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Gastrointestinal Cancer

Blood-Based Next-Generation Sequencing Analysis of Appendiceal Cancers

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

Key Words. Appendiceal Cancer • Next-generation sequencing • ctDNA

Abstract

Background. Appendiceal cancers (ACs) are rare. The genomic landscape of ACs has not been well studied. The aim of this study was to confirm the feasibility of next-generation sequencing (NGS) using circulating tumor DNA (ctDNA) in ACs and characterize common genomic alterations.

Materials and Methods. Molecular alterations in 372 plasma samples from 303 patients with AC using clinical-grade NGS of ctDNA (Guardant360) across multiple institutions were evaluated. Test detects single nucleotide variants in 54–73 genes, copy number amplifications, fusions, and indels in selected genes.

Results. A total of 303 patients with AC were evaluated, of which 169 (56%) were female. Median age was 56.8 (25–83) years. ctDNA NGS testing was performed on 372 plasma samples; 48 patients had testing performed twice, 9 patients had testing performed three times, and 1 patient had testing performed four times. Genomic alterations were defined in 207 (n = 207/372, 55.6%) samples, and 288 alterations were identified excluding variants of uncertain significance and synonymous mutations. Alterations were identified in at least one sample from 184 patients; *TP53*-associated genes (n = 71, 38.6%), *KRAS* (n = 33, 17.9%), *APC* (n = 14, 7.6%), *EGFR* (n = 12, 6.5%), *BRAF* (n = 11, 5.9%), *NF1* (n = 10, 5.4%), *MYC* (n = 9, 4.9%), *GNAS* (n = 8, 4.3%), *MET* (n = 6, 3.3%), PIK3CA (n = 5, 2.7%), and *ATM* (n = 5, 2.7%). Other low-frequency but clinically relevant genomic alterations were as follows: *AR* (n = 4, 2.2%), *TERT* (n = 4, 2.2%), *ERBB2* (n = 4, 2.2%), *SMAD4* (n = 3, 1.6%), *CDK4* (n = 2, 1.1%), *NRAS* (n = 2, 1.1%), *FGFR1* (n = 2, 1.1%), *FGFR2* (n = 2, 1.1%), *PTEN* (n = 2, 1.1%), *RB1* (n = 2, 1.1%), and *CDK6*, *CDKN2A*, *BRCA1*, *BRCA2*, *JAK2*, *IDH2*, *MAPK*, *NTRK1*, *CDH1*, *ARID1A*, and *PDGFRA* (n = 1, 0.5%).

Conclusion. Evaluation of ctDNA is feasible among patients with AC. The frequency of genomic alterations is similar to that previously reported in tissue NGS. Liquid biopsies are not invasive and can provide personalized options for targeted therapies in patients with AC. ***The Oncologist*** 2020;25:414–421

Implications for Practice: The complexity of appendiceal cancer and its unique genomic characteristics suggest that customized combination therapy may be required for many patients. Theoretically, as more oncogenic pathways are discovered and more targeted therapies are approved, customized treatment based on the patient's unique molecular profile will lead to personalized care and improve patient outcomes. Liquid biopsies are noninvasive, cost-effective, and promising methods that provide patients with access to personalized treatment.

Introduction

Appendiceal cancers (ACs) are rare and account for 0.5% of all gastrointestinal neoplasms [1]. ACs comprise different histologies, of which neuroendocrine origin (65%) and mucinous and nonmucinous adenocarcinomas (20%) are the most common. Other histologies include goblet and ex-goblet cell tumors, lymphomas, and mesenchymal sarcomas [1]. Treatments for AC depend on grade and stage. Surgical resection or debulking is the standard therapy for low-grade mucinous tumors. The role of systemic therapy (adjuvant, neoadjuvant, or palliative) is controversial, with several series demonstrating inferior outcome for chemotherapy-treated patients with low-grade tumors. For high-grade tumors, chemotherapy is based on results of trials

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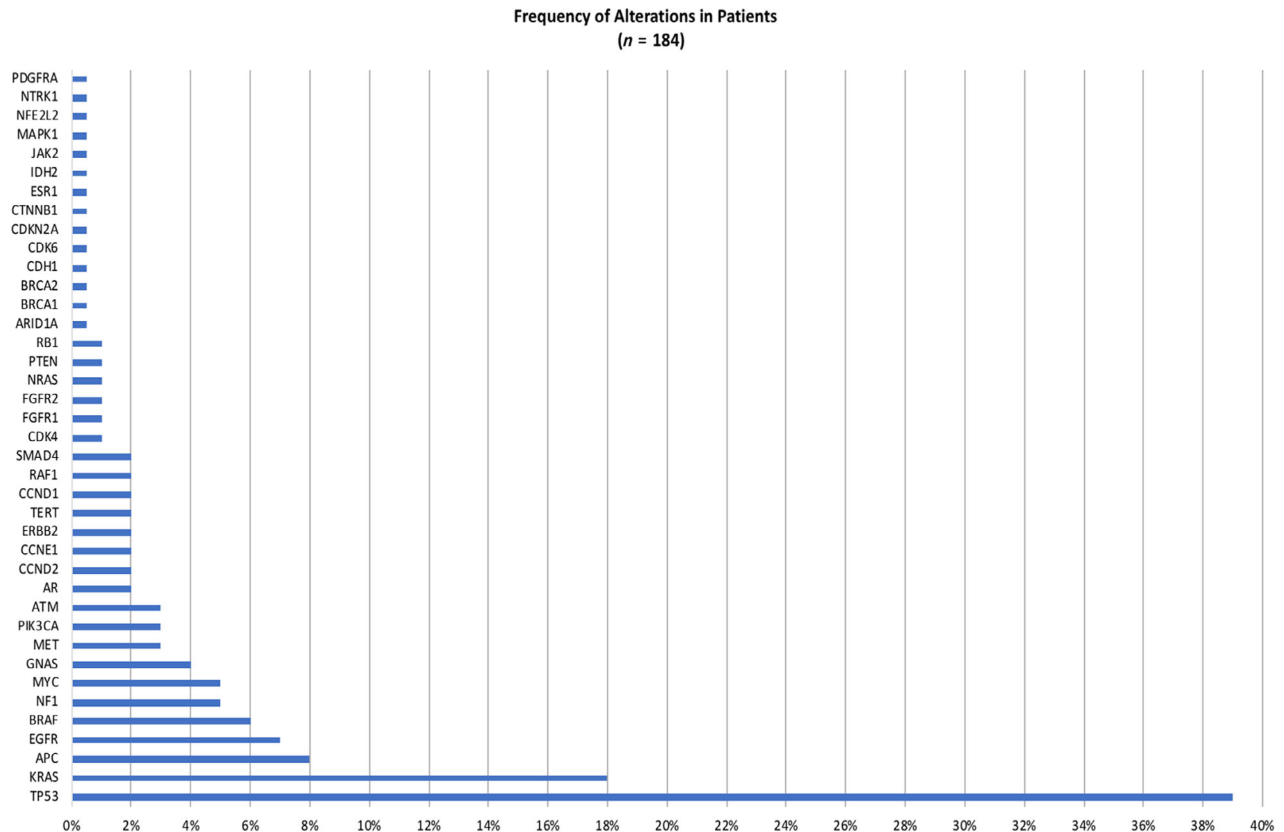

Figure 1. Prevalence of genomic alterations (variants of uncertain significance excluded).

with colorectal cancer. The lack of prospective trials in ACs has contributed significantly to this controversy [2].

Historically, AC treatment decisions are extrapolated from colorectal carcinoma (CRC) because of similarities in location and pathogenic features. Published literature suggests that some pathways, including point mutations in the *KRAS* proto-oncogene, mutations and/or deletions in the *TP53* gene (chromosome 17p), truncating mutations or deletions in the adenomatous polyposis coli *(APC*) gene (chromosome 5q), and mutations in the beta-catenin gene [3–6], are common in both CRC and ACs. Given the rarity of the disease, its heterogeneous nature, and the absence of clinical trials and genomic data for ACs, it is important to evaluate common genomic alterations that these cancers carry because clear molecular differences exist between ACs and CRCs [7–9]. A study conducted by Raghav et al. demonstrated that, compared with CRC, mutations in *BRAF*, *EGFR*, and *c-KIT* are less frequent in *AC*, *PI3K* mutations occur with similar frequency, and *KRAS* mutations occur at a higher rate [9]. The molecular profile supports the assumption that, despite their anatomic similarities, AC and CRC are two molecularly distinct tumor types [9]. Consequently, therapy for appendiceal tumors extrapolated from CRC regimens is unjustified [8].

The role of genomic profiling in patients with AC to develop and implement matched targeted therapies [10–13] has not been studied. The implementation of this approach in the clinical setting relies on the ability to biopsy tumors, perhaps multiple times, prior to the selection of new treatment regimens [14]. Tissue biopsies are invasive, expensive, associated with potential complications, and may not be feasible in the setting of peritoneal spread of the disease [14]. Spatial and temporal heterogeneity has been established between primary cancers and their metastatic lesions [15, 16], and thus primary tumor biopsies may not be the best source of material for genomic characterization of the disease [15, 17–19]. Cancer molecular profile might change with time after exposure to different cytotoxic or targeted therapies [20], which translates to the need for repeated tumor sampling, which is not feasible [15, 17–19]. All these challenges highlight the need for a method that is easily repeatable and minimally invasive such as next-generation sequencing (NGS) of circulating tumor DNA (ctDNA) [21]. ctDNA is secreted into the circulation by cancer cells, thus representing a source of tumor material representative of all disease sites. Blood testing for NGS presents a real-time, easily accessible tool for the identification of molecular biomarkers [18, 19, 22]. Because of the minimally invasive nature of a blood test, as opposed to a tissue biopsy, the reproducibility of liquid biopsies has been used for several proof-of-concept studies predicting response and resistance to treatment [23, 24], as well as prognosis and recurrence [25–27].

In this report, analysis of ctDNA through blood-based Guardant360 NGS from patients with a diagnosis of AC across various histological subtypes was evaluated. The aim was to confirm the feasibility of NGS using ctDNA in low-grade mucinous AC and characterize common alterations in the genomic profile. Furthermore, we aimed to identify whether the molecular alterations lead to the identification of potential actionable targets and combinations.

Subjects, Materials, and Methods

This was a retrospective review evaluating the molecular alterations in ctDNA samples from patients who had a diagnosis of

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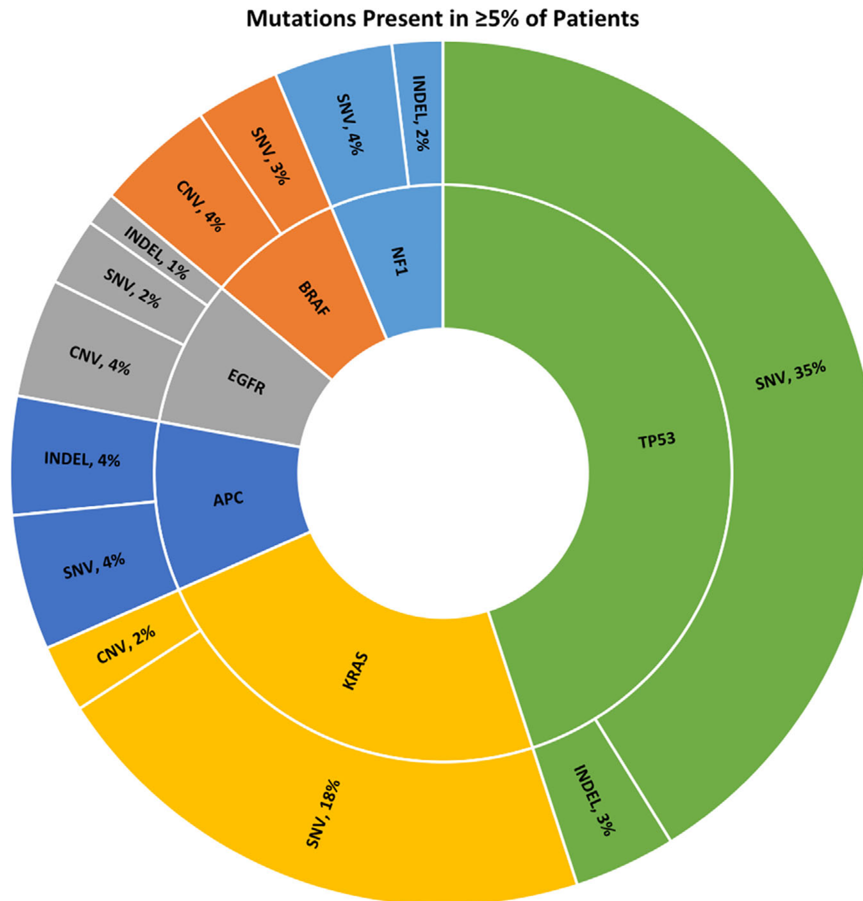

Figure 2. Types of alterations in the most commonly identified mutations (≥5% of patients, excluding variants of uncertain significance). Abbreviations: CNV, copy number variation; SNV, single nucleotide variation.

AC and underwent Guardant360 clinical-grade NGS across multiple institutions. Samples from patients with AC between the years 2014 and 2018 were analyzed. Patient-specific covariates included sex and age. Ethical approval was not required for the study; patient identity protection was maintained throughout the study in a deidentified database through a data transfer agreement between Guardant Health and Emory University, and existing data were collected in accordance with the Emory University institutional review board guidelines. All the authors contributed to reviewing and approving the final manuscript. Some patients had samples analyzed more than once but at different times. Data regarding histologic subtypes of AC were unavailable.

Next-Generation Sequencing

NGS of plasma ctDNA (liquid biopsies) was done by Guardant Health (Guardant360, http://www.guardanthealth.com/), a College of American Pathologists-accredited and Clinical Laboratory Improvement Amendments-certified laboratory. Next-generation sequencing data were interpreted by N-of-One, Inc. (Lexington, MA; www.n-of-one.com). This is a highly analytically and clinically sensitive and specific test, able to detect single molecules of tumor DNA in 10 mL blood samples and >85% of single-nucleotide polymorphisms found in tumors of patients with advanced cancers, with an analytic specificity of >99.9999% [28]. At the time of this study, the test detected single nucleotide alterations (e.g., mutations, fusions, copy number change) in a panel of 54–73 genes. This panel detects point mutations (single nucleotide variations [SNVs]) in 73 genes, indels in 23 genes, amplifications in 18 genes, and fusions in 6 genes (supplemental online Table 1). Sequencing covered all cell-free DNA, including germline found in the bloodstream (e.g., as a result of immune lysis), as well as the somatic ctDNA [28]. Germline alterations were filtered out and not reported. Guardant Health uses an internal database, COSMIC v77, dbSNP build 147, and ExAC version 0.3.1 to call pathogenic or likely pathogenic somatic mutations. Gene amplifications were reported by absolute copy number detected in plasma, as compared with normal controls from healthy patients included in each run [28].

Results

Patient Demographics

Between the years 2014 and 2018, a total of 303 patients with ACs underwent Guardant360 testing using clinical-grade NGS of ctDNA across multiple institutions, and 184 (61%) patients had at least one sample with alterations. The median age was 56.8 years (range, 25–83), with a female preponderance (56%). ctDNA NGS testing was performed on 372 plasma samples; 48 patients had testing performed twice, 9 patients had testing performed three times, and 1 patient four times. Genomic alterations were defined in 207 (n = 207/372, 55.6%) samples

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Table 1. Longitudinal study of genomic alterations in repeated blood samples

Patient number (n = 19)	Year	Gene	Alteration	Percentage[a]
1	June 2015	*KRAS*	G12V	1.65
		TP53	V272L	0.95
	April 2017	*BRCA2*	E1110K	0.23266482
		KRAS	G12V	0.33588519
		NF1	p.Val2259fs	0.11896277
			V1453D	0.55910126
		TP53	V272L	0.26401373
2	May 2016	*NF1*	G629R	0.31
	August 2016	*BRCA2*	L474L	0.15
		CCND2	AMP	0
		NF1	G629R	0.13
		PDGFRA	T223T	0.19
	December 2016	*BRCA2*	L474L	0.38428861
		CCNE1	NA	0.329072
		MTOR	N161S	0.23338677
		NF1	G629R	0.3031467
3	March 2017	*ATM*	D2708N	3.1544311
		ERBB2	I949T	0.49880917
	April 2017	*ATM*	D2708N	1.6990995
4	September 2015	*ATM*	R3008C	0.23
		MAP2K2	K68K	0.23
		TP53	R248W	0.55
	November 2015	*PDGFRA*	S851S	0.23
		TP53	R248W	0.44
	August 2017	*ATM*	p.Gly3019fs	0.176305
		TP53	R248W	2.24065
5	March 2016	*ARAF*	P200L	0.22
		NF1	R1204W	0.47
		PDGFRA	L261L	0.36
	June 2017	*ARAF*	P200L	0.372306
		NF1	R1204W	0.359565
6	June 2016	*CCND1*	AMP	0
	July 2016	*ERBB2*	T278T	0.12
7	July 2016	*APC*	H2532Y	0.14
		ARID1A	P225L	1.38
		TP53	G245V	0.47
	July 2016	*ARID1A*	P225L	1.14
		CDH1	L71L	0.1
		TP53	G245V	0.96
8	August 2016	*PDGFRA*	R981H	0.38
	August 2016	*PDGFRA*	R981H	0.22
9	September 2016	*TP53*	R273H	0.24
	March 2017	*TP53*	G325	0.11297166
			R273H	0.32542678
10	September 2016	*RET*	G592E	0.12
	October 2016	*ERBB2*	P525S	0.19
		NF1	Q1341	0.15
		RB1	S114L	0.23

(continued)

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Table 1. (continued)

Patient number (*n* = 19)	Year	Gene	Alteration	Percentage[a]
11	October 2016	*AR*	Y514C	0.31
		CDK4	A220A	0.15
		IDH2	R140Q	0.19
	February 2017	*MTOR*	L573L	0.35728586
12	November 2016	*FGFR2*	P582H	0.15
	November 2016	*RB1*	R661W	0.10031596
13	November 2016	*APC*	p.Thr1556fs	0.37954538
		KRAS	G12A	0.19769047
	December 2016	*TP53*	S241F	0.17788795
	January 2017	*IDH2*	R149W	0.15263173
14	November 2016	*EGFR*	L619L	0.10810312
			p.Cys582fs	0.09437097
		PIK3CA	G865S	1.4246402
		TP53	A159P	1.3637159
			C242S	0.21164862
			E258D	0.44184776
			P152Q	0.17850897
			V203M	0.39819554
			Y205C	0.12467888
	December 2016	*AR*	R630Q	0.30100751
		EGFR	p.Cys582fs	0.06360528
		FGFR3	P300P	0.09369497
		PIK3CA	G865S	0.79638684
		TP53	A159P	1.2249438
			A159V	0.10088058
			C242S	0.21596307
			E258D	0.19042433
			V203M	0.25117232
15	January 2017	*PTEN*	N49S	0.17563186
	June 2017	*EGFR*	F795V	0.15857818
		PTEN	N49S	0.2780047
16	February 2017	*EGFR*	A750E	0.57628694
	March 2017	*EGFR*	A750E	0.42459659
17	June 2017	*APC*	p.Glu1157fs	0.36905797
	July 2017	*APC*	p.Glu1157fs	0.660704
18	August 2017	*KRAS*	G12V	0.988142
		TP53	G245S	0.657264
	November 2017	*KRAS*	G12V	0.215241
19	December 2017	*DDR2*	I798F	0.168744
		EGFR	E967	0.113921
	March 2018	*EGFR*	E967	0.526277

[a]Percentage refers to the number of genomic alterations and/or variants found in a particular gene out of the total number of genomic alterations detected in 184 patients.

with a total of 288 alterations identified after excluding variants of uncertain significance and synonymous mutations.

Molecular Alterations

TP53 associated genes were most commonly altered (*n* = 71, 38.6%), followed by *KRAS* (*n* = 33, 17.9%), *APC* (*n* = 14, 7.6%), *EGFR* (*n* = 12, 6.5%), *BRAF* (*n* = 11, 5.9%), *NF1* (*n* = 10, 5.4%), *MYC* (*n* = 9, 4.9%), *GNAS* (*n* = 8, 4.3%), *MET* (*n* = 6, 3.3%), *PIK3CA* (*n* = 5, 2.7%), and *ATM* (*n* = 5, 2.7%). Other genomic alterations of low frequency, but clinical relevance, included *AR* (*n* = 4, 2.2%), *TERT* (*n* = 4, 2.2%), *ERBB2* (*n* = 4, 2.2%), *SMAD4* (*n* = 3, 1.6%), *CDK4* (*n* = 2, 1.1%), *NRAS* (*n* = 2,

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Table 2. Frequency of actionable mutations

Row labels	Count of gene
EGFR	15
NF1	13
PIK3CA	7
ATM	6
MET	6
AR	4
ERBB2	4
CDK4	2
FGFR1	2
FGFR2	2
BRCA1	1
BRCA2	1
CDK6	1
CDKN2A	1
IDH2	1
JAK2	1
NTRK1	1
PDGFRA	1

Alts prevalence (no variants of uncertain significance).

1.1%), *FGFR1* (*n* = 2, 1.1%), *FGFR2* (*n* = 2, 1.1%), *PTEN* (*n* = 2, 1.1%), and *RB1* (*n* = 2, 1.1%). Alterations in *CDK6*, *CDKN2A*, *BRCA1*, *BRCA2*, *JAK2*, *IDH2*, *MAPK*, *NTRK1*, *CDH1*, *ARID1A*, and *PDGFRA* were all reported once (*n* = 1, 0.5%; Fig. 1).

The alterations seen in *BRAF* with their respective frequencies are as follows: V504V (1), D594G (1), AMP (3), N581S (1), G466V (1), and V600E (1). The alterations seen in *KRAS* with their respective frequencies are as follow: E63K (1), G13D (1), Q61H (2), G12D (2), D132I (1), G12V (4), AMP (2), and G12C (1).

Regarding the types of alterations identified, no fusions were found. Only one patient had an *ERBB2* copy number variation (CNV) or amplification, as the other *ERBB2* mutations identified were SNVs. Of the *BRAF* SNVs identified, all were activating, but only one was V600E. Four patients had *MET* amplifications (CNVs; Fig. 2). Table 2 summarizes potential actionable mutations in this analysis.

Plasma-Derived ctDNA for Longitudinal Disease Monitoring

Among the 303 patients studied, 48 had testing performed twice, 9 had testing performed three times, and 1 patient had testing performed four times. By analyzing these longitudinal blood samples, we found that appendiceal tumors can gain new mutations over time that could potentially be targeted. With serial testing, we identified two patients that gained mutations in *BRCA2*, two patients that gained mutations in *MTOR*, two patients that gained mutations in *ERBB2*, one patient that gained a mutation in *IDH2*, and one patient that gained a mutation in *FGFR3*, which could all be targeted. Loss of mutations was identified in 11 of the 19 patients. These include *CCND1*, *CCND2*, *PDGFRa* (3), *ERBB2*, *MAP2K2*, *APC*, *RET*, *AR*, *CDK4*, *IDH2*, *FGFR2*, *TP53* (2), *and DDR2* (Table 1).

Correlation Between Age and Sex with Respect to NGS Results

Age and sex did not seem to correlate with mutation findings in this study. *KRAS* mutations occurred equally among men and women with a mean age of 53.5 years. Prevalence of *BRAF* mutations and *ATM* mutations were also similar between men and women with a mean age of 58.9 years and 51.7 years, respectively. In this study, *BRCA1* and *BRCA2* mutations were only seen in women with a mean age of 51 years and 60 years, respectively (supplemental online Table 2). These results need to be validated by a larger sample size in future studies to reach a statistically significant correlation.

Mutation Frequency by Histology

For this analysis, 63 of 303 patients had known histology per medical records included with the Guardant360 sample. These 63 patients had a total of 109 samples. The majority of patients had mucinous histology (52.4%), followed by adenocarcinoma (22.2%) and atypical goblet cell carcinoma (22.2%; Table 3). The number of mutation observations by gene was counted across all patients and stratified by histology (Fig. 3). Synonymous mutations, variants of uncertain significance, and genes with only one mutation across the cohort were excluded, and only alterations with potential functional and/or clinical significance were included. For patients with multiple samples, a given mutation was counted one time over the course of their sampling to include all samples in the analysis without inflating the mutation frequencies due to mutations that persist over time.

Discussion

The challenge for molecularly targeted personalized therapy in oncology, include the need for effective novel molecular tests [29] and potent targeted therapies. Extensive research demonstrates that detecting biomarker mutations regardless of cancer primary site allows for successful changes in treatments [2]. Liquid biopsies offer several advantages for molecular testing, including feasibility of repeat testing, representation of all disease sites, low expense, and minimal invasiveness. The results of ctDNA analysis have led to further research in real-time prognostics and drug-cycling applications, such as the use of *KRAS* mutations for prognosis in advanced pancreatic cancer [30], serial ctDNA biopsies to track treatment-conditional clonal evolution to cycle *EGFR* inhibitors in CRC [31], and cell-free plasma exome sequencing to identify new pathways of acquired resistance to targeted therapeutics in cancers [23].

The present analysis is the first report exploring the genetic mutations in patients with AC using ctDNA derived from liquid biopsy. The genes commonly found to have characterized alterations in patients with AC included genes known to be commonly altered in ACs and CRC such as *TP53*, *KRAS*, *APC*, *PIK3CA*, and *BRAF*. The frequencies of genomic alterations in ctDNA were similar to those previously reported in tissue NGS [9] and those previously reported in a study by Riviere et al. evaluating 42 AC liquid biopsies [14]. These findings confirm the feasibility and reliability of using ctDNA in profiling ACs.

In addition, although *KRAS*, *APC*, *PIK3CA*, and *BRAF* genes are commonly altered in CRC, the frequency of these alterations differ in ACs. Specific alterations, such as *BRAF* mutations present in 15% of CRCs, and are associated with poor

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Table 3. Mutation frequency by histology

Histology	Samples, *n* (%)
Mucinous	33 (52.4)
Adenocarcinoma	14 (22.2)
Atypical GCC	14 (22.2)
GCC	1 (1.6)
NET typical	1 (1.6)

Abbreviations: GCC, goblet cell carcinoma; NET, neuroendocrine tumors.

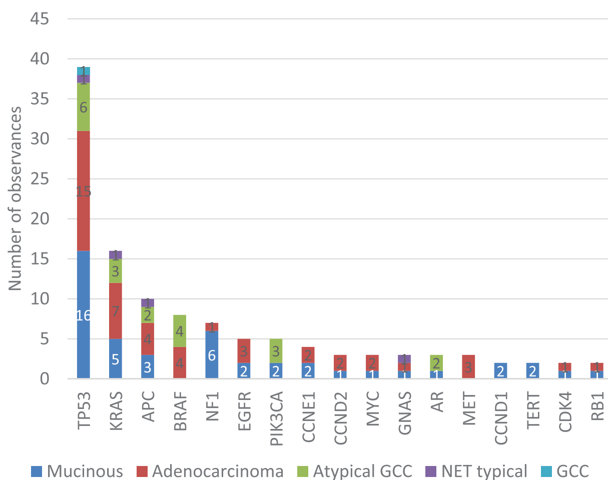

Figure 3. Mutation frequencies in genes with at least two observations across the cohort.
Abbreviations: GCC, goblet cell carcinoma; NET, neuroendocrine tumors.

prognosis [32]. In this study, *BRAF* mutation was reported in 6% of the samples of ACs. In a study of 183 patients with AC analyzed by NGS, high mutation rates were observed in *KRAS* (55%), *TP53* (40%), *GNAS* (31%), *SMAD4* (16%), and *APC* (10%) [33]. As opposed to our findings, the later study concluded that ACs exhibited higher mutation rates in *KRAS* and *GNAS* and lower mutation rates in *TP53*, *APC*, and *PIK3CA* (6%) than CRC [33]. When compared with CRC [9], our findings suggest that *KRAS* mutation is much less prevalent in ACs, at a rate of 18% in this study. In addition, several unique genetic alterations were observed in our series: *APC* (7.6%), *EGFR* (6.5%), *NF1* (5.4%), *MYC* (4.9%), *GNAS* (4.3%), *PIK3CA* (2.7%), *MET* (3.3%), and *ATM* (2.7%). Other genomic alterations found at low frequencies include *AR* (2.2%), *TERT* (2.2%), *ERBB2* (2.2%), *SMAD4* (1.6%), *CDK4* (1.1%), *FGFR1* (1.1%), *FGFR2* (1.1%), *PTEN* (1.1%), and *RB1* (1.1%). Alterations in *CDK6*, *CDKN2A*, *BRCA1*, *BRCA2*, *JAK2*, *IDH2*, *NTRK1*, *CDH1*, and *PDGFRA* were all reported once (0.5%). Some of these alterations are in clinical development as potential targets. These findings highlight the unique molecular profile of AC and necessity to research AC independent of CRC. In our population of patients with AC (n = 303), 21.2% (n = 61/288) of alterations identified could potentially be targeted by drugs approved for other cancers. This frequency is relatively high when compared with commonly profiled tumors, such as non-small cell lung cancer or CRC. Examples of these mutations with therapeutic implications include *EGFR*, *NF1*, *PIK3CA*, *MET*, *ATM*, *AR*, *ERBB2*, *CDK4*, *FGFR1*, *FGFR2*, *CDK6*, *CDKN2A*, *BRCA1*, *BRCA2*, *JAK2*, *IDH2*, *NTRK1* and *PDGFRA* (Table 2). The identification of these abnormalities would have justified using *pan-kinase*, *cyclin-D*, *PARP*, *JAK25*, and *IDH* inhibitors, which are not commonly used in ACs. The absence of standard evidence-based therapies in ACs highlights the importance of genomic profiles in identifying therapeutic options for patients.

Repeat sampling is a unique advantage of liquid biopsies over tissue based assays. In this series, 48 patients had serial profiling of ctDNA. Analysis showed gain and loss of mutations with time. This could be related to type of therapy received. A limitation to this analysis is the unavailability of the exact treatments patients received. Some of the gained mutations are targetable via agents not commonly used in ACs. The gained mutations included *BRCA2*, *mTOR*, *ERBB2*, *IDH2*, and *FGFR3*. Age and sex do not seem to be correlated with specific mutations. Liquid biopsies are easy accessible and cost-effective. Tissue biopsies are invasive and costly, with needle biopsies documented to cost over $10,000, based on a population-based national Medicare sample [34]. Furthermore, biopsy site could be a challenge to obtain a representative sample for tissue testing specifically with histologies like mucinous cancers, which are common in ACs. In lung cancer, liquid biopsies have become a standard diagnostic procedure for targetable treatments [35]. In addition, it is currently established that there is molecular heterogeneity within the primary tumor and its metastatic tumors in the same patient [15] and even within the same tumor (depending on sampling area). Therefore, ctDNA results may reflect genomic aberrations in DNA shed from the primary site and metastatic sites [10].

The current study is the largest population-based study that incorporates patients with AC who have undergone liquid biopsies. There are several limitations to our study inherent to all retrospective analyses. First, genomics data were obtained from a deidentified database and, hence, only limited clinical information was available. There were no data available regarding whether samples were obtained prior to or after medical treatment and/or surgery. There are no data to compare tissue genomics with liquid testing in this analysis. In addition, no survival data were available, and the data were limited by the coding of physicians at the different institutions. Another limitation is the different subtypes and histologies of AC, which are pathologically challenging to classify and entail different treatments. There is no standardized classification for mucinous ACs, and the challenge lies in pathologic classification of goblet and ex-goblet cancers. For the samples analyzed in this study, histology coding was only available for 63 of 303 patients, and blood-based test for microsatellite instability at the time of the analysis was not yet validated. Other mutations that are not accounted for in Guardant360 might be present. These mutations are under study regarding the optimization of detection through ctDNA. Despite these limitations, our findings have important implications. The complexity of AC and its unique genomic characteristics suggest that customized combination therapy may be required for many patients [14]. Theoretically, as more oncogenic pathways are discovered and more targeted therapies are approved, customized treatment based on the patient's unique molecular profile will lead to personalized care and improve patient outcomes [2].

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Conclusion

Evaluation of ctDNA was feasible and reliable among individuals with AC. The frequency of genomic alterations detected by ctDNA testing is similar to that previously reported in tissue NGS. Liquid biopsies are noninvasive, cost-effective, and promising methods that provide patients with access to personalized treatment. Liquid biopsies merit investigation in prospective clinical trials, specifically in rare tumors such as AC in which traditional drug development paradigms are not feasible.

Author Contributions

Conception/design: Walid L. Shaib, Katerina Zakka
Provision of study material or patients: Ali Roberts
Collection and/or assembly of data: Walid L. Shaib, Katerina Zakka, Ali Roberts
Data analysis and interpretation: Walid L. Shaib, Katerina Zakka, Ali Roberts
Manuscript writing: Walid L. Shaib, Katerina Zakka, Ali Roberts
Final approval of manuscript: Walid L. Shaib, Charles Staley III, Katerina Zakka, Ali Roberts, Mehmet Akce, Christina Wu, Olatunji B. Alese, Bassel F. El-Rayes

Disclosures

Ali Roberts: Guardant Health (E, OI). The other authors indicated no financial relationships.

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