original reports

Molecular Classification of Appendiceal Adenocarcinoma

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abstract

PURPOSE Appendiceal adenocarcinomas (ACs) are rare, histologically diverse malignancies treated as colorectal cancers despite having distinct biology and clinical behavior. To guide clinical decision making, we defined molecular subtypes of AC associated with patient survival, metastatic burden, and chemotherapy response.

PATIENTS AND METHODS A comprehensive molecular analysis was performed in patients with AC to define molecular subtypes. Associations between molecular subtype and overall survival, intraoperative peritoneal cancer index, and first-line chemotherapy response were assessed adjusting for histopathologic and clinical variables using multivariable Cox proportional hazards, linear regression, and logistic regression models.

RESULTS We defined distinct molecular lineages of mucinous appendiceal adenocarcinoma (MAAP) from co-occurring mutations in *GNAS*, *RAS*, and *TP53*. Of 164 MAAP tumors, 24 were RAS-mutant (mut) predominant (*RAS*-mut/*GNAS*-wild-type [wt]/*TP53*-wt) with significantly decreased mutations and chromosomal alterations compared with tumors with *GNAS* mutations (GNAS-mut predominant) or *TP53* mutations (TP53-mut predominant). No patient with RAS-mut predominant subtype metastatic MAAP died of cancer, and overall survival in this subgroup was significantly improved compared with patients with GNAS-mut ($P = .05$) and TP53-mut ($P = .004$) predominant subtypes. TP53-mut predominant subtypes were highly aneuploid; increased tumor aneuploidy was independently ($P = .001$) associated with poor prognosis. The findings retained significance in patients with any metastatic AC. RAS-mut predominant metastases exhibited reduced peritoneal tumor bulk ($P = .04$) and stromal invasion ($P < .001$) compared with GNAS-mut or TP53-mut predominant tumors, respectively. Patients with RAS-mut predominant MAAP responded more to first-line chemotherapy (50%) compared with patients with GNAS-mut predominant tumors (6%, $P = .03$).

CONCLUSION AC molecular patterns identify distinct molecular subtypes: a clinically indolent RAS-mut/GNAS-wt/TP53-wt subtype; a chemotherapy-resistant GNAS-mut predominant subtype; and an aggressive, highly aneuploid TP53-mut predominant subtype. Each subtype exhibits conserved clinical behavior irrespective of histopathology.

J Clin Oncol 41:1553-1564. © 2022 by American Society of Clinical Oncology

INTRODUCTION

Appendiceal adenocarcinomas (ACs) encompass diverse, rare tumors that comprise up to 1% of all gastrointestinal cancers.[1-3] ACs are conventionally defined by histology. Mucinous appendiceal adenocarcinoma (MAAP) is the most common subtype, identified by tumor cells highly enriched in surrounding mucin, while goblet cell adenocarcinoma (GCA) exhibits mixed glandular and neuroendocrine features, and intestinal or colonic-type adenocarcinoma (CTAAP) is morphologically similar to colorectal cancer.[4-8] Due to the lack of AC-specific guidelines, ACs are uniformly managed using colorectal cancer treatment paradigms. Given high AC peritoneal tropism, management often involves extensive peritoneal-based cytoreductive surgeries (CRSs) to remove visible disease.[9-11]

Although AC histologic grade is associated with overall survival (OS),[11-13] in practice, AC tumors often exhibit disease behavior that is inconsistent with histologic grade, leading to substantial management uncertainty.[13] Many patients with metastatic AC exhibit decades long disease indolence with a low peritoneal cancer index (PCI) tumor burden; these patients can experience long treatment-free intervals following CRS.[11,14-16] Other patients with similar histologic features exhibit aggressive, unresectable disease, poor OS, and low chemotherapy responsiveness.[6,11,17] Histology-based classifications are frequently amended because few shared traits adequately explain this variable biology.[6,18,19]

Increased tumor genomic complexity is associated with disease aggressiveness.[20-23] In colorectal cancer, serial accumulation of alterations in *RAS*, *APC*, *TP53*, and other WNT-signaling modifiers drive tumorigenesis and

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on October 11, 2022 and published at ascopubs.org/journal/jco on December 9, 2022: DOI https://doi.org/10.1200/JCO.22.01392

CONTEXT

Key Objective

Appendiceal adenocarcinomas (ACs) exhibit diverse clinical behavior frequently inconsistent with tumor histopathology, complicating management. It is unclear if AC genomic features are clinically relevant. The study defines molecular subtypes of AC associated with genomic complexity and patient outcomes.

Knowledge Generated

Molecular subtypes of AC defined by *RAS*, *GNAS*, and *TP53* mutations are associated with patient overall survival independently of other histopathologic and clinical characteristics. Mucinous AC that is RAS-mutant (mut) predominant exhibits minimal genomic alterations and clinical indolence compared with chemoresistant GNAS-mut predominant tumors and highly invasive, aneuploid TP53-mut predominant tumors.

Relevance *(A.H. Ko)*

This study highlights the value of performing somatic tumor profiling for this relatively rare malignancy. Different molecular subtypes of AC have distinct clinical and biological behaviors that may be useful in guiding therapeutic decision making regarding whether to offer cytoreductive surgery and/or systemic therapy for any given patient.*

*Relevance section written by *JCO* Associate Editor Andrew H. Ko, MD, FASCO.

metastatic potential.[24,25] Prior studies have demonstrated that AC is genetically distinct from colorectal cancer.[13,26,27] Given the shortcomings of histopathologic AC features to predict clinical outcomes, we determined whether co-occurring genomic alterations can identify molecular subtypes with conserved clinical behavior.

PATIENTS AND METHODS

Clinical Cohort and Sample Review

All patients with AC who underwent MSK-IMPACT[28] next-generation sequencing at the Memorial Sloan Kettering Cancer Center from April 2015 to October 2020 (Data Supplement, online only) were included in this retrospective cohort study approved by the Memorial Sloan Kettering Cancer Center Institutional Review Board (IRB). Patients with low (< 10%) tumor purity and no called somatic mutations were excluded from somatic mutational and clinical analyses. Clinical information, including disease and treatment history, was verified from the electronic medical record (Data Supplement). Histologic classification and grade were determined by a team of expert study pathologists (Data Supplement). Well-differentiated tumors lacked high cellularity, invasive implants, or significant cytologic atypia. Moderately differentiated tumors exhibited invasive implants or cytologic atypic in more than 10% of the tumor. Poorly differentiated tumors exhibited signet ring cells in more than 10% of cells (Data Supplement). GCA grade was determined per the WHO guidelines,[4] and the extent of gland formation was used for grading of CTAAP.

Molecular and clinical analyses were first performed in the most prevalent MAAP subtype and then expanded to the entire AC series. Molecular subtypes were derived within the MAAP group as this single histologic subset has a range of patient outcomes with adequate sample size for molecular interrogation.

Genomic Analysis

MSK-IMPACT hybridization capture–based next-generation sequencing was performed on matched patient tumor and blood samples (Data Supplement). The OncoKB[29] database defined clinically actionable mutations and oncogenic driver and tumor suppressor genes.

Mutational prevalence rates in key driver genes and mutational quantity were compared using Fisher's exact and Wilcoxon-Mann-Whitney statistical tests, respectively. Co-occurrence associations between molecular alterations with > 5% prevalence in MAAP tumors were assessed with pairwise Fisher's exact testing and significance was adjusted using the Benjamini-Hochberg (BH) method ($P < .05$) to define molecular subtypes (Data Supplement). Overall tumor aneuploidy was calculated using a score that sums the product of chromosome segment length by copy-number deviation from diploid (2.0; Data Supplement).[30] The FACETS algorithm was used to assess the clonality of called mutations.[31] Differences in tumor aneuploidy between molecular subtypes were assessed with Wilcoxon-Mann-Whitney tests.

Study Outcome Analysis

OS was determined using Kaplan-Meier analysis from the time of first metastasis detection to death with censoring at last follow-up. Cox proportional hazards regression models were used to calculate hazard ratios for death. Multivariable Cox models included relevant covariates,[11] such as tumor histology (when applicable), histologic grade/differentiation, patient age, CRS number (continuous variable), and first-line chemotherapy type. The proportional hazards assumption was verified to assess interactions between predictors and

log(time). The area under the curve (AUC) was calculated for separate Cox models to predict 3-year OS.

The total PCI of patients with metastatic MAAP who underwent CRS was intraoperatively scored in 13 abdominal regions (Data Supplement).[32-34] Discrete comparisons of overall PCI were performed with univariable Wilcoxon-Mann-Whitney and multivariable linear regression testing. All available surgical samples were evaluated for tumor stromal invasion using hematoxylin and eosin staining. Tumor stromal invasion prevalence was compared using Fisher's exact test.

Best radiographic tumor regression rates were compared from computed tomography or magnetic resonance imaging scans of patients with metastatic MAAP undergoing at least one full cycle of first-line chemotherapy using multivariable logistic regression (Data Supplement). In this cohort, the proportional change between the carcinoembryonic antigen (CEA) or carbohydrate antigen 19-9 (CA19-9) at baseline and around the best radiographic assessment was assessed for patients with at least one abnormal biomarker (CEA $\geq$ 10 or CA19-9 $\geq$ 40) in an exploratory analysis with Fisher's exact test. All study statistical analyses were performed using R (v4.0.0; R Studio, Boston, MA). Study data are publicly available at cBioPortal.[35]

Ethics Approval

All patients in the cohort had previously signed a consent form (IRB 12-245) for genomic analysis of their tumors. This study was reviewed and approved by the MSK IRB/Privacy Board and was granted a waiver of consent documentation (IRB retrospective research protocol 21-105).

RESULTS

Patient Cohort Characteristics

Of the 288 unique patient tumor samples, 15 were excluded for low tumor purity (< 10%: Data Supplement). The final study cohort included 273 patients with mucinous adenocarcinomas (MAAP; n/N = 164/273), GCAs (n/N = 72/273), and CTAAPs (n/N = 37/273). Most patients (n/N = 245/273) exhibited metastatic or unresectable disease (Data Supplement). Sample sequencing coverage and tumor purity did not meaningfully differ between subtypes (Data Supplement).

Somatic Mutational Analyses

KRAS, *GNAS*, and *TP53* were frequently mutated in AC (Fig 1A). MAAP tumors exhibited the highest prevalence of *KRAS* (79%, n = 130/164) and *GNAS* (52%, n = 85/164) mutations, while CTAAP tumors exhibited higher frequencies of *TP53* (54%, n = 20/37) alterations and total nonsynonymous mutations per patient (Fig 1A, Data Supplement). All tumors were mismatch repair proficient; median tumor mutational burden was highest in CTAAP (4.4) compared with MAAP (3) and GCA (1.9).

Genomic alterations with an OncoKB database[29] defined level 1 (US Food and Drug Administration [FDA]–recognized biomarker predictive of response to an FDA-approved drug) or level 3B (standard of care or investigational biomarker predictive of response to an FDA-approved or investigational drug in another indication) mutations in nearly 25% of patients (Fig 1B). A level 1 *NTRK1* fusion biomarker was identified in one MAAP tumor,[36] while 6% (n = 10/164) of MAAP exhibited *KRAS* G12C mutations. *BRAF* V600E mutations were rarely observed (1%: n = 3/273).

MAAP Molecular Subtypes

Conserved mutational patterns were evaluated in MAAP tumors (n = 164); the largest AC histologic subtype with diverse tumor grades and clinical outcomes. Ten different genes were pathogenically altered in at least 5% in MAAP tumors (Fig 2A). *GNAS*, *RAS* (*KRAS* and *NRAS*), or *TP53* mutations were found in 89% (n = 146/164) of MAAP tumors

GNAS and *RAS* mutations significantly (BH-corrected $P = .006$) co-occurred. Of 85 patients with *GNAS*-mutant (mut) tumors, 91% of these tumors (77/85) were also *RAS*-mut. *GNAS* and *TP53* mutations showed near-mutual exclusivity (BH-corrected $P < .001$; Fig 2A); only one tumor (1/85) had *GNAS* and *TP53* alterations with no *RAS* mutation.

Of 164 MAAP tumors, 135 (82%) were evaluable for FACETS[31] clonality assessment to examine mutant allele fractions and estimate timing of these recurrent alterations. The majority (69%: 57/83) of clonal *RAS*-mut tumors were *TP53* wild-type (wt), while only 26% (9/35) of clonal *TP53*-mut tumors were *RAS*-wt (Data Supplement), suggesting that when *RAS* and *TP53* mutations co-occur, the *RAS* mutation usually occurs first. Similarly, only 12% (7/59) of *GNAS*-mut clonal tumors were *RAS*-wt, indicating that *GNAS* mutations tend to occur within a background of previously altered *RAS*. No tumors with *TP53* or *GNAS* subclonal mutations were *RAS*-wt, consistent with generally early *RAS* mutation acquisition and subsequent *TP53* or *GNAS* alteration.

On the basis of these associations, we classified four distinct molecular subtypes (Fig 2B): (1) RAS-mut predominant (*RAS*-mut, all *TP53*-wt/*GNAS*-wt: n = 24/164), (2) GNAS-mut predominant (*GNAS*-mut/*RAS*-wt or *GNAS*-mut/*RAS*-mut, all *TP53*-wt: n = 76/164), (3) TP53-mut predominant (all *TP53*-mut tumors: n = 46/164), and (4) triple-negative (all *RAS*/*GNAS*/*TP53*-wt: n = 18/164).

RAS-Mut Predominant MAAP Demonstrates Minimal Molecular Complexity

Molecular subtypes were all composed of diverse tumor grades and similar patient characteristics (Fig 2B, Data Supplement). RAS-mut predominant MAAP exhibited significantly ($P < .05$) fewer nonsynonymous alterations per patient and fewer median OncoKB-defined pathogenic oncogene mutations compared with all subtypes except triple-negative (Fig 2C). TP53-mut predominant MAAP had

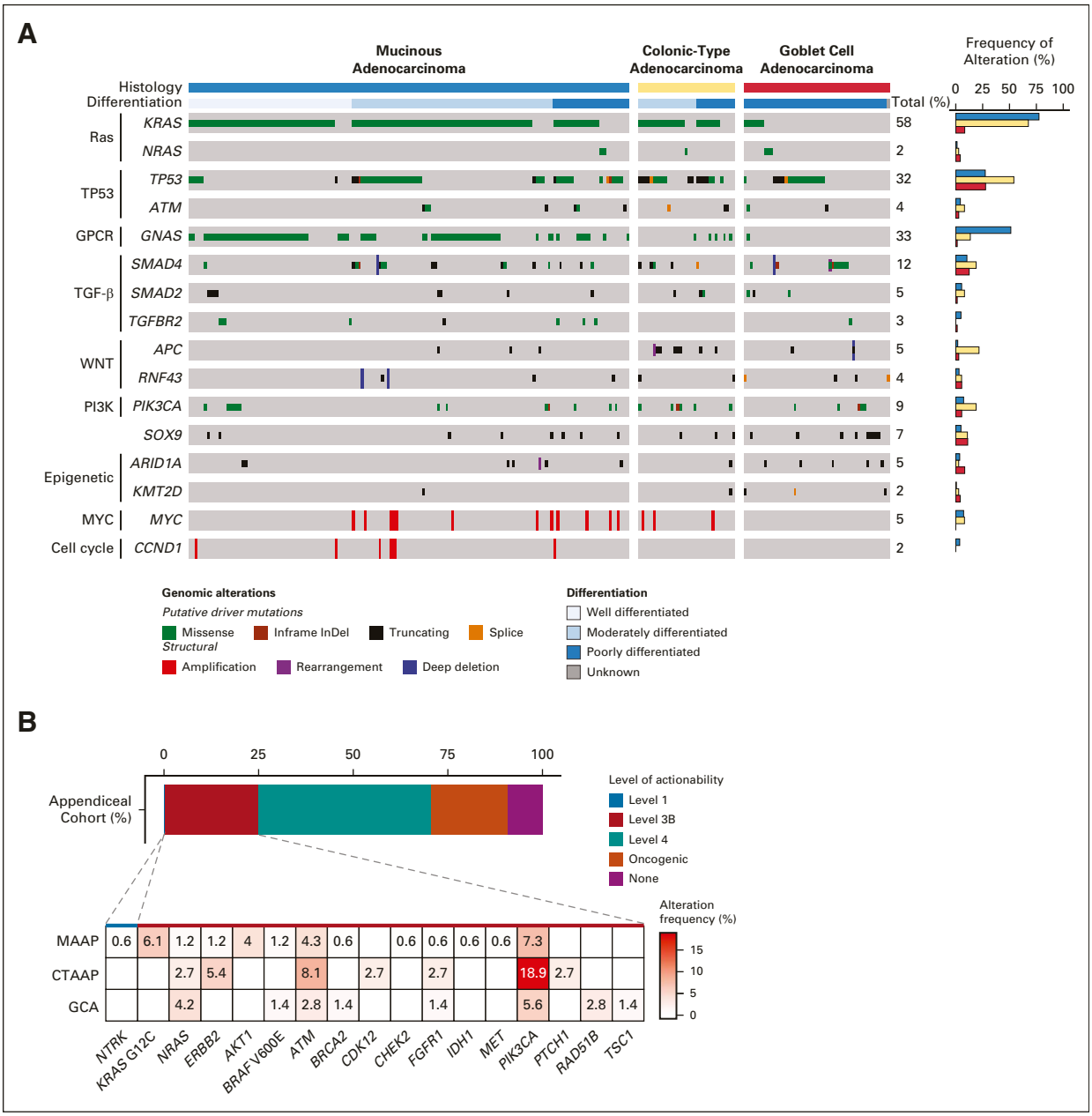

FIG 1. Mutational landscape of AC. (A) Overview of mutational type and prevalence in all study tumors (N = 273) assessed through MSK-IMPACT hybrid capture–based sequencing platform. Tumors with sample purity ≤ 10% with no called synonymous or nonsynonymous mutations were not included in the analysis. (B) Prevalence of OncoKB-predicted clinically actionable alterations in AC. Numbers and red shading intensity represent the frequency of the mutation alterations in the respective AC histology. AC, appendiceal adenocarcinoma; CTAAP, colonic-type adenocarcinoma; GCA, goblet cell adenocarcinoma; MAAP, mucinous appendiceal adenocarcinoma.

significantly higher alterations in tumor suppressor genes per patient and increased aneuploidy scores (Fig 2C). Calculated tumor aneuploidy scores strongly correlated with tumor fraction genome altered (Pearson's r = 0.91; Data Supplement).

RAS-Mut Predominant Tumors Confer a Highly Favorable Prognosis in Metastatic MAAP

We assessed whether molecular subtypes prognosticate OS in patients with metastatic MAAP. All mortality events were cancer-related except for four instances (three of the four were indeterminate). Patients with RAS-mut predominant tumors (n = 21/149) exhibited significantly improved median OS (not reached [NR]) compared with patients with GNAS-mut predominant (NR: $P = .05$), triple-negative (50 months: $P = .01$), and TP53-mut predominant (35 months: $P = .004$) subtypes (Fig 2D). No cancer-related mortality events were witnessed in patients with RAS-mut predominant MAAP after a median follow-up of 54 months (one patient died of COVID-19 disease).

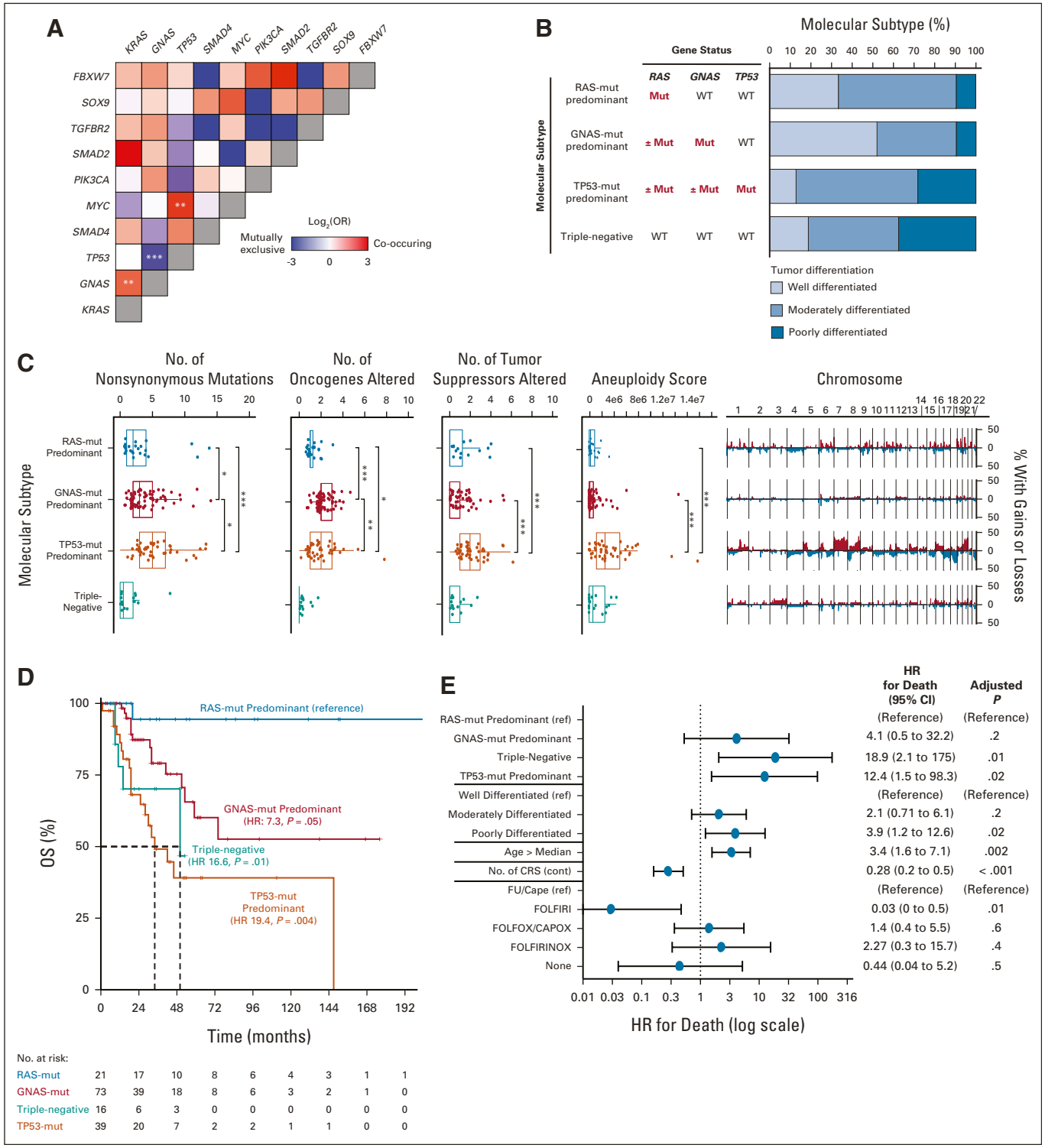

FIG 2. Co-occurrence patterns of driver mutations define prognostically relevant molecular subtypes in MAAP associated with varying molecular complexity. (A) Associations between prevalent driver mutations in MAAP tumors (n = 164) were assessed using the Fisher's exact method with Benjamini-Hochberg multiplicity correction and a significant FDR indicated by asterixis (**FDR-corrected $P < .01$, ***FDR-corrected $P < .001$). (B) Proportion of molecular subtypes with well, moderately, or poorly differentiated tumors. (C) Number of nonsynonymous mutations, pathogenic mutations in OncoKB-defined oncogenes, and pathogenic mutations in OncoKB-defined tumor suppressors organized by molecular subtype with aneuploidy score and copy-number alteration plot (n = 164). Data are represented as median with interquartile ranges. Aneuploidy scores were calculated as per the Data Supplement. Percent gain (red) or loss (blue) of chromosomal segments are represented. Brackets indicate statistically significant differences (*$P < .05$, **$P < .01$, ***$P < .001$) between compared samples using Wilcoxon-Mann-Whitney testing. Triple-negative subtype statistical comparisons are not shown. (D) OS from time of documented metastasis (continued on following page)

FIG 2. (Continued). to death or censoring in patients with metastatic MAAP (n = 149) stratified by molecular subtype. HRs are determined by Cox univariable proportional hazards regression. (E) Multivariable Cox regression model of OS in patients with metastatic MAAP. HRs for death are shown with adjusted *P* values. Patients who received other chemotherapy types are included in the analysis, but the other chemotherapy covariate is not visually represented (n = 2 patients). Chemotherapy types are summarized in the Data Supplement. Cape, capecitabine; CAPOX, capecitabine and oxaliplatin; CRS, cytoreductive surgery; FDR, false discovery rate; FOLFIRI, FU, leucovorin, and irinotecan; FOLFIRINOX, FU, leucovorin, oxaliplatin, and irinotecan; FOLFOX, FU, leucovorin, and oxaliplatin; FU, fluorouracil; HR, hazard ratio; MAAP, mucinous appendiceal adenocarcinoma; OR, odds ratio; OS, overall survival; ref, reference; WT, wild-type.

In a multivariable Cox regression analysis, RAS-mut predominant status was independently associated with significantly reduced mortality compared with the TP53-mut predominant and triple-negative subtypes (Fig 2E). Patients with poorly differentiated tumors exhibited worse median OS (19 months) compared with patients with well-differentiated tumors (OS median NR: $P = .02$; Fig 2E). However, in a separate stratified exploratory analysis, patients with RAS-mut predominant tumors exhibited high survival regardless of differentiation (Data Supplement). Overall, a multivariable model including molecular subtypes demonstrated a superior AUC for 3-year OS evaluation (AUC, 0.75) compared with a conventional histopathologic model including only tumor differentiation (AUC, 0.67) for patients with metastatic MAAP (Table 1).

We further evaluated whether survival differences between molecular subtypes may be explained by tumor aneuploidy. TP53-mut predominant metastatic MAAP tumors composed 31% (46/149) of all analyzed tumors, yet constituted 63% (24/38) of tumors with the highest (75th percentile or above) aneuploidy scores (Data Supplement). High tumor aneuploidy was significantly associated with death in univariable (hazard ratio for death, 3.7; 95% CI, 1.9 to 6.9) and multivariable analyses irrespective of tumor grade (Data Supplement).

MAAP-Derived Molecular Subtypes Are Prognostic for Patients With Any Subtype of Metastatic AC

We applied the molecular subtypes derived in MAAP to the broader cohort of all patients with metastatic AC, including CTAAP and GCA (n = 244; Fig 3A). Approximately 11% of MAAP (16/149), 59% of GCA (36/61), and 11% (4/35) of CTAAP tumors were triple-negative (Data Supplement). Patients with RAS-mut predominant tumors exhibited significantly improved median OS (NR) compared with patients with triple-negative (42 months: $P = .002$), and TP53-mut predominant (35 months, $P < .001$) tumors independent of other

TABLE 1. Model Prediction of 3-Year Overall Survival in Patients With Metastatic Mucinous Appendiceal Adenocarcinoma

				Model 1	Model 2	Model 3	Model 4
				Differentiation + RAS	Differentiation + TP53	Differentiation + GNAS	Complex Cluster
Single Predictor Models				**(AUC, 0.70)**	**(AUC, 0.70)**	**(AUC, 0.68)**	**(AUC, 0.75)**
Predictor	Levels	HR (95% CI)	AUC	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Differentiation	WD	(Reference)	0.67	(Reference)	(Reference)	(Reference)	(Reference)
	MD	3.2 (1.2 to 8.6)		3.2 (1.2 to 8.4)	2.7 (1.0 to 7.4)	3.1 (1.2 to 8.4)	2.8 (1.0 to 7.8)
	PD	8.2 (2.9 to 22.8)		8.7 (3.1 to 24.5)	6.1 (2.1 to 17.8)	8.0 (2.9 to 22.5)	5.9 (2.0 to 17.2)
RAS	WT	(Reference)	0.6	(Reference)			
	Mut	0.32 (0.16 to 0.65)		0.3 (0.2 to 0.6)			
TP53	WT	(Reference)	0.62		(Reference)		
	Mut	3.0 (1.6 to 5.7)			2.1 (1.1 to 4.1)		
GNAS	WT	(Reference)	0.54			(Reference)	
	Mut	0.73 (0.39 to 1.4)				0.9 (0.5 to 1.6)	
Molecular subgroup	RAS-mut	(Reference)	0.70			(Reference)	(Reference)
	GNAS-mut	7.4 (1.0 to 56.0)					7.6 (1.0 to 58.1)
	TP53-mut	19.4 (2.6 to 147)					13.7 (1.8 to 104)
	Triple-negative	16.6 (1.9 to 144)					14.1 (1.6 to 122)

NOTE. HRs and 95% CIs were determined for respective univariable (single predictor models) and multivariable models using Cox proportional hazards regression. *RAS*, *TP53*, and *GNAS* covariates represent individual mutations in these discrete genes, while molecular subtype is defined as a separate covariate. Multivariable models are enumerated models 1-4 with HRs reported for the covariates included in the respective models. AUC for assessment of 3-year overall survival was determined for each univariable and multivariable model.

Abbreviations: AUC, area under the curve; HR, hazard ratio; MD, moderately differentiated; Mut, mutated; PD, poorly differentiated; WD, well differentiated; WT, wild-type.

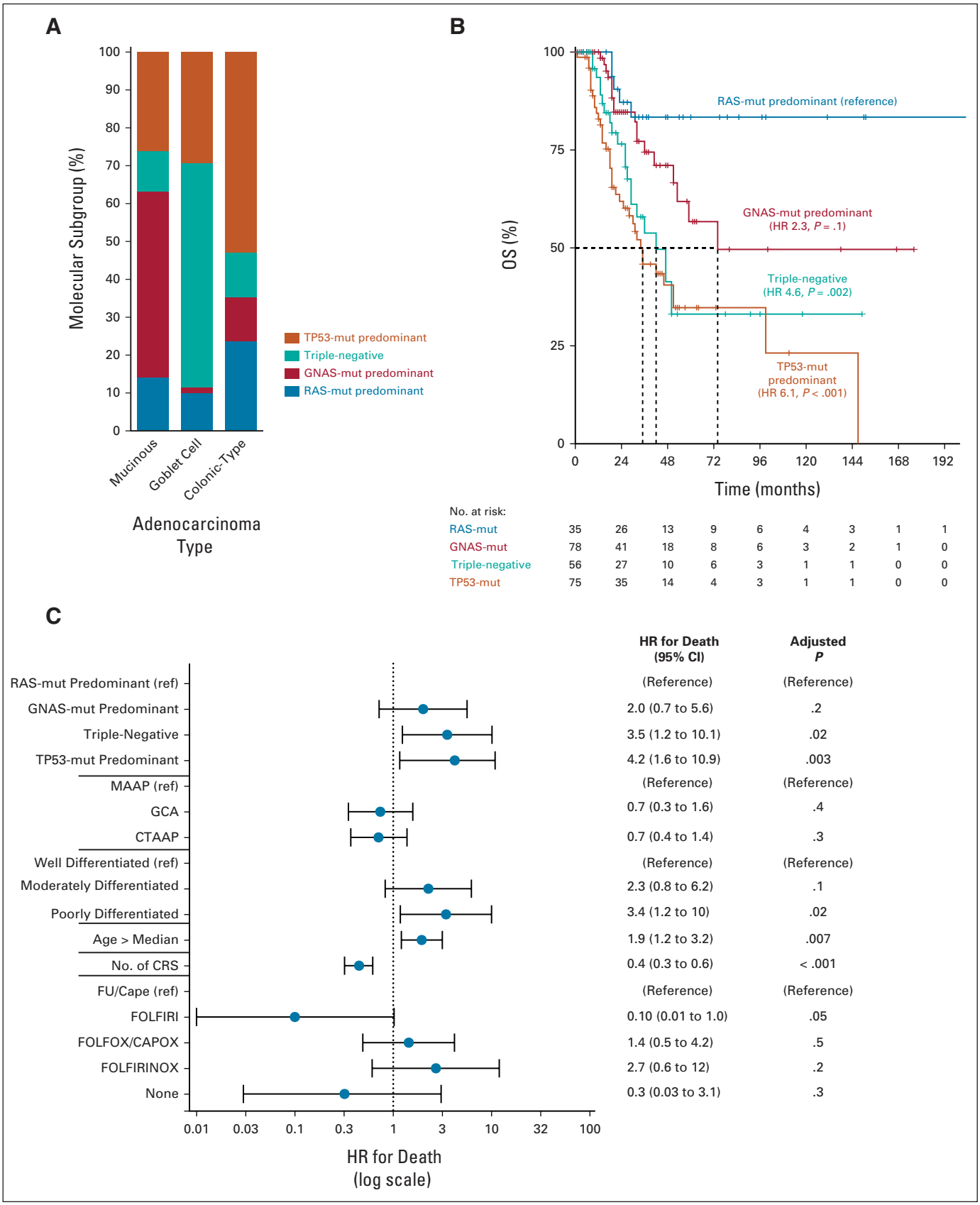

FIG 3. Molecular subtypes are prognostic for overall survival in all patients with metastatic appendiceal adenocarcinoma. (A) Composition of genomic subgroups for each metastatic appendiceal adenocarcinoma histology type. (B) OS of patients with metastatic appendiceal adenocarcinoma stratified by (continued on following page)

FIG 3. (Continued). molecular subtype. (C) Multivariable Cox regression analysis of patients with metastatic appendiceal adenocarcinoma. HRs, 95% CIs, and *P* values were determined from univariable (B) and multivariable (C) Cox proportional hazards regression models. Chemotherapy types are summarized in the Data Supplement. Cape, capecitabine; CAPOX, capecitabine and oxaliplatin; CRS, cytoreductive surgery; CTAAP, colonic-type appendiceal adenocarcinoma; FOLFIRI, FU, leucovorin, and irinotecan; FOLFIRINOX, FU, leucovorin, oxaliplatin, and irinotecan; FOLFOX, FU, leucovorin, and oxaliplatin; FU, fluorouracil; GCA, goblet cell adenocarcinoma; HR, hazard ratio; MAAP, mucinous appendiceal adenocarcinoma; mut, mutant; OS, overall survival; ref, reference.

clinical features (Fig 3B). The findings retained significance independent of histologic and other clinical covariates in a multivariable analysis (Fig 3C). Tumor histology was not a significant predictor of OS (Fig 3C). Multivariable models incorporating molecular subtypes demonstrated improved prognostication of 3-year OS (AUC 0.71) compared with prognostic models with only tumor histology (AUC 0.59) or tumor differentiation (AUC 0.66; Data Supplement).

Akin to MAAP, in patients with all metastatic AC, tumor aneuploidy was highly associated with poor OS independent of histopathology (Data Supplement). Highly aneuploid tumors were disproportionately (61%; 37/61) TP53-mut predominant (Data Supplement).

Complex MAAP Molecular Subtypes Demonstrate Increased Peritoneal Metastatic Burden and Destructive Stromal Invasion

To investigate the biology underlying survival disparities between molecular subtypes, we assessed the intraoperative PCI of 81 patients with metastatic MAAP who underwent CRS. PCI metastatic burden scores were significantly lower in RAS-mut predominant tumors (PCI, 13) compared with a combined group of tumors with complex GNAS-mut and TP53-mut predominant subtypes (PCI, 21, $P = .04$) in univariable (Fig 4A) and multivariable (Fig 4B) analyses. Patients with GNAS-mut predominant tumors exhibited the highest median PCI of 21 (Data Supplement).

In metastatic tumor specimens obtained from patient CRS, four of 20 (25%) evaluable RAS-mut predominant metastatic tumors exhibited destructive stromal invasion. By contrast, destructive stromal invasion was seen in 29 of 32 (91%) TP53-mut predominant metastatic tumors (odds ratio [OR] for invasion, 34.3; 95% CI, 6.4 to 273; $P < .0001$; Fig 4C). Representative images depict RAS-mut predominant metastatic tumors in abdominal mucin pools (Fig 4D) and adhered to the surface of peritoneal fibrous tissue without marked stromal destruction (Fig 4E) in comparison with aggressive omental stromal invasion by TP53-mut predominant tumors (Figs 4F and 4G).

First-Line Chemotherapy Response

We next evaluated whether molecular subtypes predicted clinical response to first-line therapy. Forty-nine patients with metastatic MAAP received first-line chemotherapy (Fig 4H). In a multivariable logistic regression model, only one patient of 19 patients with GNAS-mut predominant (5%) exhibited a radiographic disease response to chemotherapy compared with 50% (3/6) of patients with RAS-mut predominant (adjusted OR of disease response, 0.03; 95% CI, 0.00 to 0.5; $P = .03$; Fig 4H, Data Supplement).

Fourteen of 49 (29%) of patients had at least one abnormal CEA or CA19-9 biomarker measured before chemotherapy and another timed at their best radiographic response disease assessment (Data Supplement). Only one patient of seven (14%) with a GNAS-mut predominant tumor exhibited at least 10% decrease from baseline CA19-9 or CEA during first-line therapy, compared with 6/7 (86%) of tumors with other subtypes (OR for 10% biochemical decrease, 0.04; 95% CI, 0.001 to 0.80; $P = .04$; Fig 4I, Data Supplement).

Finally, we integrated findings from our study analyses of patients with metastatic MAAP (Fig 5). Molecular subtypes identified discrete tumor groups with progressively advanced tumor aneuploidy that was inversely correlated with 3-year OS (Pearson's $r = -0.94$).

DISCUSSION

In agreement with other studies,[13,26,27] we found that activating mutations in *GNAS* and *RAS* are common in AC with relatively fewer *TP53* mutations compared with sporadic colorectal cancer.[25] Our results demonstrate that the clinical course of AC is firmly linked to specific mutational profiles. The profiles show that *RAS* mutations likely occur early in MAAP oncogenesis followed by acquisition of mutations in *GNAS* or *TP53* in a near mutationally exclusive pattern. Analysis of molecular subtypes defined by these mutational patterns reveals the associated progressive aneuploidy and adverse patient outcomes that accompany this mutational evolution.

RAS-mut predominant MAAP is molecularly young, with few nonsynonymous mutations, pathogenic oncogene activations, tumor suppressor disruptions, and copy-number alterations. Two thirds (14/21) of metastatic RAS-mut predominant MAAP tumors have conventionally high-risk moderate or poor histologic differentiation, yet RAS-mut predominant tumors exhibit remarkably low mortality in multivariable models of patients with metastatic MAAP and separately in all AC, including CTAAP and GCA. These RAS-mut predominant ACs demonstrate clinical indolence more akin to low-grade appendiceal mucinous neoplasms and intraductal pancreatic mucinous neoplasms that similarly exhibit *RAS* driver mutations, minimal genomic complexity, and rare progression to overt malignancy.[37-41] Minimal microscopic stromal invasion of RAS-mut predominant tumors further suggests that RAS-mut predominant tumors are indolent and may not be clinically aggressive. Thus, patients with RAS-mut predominant AC may be able to avoid chemotherapy and undergo surgical resection alone with a prolonged disease-free interval.

GNAS-mut predominant MAAP have a higher number of somatic alterations, higher peritoneal disease burden, and

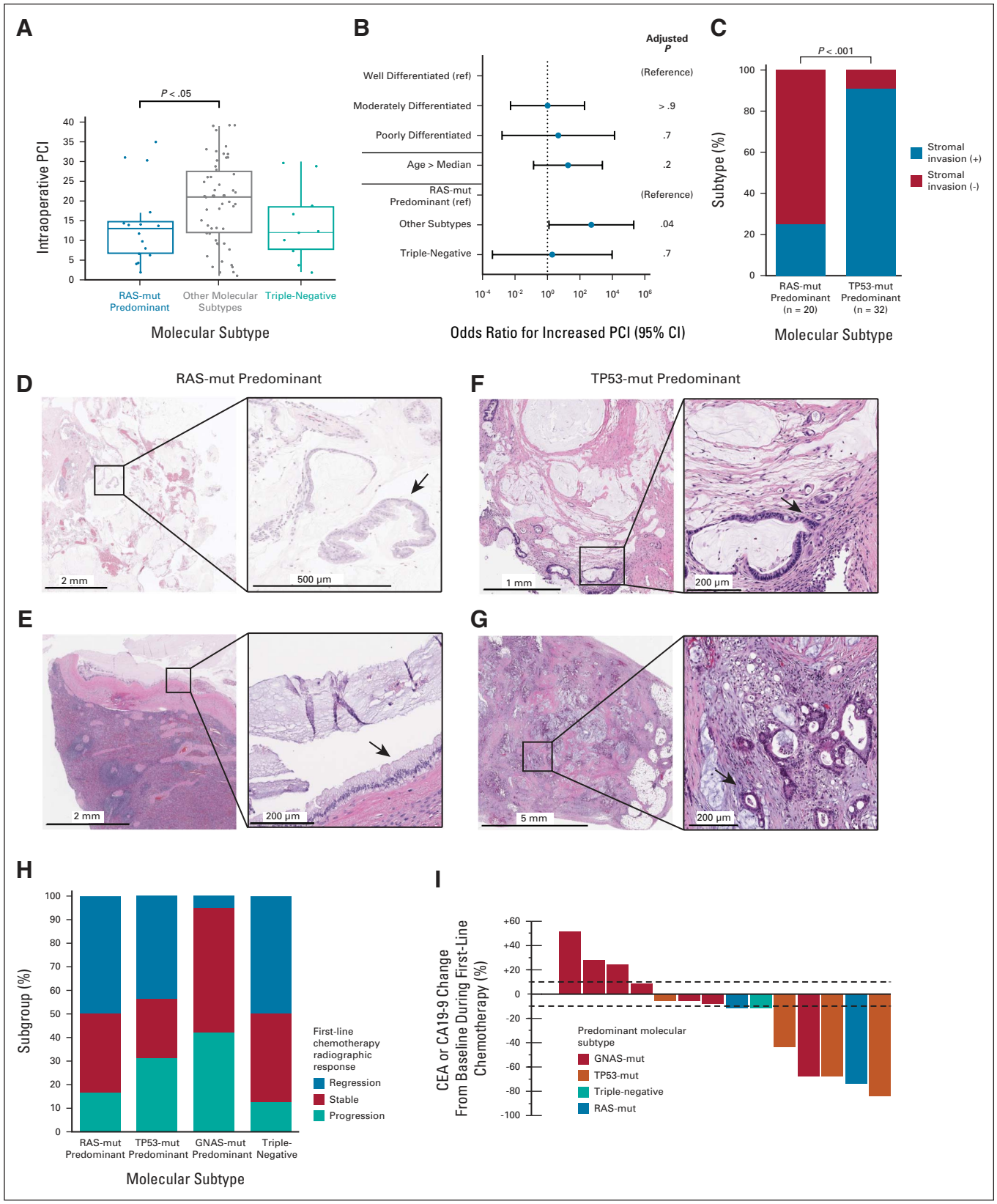

FIG 4. RAS-mut predominant mMAAP exhibits decreased stromal destruction and increased response to chemotherapy compared with other subtypes. (A) Tumor PCI was assessed intraoperatively in patients with metastatic MAAP who underwent a cytoreductive surgery (n = 81). Scores are represented as median with interquartile ranges for RAS-mut predominant subtype tumors versus the other molecular subtypes. *P* value determined by Wilcoxon-Mann-Whitney statistical testing. (B) Multivariable linear regression to determine odds ratios for increased PCI on the basis of various study (continued on following page)

FIG 4. (Continued). covariates. (C) Proportion of assessed intraoperative mMAAP samples exhibiting destructive stromal invasion organized by molecular subgroup with a *P* value representing statistical significance with Fisher's exact testing. (D-G) Representative tumor sections from intraoperative samples of different patients with mMAAP. RAS-mut predominant MAAP cells were typically observed surrounded by peritoneal-adjacent mucin pools (D) or adherent to the surface of tissues without destructive stromal invasion (splenic tissue, E). By contrast, TP53-mut predominant cells exhibited destructive stromal invasion of peritoneal tissue and organs such as the visualized omentum (F and G). Arrows indicate tumor tissue and scaling bars provide magnification orientation. (H) Radiographic best overall disease response to first-line therapy in patients with mMAAP (n = 49). (I) Percent change in CEA or CA19-9 biochemical markers from the beginning of therapy to the time of radiographic best response scan in patients with mMAAP (n = 14). Bars are colored by molecular subgroup. Only patients with at least one historically-elevated tumor marker and markers evaluated pretreatment and periradiographic scan were included. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; MAAP, mucinous appendiceal adenocarcinoma; mMAAP, metastatic MAAP; mut, mutant; PCI, peritoneal cancer index.

reduced radiographic and biochemical responses to chemotherapy. *GNAS* mediates cyclic adenosine monophosphate and protein kinase A signaling, which activates downstream mucin production and may, similar to pancreatic cancer, create a microenvironment that supports metastasis and drug resistance.[37,39,42-44] Given the potential for chemoresistance and high metastatic bulk, systemic therapy may be less effective for patients with GNAS-mut predominant tumors. Further investigation is needed to define the appropriateness of CRS in *GNAS*-mut tumors. GNAS-mut predominant tumors showed relatively indolent growth compared with more aneuploid subtypes, such as triple-negative or TP53-mut predominant. Surgeons may be inclined to pursue CRS despite higher initial tumor peritoneal bulk in *GNAS*-mut tumors, given the possibility that disease recurrence may be delayed in this less aggressive molecular type.

The TP53-mut predominant MAAP subtype exhibits the highest burden of total nonsynonymous mutations and aneuploidy, as well as a highly invasive histologic phenotype. Patients with this aggressive TP53-mut predominant AC suffer the worst survival outcomes, will likely require systemic treatment, and may recur quickly after surgical intervention.

Our study has several limitations. AC is rare, hindering statistical power and validation in a completely independent cohort. Sequencing data were manually reviewed to ensure adequate coverage, yet it is possible that low-frequency alterations fell below the limit of detection. Low alteration detection mainly affects the minority of analyzed metastatic tumors classified as triple-negative (*RAS*-wt/*GNAS*-wt/*TP53*-wt). Despite similar purity and sequencing coverage to the other subtypes, triple-negative samples have few recurrent mutations and may be driven by copy-number changes. Further investigation is needed to resolve these tumors within the molecular and biological trends seen in the majority of MAAP.

Our findings offer a new perspective on molecular complexity as a driver of AC metastatic aggressiveness and demonstrate the potential value of key mutational patterns in the clinical management of this disease.

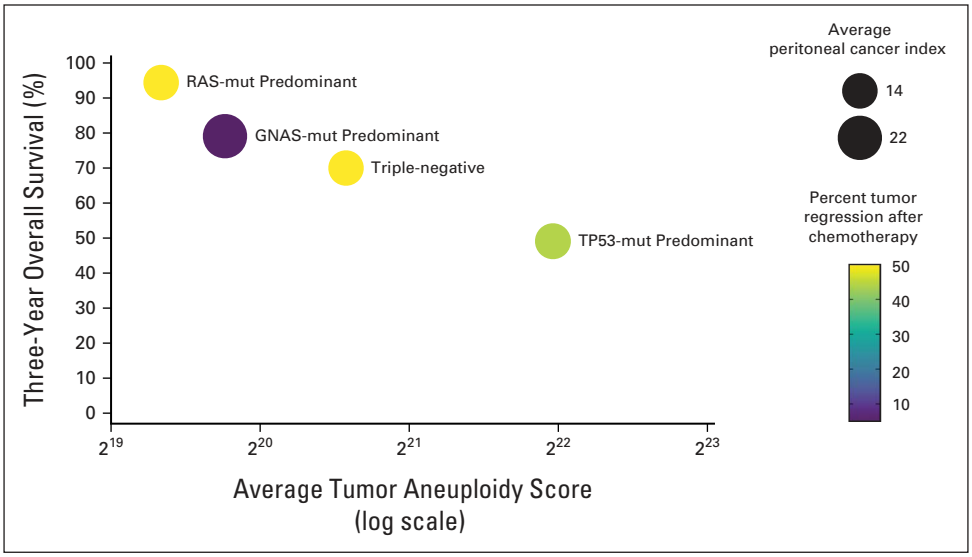

FIG 5. Clinical outcomes summary for patients with metastatic MAAP organized by molecular subtype. Three-year overall survival was calculated using Kaplan-Meier analysis for all patients with metastatic mucinous appendiceal adenocarcinoma (n = 149). Molecular subtypes are represented with corresponding average tumor aneuploidy score (Data Supplement). The average peritoneal cancer index (scaled dot size) and the percent of tumors per subtype with radiologic tumor regression after chemotherapy (dot color) are represented from each respective study analysis. MAAP, mucinous appendiceal adenocarcinoma.

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SUPPORT

Supported by the Glades Foundation (A.C.), T32-CA009512 funding from the National Institutes of Health (M.B.F.), the Conquer Cancer—Boehringer Ingelheim Endowed Young Investigator Award in Gastrointestinal Cancer (M.B.F.), National Cancer Institute grant NCI-P30 CA 008748, and by the Marie-Josée and Henry R. Kravis Center for Molecular Oncology (L.A.D.J.). The Conquer Cancer Foundation is administered by the American Society of Clinical Oncology.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.22.01392.

DATA SHARING STATEMENT

All study statistical analyses were performed using R (v4.0.0). Study data are publicly available at https://www.cbioportal.org/study/summary?id=appendiceal_msk_2022.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Molecular Classification of Appendiceal Adenocarcinoma

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/authors/author-center.

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Patents, Royalties, Other Intellectual Property: US-2010041048-A1—Circulating Mutant DNA to Assess Tumor Dynamics, US-2015344970-A1—Personalized Tumor Biomarkers, WO-2010118016-A2—Digital quantification of DNA methylation, US-2005202465-A1—Thymidylate synthase gene and metastasis, US-2014227271-A1—Somatic mutations in ATRX in brain cancer, WO-2012094401-A2—Genes frequently altered in pancreatic neuroendocrine tumors, US-2013323167-A1—Detecting and treating solid tumors through selective disruption of tumor vasculature, EP-2912468-B1—Papanicolaou test for ovarian and endometrial cancers, US-9976184-B2—Mutations in pancreatic neoplasms, US-2017267760-A1—Checkpoint Blockade and Microsatellite Instability, US-2018171413-A1—Head and neck squamous cell carcinoma assays, US-2018171413-A1—Head and neck squamous cell carcinoma assays, US-2018171413-A1—Head and neck squamous cell carcinoma assays, US-2018086832-A1—HLA-restricted epitopes encoded by somatically mutated genes, US-2018258490-A1—Assaying ovarian cyst fluid, US-2016208340-A1—TERT Promoter Mutations in Urothelial Neoplasia, US-2015252415-A1—ARID1B and neuroblastoma, WO-2018071796-A2—Compositions and methods for identifying functional anti-tumor t cell responses, EP-3322824-A1—Detection of tumor-derived DNA in cerebrospinal fluid, US-2016273049-A1—Systems and methods for analyzing nucleic acid (Inst), US-2018135044-A1—Non-unique barcodes in a genotyping assay (Inst), US-2017016075-A1—Neoantigen analysis (Inst)
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No other potential conflicts of interest were reported.