma histologies. Current treatment involves surgical resection or debulking, but no standard exists for adjuvant chemotherapy or treatment for metastatic disease.

**Methods:** Samples were identified from approximately 60,000 global tumors analyzed at a referral molecular profiling CLIA-certified laboratory. A total of 588 samples with appendix primary tumor sites were identified (male/female ratio of 2:3; mean age =55). Sixty-two percent of samples were adenocarcinomas (used for analysis); the rest consisted of 9% goblet cell, 15% mucinous; 6% pseudomyxoma, and less than 5% carcinoids and 2% neuroendocrine. Tests included sequencing [Sanger, next generation sequencing (NGS)], protein expression/immunohistochemistry (IHC), and gene amplification [fluorescent in situ hybridization (FISH) or CISH].

**Results:** Profiling across all appendiceal cancer histological subtypes for IHC revealed: 97% BRCP, 81% MRP1, 81% COX-2, 71% MGMT, 56% TOPO1, 5% PTEN, 52% EGFR, 40% ERCC1, 38% SPARC, 35% PDGFR, 35% TOPO2A, 25% RRM1, 21% TS, 16% cKIT, and 12% for TLE3. NGS revealed mutations in the following genes: 50.4% *KRAS*, 21.9% *P53*, 17.6% *GNAS*, 16.5% *SMAD4*, 10% *APC*, 7.5% *ATM*, 5.5% *PIK3CA*, 5.0% *FBXW7*, and 1.8% *BRAF*.

**Conclusions:** Appendiceal cancers show considerable heterogeneity with high levels of drug resistance proteins (BCRP and MRP1), which highlight the difficulty in treating these tumors and suggest an individualized approach to treatment. The incidence of low TS (79%) could be used as a backbone of therapy (using inhibitors such as 5FU/capecitabine or newer agents). Therapeutic options include TOPO1 inhibitors (irinotecan/topotecan), EGFR inhibitors (erlotinib, cetuximab), PDGFR antagonists (regorafenib, axitinib), MGMT (temozolomide). Clinical trials targeting pathways involving *KRAS*, *p53*, *GNAS*, *SMAD4*, *APC*, *ATM*, *PIK3CA*, *FBXW7*, and *BRAF* may be also considered. Overall, appendiceal cancers have similar patterns in their molecular profile to pancreatic cancers (can we say this, any statistical analysis done?) and have differential expression from colorectal cancers. These findings indicate the need to evaluate patient samples for patterns in marker expression and alteration, in order to better understand the molecular biology and formulate a personalized therapy approach in these difficult to treat cancers (supported by a grant from Caris Life Sciences).

**Keywords:** Appendiceal cancer; fluorescent in situ hybridization (FISH); next generation sequencing (NGS)

Submitted Oct 12, 2016. Accepted for publication Nov 25, 2016.

doi: 10.21037/jgo.2017.01.14

View this article at: <http://dx.doi.org/10.21037/jgo.2017.01.14>

## Introduction

Cancers arising from the appendix are rare. In reports of appendectomy specimens, the incidence of malignancy has been reported to be between 0.58% to 0.9% (1,2). The histological spectrum is quite varied, with recent reports through large database studies indicating adenocarcinoma being the most common subtype (3,4). Other histological variants include carcinoid or neuroendocrine and a mixed histology tumor of both carcinoid and adenocarcinoma subtype termed goblet cell carcinoma (5). Finally, another epithelial variant of appendiceal cancer is pseudomyxoma peritonei (PMP), a mucinous neoplasm that clinically presents as gelatinous ascites (6,7). Due to the rarity of these malignancies limited prospective trials exist guiding management. In general, treatment involves surgery for all histological subtypes. For patients with carcinoid appendiceal tumors and goblet cell, appendectomy may be considered in lesions less than 2 cm in size. For lesions 2 cm or more or those with higher grade carcinoid or lymph node involvement right colectomy is indicated (8). For patients with appendiceal adenocarcinoma (<2 cm in size), there is great debate on pursuing simple appendectomies versus hemicolectomy and frequently depends upon opinion (9-11). In patients with PMP, standard treatment involves repeated surgical debulking for symptomatic disease (12). Some clinicians also add intraperitoneal hyperthermia (IPH) treatment or intraperitoneal hyperthermic chemotherapy (IPHC) (13-15).

Outside of localized treatment interventions for appendiceal malignancies, treatment options tend to follow treatment for colorectal malignancies with limited clinical trial data. A recent report from a single center, observational study used FOLFOX-4 as treatment for unresectable or relapsed PMP (7). Furthermore, MGMT methylation was assessed in 42% of these patients, indicating a potential response benefit to temozolomide (7). For carcinoid tumors of the appendix, guidelines recommend treatment to other gastrointestinal neuroendocrine tumors (16). A recent multi-institutional review confirmed current consensus to follow colorectal cancer treatment for appendiceal adenocarcinomas (17). We conducted an analysis of 588 patients with a diagnosis of appendiceal cancer across various histological subtypes who underwent molecular profiling through Caris Life Sciences to look for potential actionable targets and combinations for therapy. Molecular profiling has been used effectively in other cancers to identify novel treatment options. Only two studies to date have reviewed the molecular profile of

appendiceal cancers; those studies had very few patients (n=38 and 149) (18,19), and the analysis was limited to gene mutations. Our analysis encompassed multiple profiling platforms from gene alterations to gene amplification and protein expression levels, and 588 patients were evaluated, almost four times more than the previous studies.

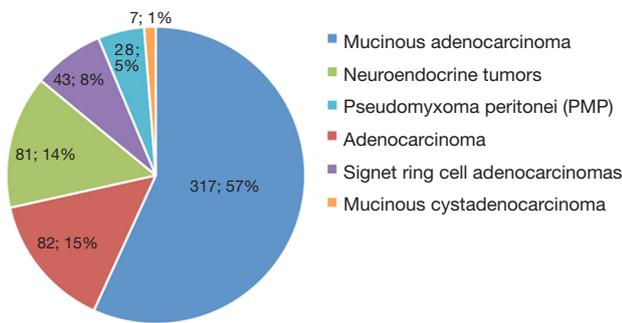
## Methods

### Materials

Data for the 588 specimens with appendix primary malignancies profiled on at least one platform by Caris Life Sciences from 2006 through 2014 were included. Formalin fixed paraffin-embedded (FFPE) samples were sent for analysis from treating physicians around the world (59 countries). The specific histology was extracted from paperwork submitted by the treating physician. Tumors were initially verified by a board certified pathologist for sufficient tumor presence and to confirm the histology. Samples were subsequently analyzed using one or more of the profiling platforms as described below. Biomarkers for analysis varied by case, dependent on tissue availability, physician preference, technology standards over the course of the study, and their potential to be targeted therapeutically and/or based on clinical evidence of a utility in other solid tumors. Carcinosarcomas and adenomas were excluded from analysis. No clinical data, with the exception of basic demographics, were available for this analysis. Biomarkers for analysis were selected based on their potential to be targeted therapeutically and/or based on clinical evidence of a utility in other solid tumors. In accordance with institutional IRB guidelines, because patient identity protection was maintained throughout the study and involves the collection of existing data, the study was considered IRB exempt.

### Immunohistochemistry (IHC)

Protein expression was determined by IHC analysis, using commercially available detection kits and automated staining techniques (Benchmark XT, Ventana, and Autostainer Link 48, Dako). Antibodies used included: androgen receptor (AR), topoisomerases 1 and 2 (TOPO1, TOPO2A) (Leica Biosystems); estrogen receptor (ER), progesterone receptor (PR), cMET, Human Epidermal Growth Factor Receptor 2 (HER2) (Ventana); cKIT, epidermal growth factor receptor (EGFR), phosphatase and tensin homolog



**Figure 1** Distribution of cases by histologic subtype.

(PTEN) (Dako), O(6)-methylguanine-methyltransferase (MGMT), P-glycoprotein (PGP), thymidylate synthase (TS) (Invitrogen); transducin-like enhancer of split 3 (TLE3, Santa Cruz); ribonucleotide reductase M1 (RRM1, Protein Tech); SPARC (monoclonal, R&D Systems; polyclonal, Exalpa), tubulin beta-3 chain (TUBB3) (Covance), Excision Repair Cross-Complementation Group 1 (ERCC1, Abcam), platelet derived growth factor receptor alpha (PDGFRA, Thermo), Programmed cell death protein 1 (PD-1) and Programmed death-ligand 1 (PD-L1) (BD Pharmingen and R&D Systems). IHC thresholds previously validated in other cancers were used, as previously described (20), as cutoffs are not established in appendiceal cancers.

### *In situ hybridization*

Fluorescent in situ hybridization (FISH) was used for evaluation of the *HER-2/neu* (*HER-2/CEP17* probe; *HER-2/CEP17* ratio >2.2 was considered amplified), *EGFR* (*EGFR/CEP7* probe *EGFR/CEP7* ratio  $\geq 2$ , or  $\geq 15$  *EGFR* copies per cell in  $\geq 10\%$  of analyzed cells was considered amplified), *TOP2A* (*TOP2/CEP17* probe; *TOP2A/CEP17* ratio  $\geq 2.0$  was considered amplified), *cMET* (*cMET/CEP7* probe; *cMET/CEP7* ratio  $\geq 5$  was considered amplified). *HER-2/neu* and *cMET* status were more recently evaluated by chromogenic *in situ* hybridization (INFORM *HER-2* Dual ISH DNA Probe Cocktail; commercially available *cMET* and chromosome 7 DIG probe; Ventana), and used the same scoring system as for FISH.

### *Mutational analysis*

#### **Sanger sequencing**

Prior to the availability of CLIA certified NGS, mutation analysis by Sanger sequencing included selected regions of *BRAF*, *KRAS*, *cKIT*, and *EGFR* genes and was performed

by using M13-linked PCR primers designed to amplify targeted sequences. PCR products were bi-directionally sequenced using the BigDye Terminator v1.1 chemistry, analyzed using the 3730 DNA Analyzer (Applied Biosystems). Sequence traces were analyzed using Mutation Surveyor software v3.25 (Soft Genetics).

#### **Next generation sequencing (NGS)**

Direct sequence analysis was performed on genomic DNA isolated from FFPE tumor samples using the Illumina MiSeq platform. Average sequencing depth was >1,000 $\times$ . Specific regions of 47 genes were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.

Complete information on thresholds and specific reagents are available at: <http://www.carismolecularintelligence.com> (20). The variant call was based on nomenclature defined by the ACMGG. Mutations were defined as clinically actionable if the mutation was one for which there is an approved agent available to target, even if the agent is approved for a different tumor type, as well as any clinical trial based on that alteration.

#### *Statistical analysis*

The patient population and profiling data were characterized using standard descriptive statistics. When comparing data across the subtypes, groups with less than five cases were not considered. For chemotherapy protein biomarkers, overexpression or loss in at least 60% of samples in a particular subtype were considered clinically significant (mean selected as cutoff).

## **Results**

The cases were categorized into histologic subtypes, utilizing information in pathology reports and subsequent pathology review of H&E slides, prior to analysis of molecular patterns in the test results. The majority of cases were adenocarcinomas, at 57% [317] of the total cases (*Figure 1*). Basic demographic comparisons identified similarities and differences in gender and age distribution by subtypes (*Table 1*). Specific differences by technology are described by section, followed by an evaluation of the overall differences identified between subtypes.

#### *Protein expression*

Expression of proteins varied by subtype (*Table 2*). Specific

**Table 1** Demographic comparisons by histologic subtype

Subtype	Total cases	Percent metastatic when profiled (%)	Median age (years)	Age range (years)	Gender, n [%]	
					Female	Male
Mucinous adenocarcinoma	317	88.6	55	20–88	191 [60]	126 [40]
Adenocarcinoma	82	84.1	56	28–85	43 [52]	39 [48]
Neuroendocrine tumors	81	71.6	56	23–80	49 [61]	32 [39]
Signet ring cell adenocarcinomas	43	83.7	53	27–70	30 [70]	14 [30]
Pseudomyxoma peritonei (PMP)	28	64.3	57	31–77	16 [57]	12 [43]
Mucinous cystadenocarcinoma	7	42.9	57	40–67	4 [57]	3 [43]

patterns included overexpression of epidermal growth factor receptor (EGFR) in 74% of cases, with a predominance of adenocarcinomas and PMP's. TOPO2A was overexpressed on an average in 38% of cases, with the highest rate of in adenocarcinomas (69%). Mismatch repair proteins, MLH1, MSH2, MSH6, and PMS2, were overexpressed in 100% of 75 cases tested. No microsatellite instability was identified in 39 cases tested across subtypes. PR was overexpressed most frequently in PMP (11%) while AR and ER were only aberrated in 2 of >500 cases tested. Other identified aberrations in protein expression included the over-expression of PD-L1 or PD-1 tumor infiltrating lymphocytes in 3% and 31% of cases, respectively and overexpression of cKIT in 18% of mucinous, 16% of adenocarcinoma, and 53% of pseudomyxoma tissues.

### *In situ hybridization*

A single neuroendocrine case was identified with an increase in EGFR copy number, out of 43 cases tested. No amplifications were identified in TOP2A (n=24), HER2 (n=246), or cMET (n=194) (data not shown).

### *Gene sequencing*

Patterns in a majority of the genomic alterations were different across subtypes. The adenocarcinomas had higher frequency of alterations in *APC*, *BRAF*, *BRCA2*, *SMAD4*, and *TP53* compared to all other subtypes (Table 3), while PMP had the highest incidence of *GNAS* and *KRAS* alterations. Alterations in the PI3 kinase pathway (*AKT1*, *PIK3CA*, and *PTEN*) were infrequently identified across subtypes, with 11% *PIK3CA* alterations in PMP. *BRCA2* mutations were identified in 3 of 8 adenocarcinomas tested. Notably, other targetable mutations were found

in individual cases, including three cKIT mutations (two mucinous and one neuroendocrine), an *ERBB2* mutation, and a *BRCA1* mutation.

Overall differences were observed between the subtypes, in both patterns of gene mutations and protein expression levels. The mucinous adenocarcinoma, adenocarcinoma, and PMP were tumors more likely to exhibit *KRAS* and *GNAS* mutations (65%, 47%, and 83%, respectively, compared to only 9% in neuroendocrine and 7% in signet ring cell adenocarcinomas). Notably all subtypes, except for PMP, harbored *TP53* mutations. Only mucinous adenocarcinomas harbored *ATM* mutations, and only mucinous adenocarcinomas and adenocarcinomas harbored *BRCA2* mutations (a limited number of cases were tested). *APC* mutations were found at a significantly higher percent in adenocarcinomas, compared to all other subtypes ( $P<0.001$ ), and *FBXW7* was found between 5–10% in three subtypes (Mucinous, adenocarcinoma, and signet ring) and never in two subtypes (neuroendocrine and PMP). While *SMAD4* was found at 22% in adenocarcinomas, it was identified at >10% in all subtypes. *BRAF* mutations were identified in 8% of adenocarcinomas, in 2% of mucinous adenocarcinomas and not identified in any other subtypes.

## Discussion

Molecular profiling of appendiceal cancers suggests a number of treatment options. Treatments based upon the IHC expression of these proteins in appendiceal adenocarcinomas include the use of 5-FU (low TS) that may be combined with irinotecan (due to high TOPO1). Gemcitabine (low RRM1) along with taxanes such as paclitaxel, albumin-bound paclitaxel, docetaxel (low TUBB3, high TLE3) may also be considered as a therapeutic option for appendiceal adenocarcinomas.

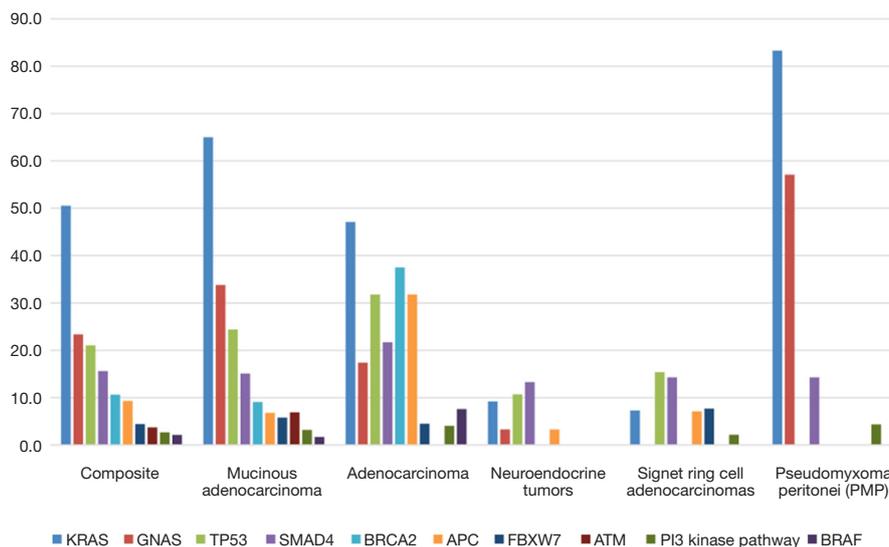
**Table 2** Immunohistochemical analysis of protein expression, as indicated by histologic subtype. Number (N) of patients tested in each subtype is identified in parentheses. Expression of the biomarker below the threshold is considered predictive of response to therapy

Histologic subtype	Totals	Immunohistochemistry																											
		Fragment analysis	FA MSI	AR	BCRP	cKIT	cMET	EGFR	ER	ERCC-1 <sup>§</sup>	HER2	MGMT <sup>§</sup>	MILH1	MRP1	MSH2	MSH6	PDGFR	PD-1	PD-L1	PGP	PMS2	PR	PTEN <sup>§</sup>	RRM1 <sup>§</sup>	SPARC	TLE3	TOP2A	TOPO1	TS <sup>§</sup>
Mucinous adenocarcinoma (n=317)	Subtotal positive	0	1	58	28	63	13	0	99	1	207	36	131	36	36	26	9	1	153	36	2	180	62	112	17	92	170	61	32
	Total cases	16	288	60	160	131	19	291	190	293	287	36	155	36	36	65	37	37	253	36	290	300	276	292	131	254	279	284	95
	% cases positive	0	0.3	96.7	17.5	48.1	68.4	0	52.1	0.3	72.1	100	84.5	100	100	40	24.3	2.7	60.5	100	0.7	60	22.5	38.4	13	36.2	60.9	21.5	33.7
	Subtotal positive	0	0	9	6	22	10	0	6	1	49	14	27	14	14	3	6	1	34	14	0	34	23	26	10	42	33	12	9
Adenocarcinoma (n=82)	Total cases	7	76	9	37	40	12	76	44	77	73	14	32	14	14	13	14	14	65	14	76	75	69	76	41	61	67	69	31
	% cases positive	0	0	100	16.2	55	83.3	0	13.6	1.3	67.1	100	84.4	100	100	23.1	42.9	7.1	52.3	100	0	45.3	33.3	34.2	24.4	68.9	49.3	17.4	29
	Subtotal positive	0	1	10	1	15	2	2	13	0	40	12	20	12	11	1	7	0	25	12	1	46	17	24	8	22	34	13	7
	Total cases	9	74	11	34	39	4	74	39	73	74	12	34	12	11	11	14	14	68	12	74	77	70	74	40	65	68	69	34
Neuroendocrine tumors (n=81)	% cases positive	0	1.4	90.9	2.9	38.5	50	2.7	33.3	0	54.1	100	58.8	100	100	9.1	50	0	36.8	100	1.4	59.7	24.3	32.4	20	33.8	50	18.8	20.6
	Subtotal positive	0	0	3	1	4	2	0	11	0	29	7	15	7	7	0	2	1	10	7	0	23	9	10	1	9	22	6	1
	Total cases	5	39	3	21	19	3	39	23	40	39	7	21	7	7	3	7	7	37	7	39	42	37	40	19	37	38	39	16
	% cases positive	0	0	100	4.8	21.1	66.7	0	47.8	0	74.4	100	71.4	100	100	0	28.6	14.3	27	100	0	54.8	24.3	25	5.3	24.3	57.9	15.4	6.3
Pseudomyxoma peritonei (PMP) (n=28)	Subtotal positive	0	0	7	8	6	9	0	6	0	20	6	11	6	6	6	0	0	22	6	3	18	2	9	3	5	15	4	3
	Total cases	2	27	7	15	12	11	27	20	27	25	6	12	6	6	10	6	6	24	6	27	24	23	28	14	23	24	25	10
	% cases positive	0	0	100	53.3	50	81.8	0	30	0	80	100	91.7	100	100	60	0	0	91.7	100	11.1	75	8.7	32.1	21.4	21.7	62.5	16	30
	Subtotal positive	0	0	1	0	0	0	5	0	5	0	4	0	0	0	0	0	0	5	0	0	4	4	2	1	1	4	3	0
Mucinous cystadenocarcinoma (n=7)	Total cases	0	7	1	5	2	0	7	7	7	7	0	5	0	1	0	0	0	6	0	7	7	7	7	2	6	7	7	0
	% cases positive	-	0	100	0	0	-	0	71.4	0	71.4	-	80	-	0	-	-	-	83.3	-	0	57.1	28.6	50	16.7	57.1	42.9	-	
	Subtotal positive	0	2	88	44	110	36	2	140	2	350	75	208	75	74	36	24	3	249	75	6	305	117	183	40	171	278	99	52
	Total cases	39	511	91	272	243	49	514	323	517	505	75	259	75	74	103	78	78	453	75	513	525	482	517	247	446	483	493	186
Composite (n=558)	% cases positive	0	0.4	96.7	16.2	45.3	73.5	0.4	43.3	0.4	69.3	100.0	80.3	100.0	100.0	35.0	30.8	3.8	55.0	100.0	1.2	58.1	24.3	35.4	16.2	38.3	57.6	20.1	28.0
	Subtotal positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Immunohistochemical analysis of protein expression, as indicated by histologic subtype. Number (N) of patients tested in each subtype is identified in parentheses. Expression of the biomarker below the threshold is considered predictive of response to therapy. §, proteins for which underexpression or loss are measured as opposed to overexpression.

**Table 3** Frequency of genomic alterations (either Sanger or next generation sequencing) by histologic subtype. Genes with no mutations identified in hot spots interrogated included: *ALK, BRCA1, CSF1R, CTNNB4, EGFR, ERBB4, FGFR1, FGFR2, FLT3, GNAI1, GNAQ, HNF1A, HRAS, IDH1, JAK2, KDR, MPL, NPM1, NRAS, PDGFRA, PTPN11, RET, SMARCB1, VHL*

Histologic subtype	Totals	ABL1	AKT1	APC	ATM	BRAF	CDH1	cKIT	cMET	ERBB2	FBXW7	GNAS	JAK3	KRAS	MLH1	PIK3CA	PTEN	RBI	SMAD4	SMO	STK11	TP53
Mucinous adenocarcinoma	Subtotal positive	1	1	6	6	4	0	2	2	1	5	27	2	171	2	7	1	0	13	1	1	20
	Total cases	83	85	88	87	239	88	99	88	84	86	80	88	263	88	114	83	88	86	77	80	82
	% cases positive	1.2	1.2	6.8	6.9	1.7	0	2	2.3	1.2	5.8	33.8	2.3	65	2.3	6.1	1.2	0	15.1	1.3	1.3	24.4
Adenocarcinoma	Subtotal positive	0	1	7	0	5	0	0	1	0	1	4	1	32	0	1	1	0	5	0	0	7
	Total cases	22	23	22	23	66	23	29	23	22	22	23	23	68	23	29	22	23	23	19	22	22
	% cases positive	0	4.3	31.8	0	7.6	0	0	4.3	0	4.5	17.4	4.3	47.1	0	3.4	4.5	0	21.7	0	0	31.8
Neuroendocrine tumors	Subtotal positive	0	0	1	0	0	0	1	0	0	0	1	0	6	1	0	0	0	4	1	1	3
	Total cases	30	30	30	30	60	30	35	30	30	30	30	30	65	30	37	30	30	30	27	30	28
	% cases positive	0	0	3.3	0	0	0	2.9	0	0	0	3.3	0	9.2	3.3	0	0	0	13.3	3.7	3.3	10.7
Signet ring cell adenocarcinomas	Subtotal positive	0	0	1	0	0	1	0	0	0	1	0	0	3	0	0	1	1	2	0	0	2
	Total cases	14	14	14	14	37	14	17	14	13	13	14	14	41	14	18	14	14	14	13	13	13
	% cases positive	0	0	7.1	0	0	7.1	0	0	0	7.7	0	0	7.3	0	0	7.1	7.1	14.3	0	0	15.4
Pseudomyxoma peritonei (PMP)	Subtotal positive	0	0	0	0	0	0	0	0	0	0	4	0	15	0	1	0	0	1	0	0	0
	Total cases	7	7	7	7	13	7	8	7	7	7	7	7	18	7	9	7	7	7	7	7	7
	% cases positive	0	0	0	0	0	0	0	0	0	0	57.1	0	83.3	0	11.1	0	0	14.3	0	0	0
Mucinous cystadenocarcinoma	Subtotal positive	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
	Total cases	0	0	0	0	5	0	0	0	0	0	0	0	6	0	1	0	0	0	0	0	0
	% cases positive	-	-	-	-	0	-	-	-	-	-	-	-	100	-	0	-	-	-	-	-	-
Composite	Subtotal positive	1	2	15	6	9	1	3	3	1	7	36	3	233	3	9	3	1	25	2	2	32
	Total cases	156	159	161	161	420	162	188	162	156	158	154	162	461	162	208	156	162	160	143	152	152
	% cases positive	0.6	1.3	9.3	3.7	2.1	0.6	1.6	1.9	0.6	4.4	23.4	1.9	50.5	1.9	4.3	1.9	0.6	15.6	1.4	1.3	21.1



**Figure 2** Distribution of gene alterations by subtype (mucinous cystadenocarcinoma not shown, due to limited number of cases tested, although it is noted that 6/6 cases had a KRAS mutation).

Due to the low expression ERCC1 seen in appendiceal adenocarcinomas, platinum therapeutics such as cisplatin or oxaliplatin may be combined with either 5-FU or gemcitabine. The adenocarcinomas have a consistent pattern of expression by IHC. In examining mutations in appendiceal adenocarcinoma, KRAS was the most frequent mutation in the mucinous and colonic type adenocarcinoma specimens (85–100% *vs.* a CRC incidence of 30–50%). Of note was the relative frequency of BRCA2 mutations at 37.5% in the eight colonic type appendiceal adenocarcinomas tested. These mutations may give rise for several different therapeutic treatment options, including those utilizing platinum based therapy (21) and PARP (poly (ADP-ribose) polymerase) inhibition (22). Another difference seen in *Figure 2* between colonic-type appendiceal adenocarcinoma and mucinous and signet ring cell is the high mutation rate in APC, a protein involved in the WNT pathway and is seen in individuals with colon cancer and has become a source of agents targeting this pathway in clinical trials (23).

In the neuroendocrine class of appendiceal malignancies, the low expression of and MGMT and TS by IHC can be used as a basis to consider the combination of capecitabine and temozolomide, which has been shown to be beneficial in individuals with neuroendocrine tumors (24). The appendiceal neuroendocrine tumors may also be sensitive to gemcitabine (low RRM1) along with taxanes (low TUBB3, high TLE3). Hot spot mutation analysis yielded

few mutations above 10% of specimens with the greatest being SMAD4 and TP53, which currently do not have FDA approved treatments targeting these mutations.

PMP tumor analysis showed low expression of ERCC1 and RRM1 which gemcitabine and cisplatin may be considered if localized therapy is not an option. The low expression of TS also points to the consideration of 5FU and oxaliplatin as an option for therapy. Combination therapy with 5-FU and irinotecan may also be a consideration due to the high expression of TOPO1 in these tumors. Of note, there was no positive expression of either PD-1 or PD-L1 in these tumors along with 100% expression of the mismatch-repair proteins in the samples analyzed. PMP tumor mutations were significant in the presence of KRAS (83.3%), GNAS (57.1%), SMAD4 (14.3%) and PI3KCA (11.1%) mutations, both of which do not have approved targeted therapy options outside of a clinical trial.

Microsatellite instability, seen in 15% of colorectal cancers (25) was not seen in appendiceal cancers. The mismatch repair markers, MLH1, MSH2, MSH6, and PMS2 were overexpressed in 100% of appendiceal cancers tested across subtypes. A recent study showed benefit in checkpoint immune blockade in individuals whose tumors were mismatch-repair deficient (26). Despite the presence of PD-1 and PD-L1 expression seen in appendiceal adenocarcinomas, the normal expression of mismatch-repair proteins points against consideration of immune

checkpoint blockade.

The high incidence of cKIT overexpression and number of cKIT mutations across appendiceal cancers, would suggest that treatment with anti cKIT therapies, such as imatinib, may be beneficial in appendiceal cancers, as has been shown in other rare cancers (27).

## Conclusions

Recent advances in therapeutics developed to target aberrations in known oncogenic genes have revolutionized medical oncology. Recognition of patterns in biomarker aberrations in different cancers is informing changes in guidelines. Analysis of 588 tumor samples from patients with appendiceal cancer, identified genomic alteration patterns, patterns in gene expression, and aberrations in protein expression that distinguish appendiceal cancers from colorectal cancers and distinguish subtypes of appendiceal cancers. Patterns of genomic alterations in *APC*, *GNAS*, and *SMAD4* in the neuroendocrine tumors most closely resembled alterations documented in pancreatic adenocarcinomas (28), with the exception of *KRAS* alterations being significantly lower). The incidence of *KRAS* alterations in PMP was most similar to rates in pancreatic cancers, while the incidence of *APC* and *KRAS* alterations in adenocarcinomas were similar to those in colorectal cancers. Identification of 37% of cases (n=8) with a *BRCA2* mutation suggests that a subset of appendiceal adenocarcinomas may have a familial predisposition. These data suggest new avenues for molecularly directed therapies in appendiceal cancers. Overall, this study identified actionable alterations in 99% of cases tested. As knowledge increases of oncogenic pathways and more targeted therapies are approved, continuing to personalize treatment based on the patient's unique molecular profile will improve the outcomes.

## Acknowledgements

None.

## Footnote

*Conflicts of Interest:* J Kimbrough: Employee Caris Life Sciences. The other authors have no conflicts of interest to declare.

*Ethical Statement:* In accordance with institutional IRB

guidelines, because patient identity protection was maintained throughout the study and involves the collection of existing data, the study was considered IRB exempt.

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**Cite this article as:** Borazanci E, Millis SZ, Kimbrough J, Doll N, Von Hoff D, Ramanathan RK. Potential actionable targets in appendiceal cancer detected by immunohistochemistry, fluorescent in situ hybridization, and mutational analysis. *J Gastrointest Oncol* 2017;8(1):164-172. doi: 10.21037/jgo.2017.01.14