

ORIGINAL ARTICLE

Genomic profiling unlocks new treatment opportunities for ampullary carcinoma

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Background: Ampullary carcinoma (AC) is a rare disease with an abysmal prognosis and few treatment options. The molecular landscape and its therapeutic implications remain inadequately understood. This study aims to provide a clinical and genomic characterization of AC and explore opportunities for precision oncology.

Materials and methods: We carried out a retrospective analysis of clinical and genomic features in patients with AC treated in our institution. Gene mutations were categorized into molecular pathways, and potentially targetable alterations were classified according to the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT). Key molecular findings were validated in an external cohort.

Results: We included 78 patients with a median age of 66 years; 51.6% were women, and most were treated with surgery (81.2%). Histologically, they were classified as pancreaticobiliary (58.3%), intestinal (INT, 33.3%), and mixed (8.3%). The percentages of patients diagnosed at stages I, II, III, and IV disease were 18.8%, 23.4%, 32.8%, and 25.0%, respectively. Of note, the INT subtype was enriched in transforming growth factor- β pathway alterations (25.9% versus 6.1%, $P = 0.03$). Potentially actionable molecular alterations were found in 52% of the patients. Importantly, $KRAS^{WT}$ tumors were enriched in potentially targetable alterations ESCAT I-IIIa both in our cohort (37.2% versus 9.4%, $P = 0.006$) and external validation cohort (23.0% versus 9.3%, $P = 0.01$), including 25.6% $ERBB2$ amplification/mutation, 14.0% homologous recombination deficiency status, and 7.4% microsatellite instability status. Six patients received matched targeted therapies after progression to chemotherapy, with a response rate of 50% and two patients surviving for >1 year.

Conclusions: AC patients are enriched in targetable alterations, especially $KRAS^{WT}$ tumors, and could particularly benefit from precision oncology-based approaches.

Key words: ampullary carcinoma, rare cancer, precision medicine, genomic profiling, ESCAT alterations, targeted therapies

INTRODUCTION

Ampullary carcinoma (AC) is a rare and aggressive malignancy that originates from the ampulla of Vater. It represents ~0.2% of all gastrointestinal cancers and ~7% of all periampullary cancers.¹⁻³ The prognosis for AC is generally poor, with a 5-year survival rate of around 30%, despite the initial resectability of >50% of these tumors.^{2,4-6} AC can be classified into three histopathological subtypes: intestinal

(INT), pancreaticobiliary (PB), and mixed (MIX) types.^{2,3} Clinical guidelines typically recommend treating the INT type similarly to colon cancer, while the PB and MIX types are treated as pancreatic adenocarcinoma or biliary tract carcinoma.⁷

The rarity and heterogeneity of AC has posed significant challenges, leading to a lack of large phase III trials specifically targeting this disease, and most of the largest trials in biliary tract cancer excluded specifically AC.^{8,9} The ESPAC-3 trial, a phase III study, aimed to assess the efficacy of adjuvant chemotherapy in patients with periampullary tumors, including AC. This trial found that adjuvant chemotherapy improves overall survival (OS) when prognostic factors are considered, recommending gemcitabine or fluoropyrimidines as the optimal adjuvant treatment

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strategy.¹⁰ Regarding the advanced setting, the ABC-02 trial, which included patients with advanced biliary tract tumors at first line, also AC, demonstrated a significant improvement in OS with the combination of cisplatin and gemcitabine compared with gemcitabine alone.¹¹ Equally, the ABC-06 trial, which included advanced biliary tract tumors at second line, including AC, showed a slight benefit of FOLFOX compared with active symptom control.¹² Despite these findings, there is still no consensus in routine clinical practice regarding the ideal systemic chemotherapy regimens.^{7,13}

Genomic characterization of AC poses unique challenges. Although some studies have yielded intriguing findings, the small sample size and lack of external validation have prevented these findings from influencing clinical practice.¹⁴ Current research indicates that AC involves a complex interplay of genetic alterations across key oncogenic pathways, including the WNT/ β -catenin, phosphoinositide 3-kinase/protein kinase B, TP53, and transforming growth factor- β (TGF- β) pathways.¹⁴⁻¹⁸ Despite these advances, significant gaps remain in our understanding of the genomic drivers and therapeutic vulnerabilities of AC. This lack of knowledge hinders the development of effective targeted therapies and personalized treatment approaches.¹⁹

In this study, we sought to provide a thorough overview of the genomic landscape of AC by analyzing the clinical and molecular characteristics of our cohort and validating key findings in an independent, publicly available cohort. By highlighting the molecular alterations that drive ampullary tumorigenesis and metastasis, we aim to pave the way for precision medicine approaches and targeted therapies to combat this aggressive malignancy. Through our research, we hope to contribute to advancing knowledge and developing more effective treatment strategies for patients suffering from this disease.

MATERIALS AND METHODS

Study design and patient population

In this retrospective descriptive cohort study, we reviewed all patients with newly diagnosed AC at Vall d'Hebron Institute of Oncology (VHIO) from 2008 to 2023. We defined the 'overall cohort' as all patients with a newly diagnosed AC, irrespective of stage, and the 'metastatic cohort' as all patients who developed metastasis during the course of the disease. Patients were followed up until 19 September 2023, or until the date of death or last follow-up. For the 'overall cohort', we collected baseline variables and evolutionary events at the time of initial diagnosis, irrespective of stage. For the 'metastatic cohort', we collected variables at the time of the first evidence of metastasis. A complete list of the baseline and evolutionary variables collected for both cohorts can be found in [Supplementary Table S1](https://doi.org/10.1016/j.esmoop.2025.104480), available at <https://doi.org/10.1016/j.esmoop.2025.104480>. The clinical stage was recorded according to TNM (tumor—node—metastasis) eighth edition classification. The study was approved by the Ethics Committee of the Vall d'Hebron University

Hospital, which waived the need for written informed consent from the patients due to the retrospective nature of this study. The study was carried out in accordance with the Helsinki Declaration, and its later amendments were reported following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. For the validation cohort, we downloaded the Baylor College of Medicine dataset from cBioPortal (hereafter referred to as the 'BCM cohort').^{15,20}

Definition of endpoints

For the 'overall cohort', OS was defined as the time from histological diagnosis of AC to date of death from any cause. Disease-free survival (DFS) was defined as the time from diagnosis to the time of recurrence or death. For the 'metastatic cohort', OS was defined as the time from treatment start to death from any cause. Progression-free survival (PFS) was defined as the time from initiation of treatment to the time of progression or death, whichever occurred first. Tumor response was determined following the RECIST 1.1 guidelines.²¹ Overall response rate (ORR) was defined as the proportion of complete (CR) or partial responses (PR), disease control rate as the proportion of CR, PR, and stable disease (SD), and clinical benefit rate as the proportion of CR, PR, and ongoing SD for at least 6 months.

Immunohistochemistry analysis

The subtyping of adenocarcinomas into INT, PB, and MIX was based on a combination of histological and immunohistochemistry (IHC) features, according to the criteria published by Ang et al.²² IHC subtyping, using the markers MUC1, MUC2, CDX2, and CK20, was as follows: INT was defined as positive for CK20 or CDX2 or MUC2 and negative for MUC1; or positive for CK20, CDX2, and MUC2, irrespective of MUC1 staining pattern. PB was defined as positive for MUC1 and negative for CDX2 and MUC2, regardless of CK20 staining pattern. MIX was defined as other combinations of phenotypes, including negative for all stains²² ([Supplementary Table S2](https://doi.org/10.1016/j.esmoop.2025.104480), available at <https://doi.org/10.1016/j.esmoop.2025.104480>).

Next-generation sequencing analysis

All patients with available tissue-based mutational testing were included in the molecular analysis. Over the study period, two different in-house sequencing panels were used: an amplicon-based next-generation sequencing (NGS) test (MiSeq Illumina) targeting hotspot mutations across 59 common oncogenes and tumor²³ suppressors in AC and a 450-gene capture panel (VHIO-300 panel).²⁴ Additionally, we included those patients with results from a commercially available test at Caris Life Sciences or Foundation Medicine.

To define molecular actionability, we classified molecular alterations following the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT) guidelines.²⁵ For this study, we focused only on ESCAT categories I, II, and IIIA ([Supplementary](https://doi.org/10.1016/j.esmoop.2025.104480)

Table S3, available at <https://doi.org/10.1016/j.esmoop.2025.104480>.

Statistical analysis

For descriptive purposes, continuous variables were summarized as medians and range, while categorical variables were represented as absolute values and percentages. We used a chi-square test to compare the clinical and molecular features of INT, PB, and MIX subtypes. We estimated median follow-up by applying the reverse Kaplan–Meier method. We used the Kaplan–Meier method to estimate the survival function. In the resected cohort, we compared the DFS between IHC subgroups and the group that received adjuvant chemotherapy versus the others. In the metastatic cohort, we compared the OS between IHC subgroups and the patients who received matched therapy according to ESCAT alterations versus those who did not. *P* values were reported for descriptive purposes and were unadjusted for multiple hypothesis testing. Given the study’s retrospective nature, we did not formally calculate the sample size. We conducted all analyses using R version 4.0.2 or higher (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical characteristics

To study AC’s clinical and molecular characteristics, we assembled a cohort of patients diagnosed with AC from 2008 to 2023 at VHIO. To address treatment response and survival in the metastatic setting, all patients who developed metastases during the course of the disease were included in the ‘metastatic cohort’ (Figure 1).

Since 2008, we identified 78 eligible patients from the internal record of our institution. Clinical baseline characteristics of AC are shown in Table 1. The median age at diagnosis was 68.0 years (range 43.1–83.4 years), and the gender ratio was similar, 1.05 : 1 female/male. We used the TNM eighth edition and identified stages I, II, III, and IV in 9.0%, 11.5%, 37.2%, and 28.2% of patients, respectively. Moreover, 74.4% of the patients underwent surgery, and the recurrence rate was 66.0%. Importantly, during the course of the disease, 54 patients (69.2%) developed metastatic disease, with 70.4% of these cases involving liver metastasis.

We also reviewed the histological subtype classification in 63 patients according to The Royal College of Pathologist Dataset (2019).²² We found that 53.5% of these cases exhibited a PB phenotype, 26.8% showed an INT phenotype, and only 5.6% displayed a MIX phenotype, which is consistent with other reports.^{26,27}

Survival outcomes of AC

Next, we investigated whether the clinical stage and histological subtypes of AC correlate with prognosis in our cohort. We found that the median OS in this study was 40.8 months [95% confidence interval (CI) 30.7–58.2 months].

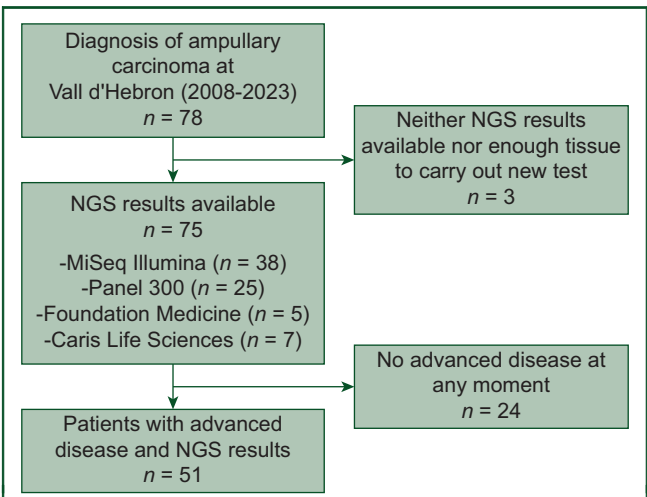


Figure 1. Flowchart of the included patients, including availability of NGS and the specific panel analyzed. NGS, next-generation sequencing.

Interestingly, we observed significant differences in OS according to clinical stage ($P < 0.001$), but not according to histological subtype ($P = 0.53$) (Figure 2A–C). Among the 58

Table 1. Baseline clinical and pathological characteristics of AC	
	Overall (n = 78)
Age at diagnosis, years, median (range)	68.0 (43.1–84.3)
Gender, n (%)	
Female	40 (51.3)
Male	38 (48.7)
Histological subtype, n (%)	
Pancreatobiliary	38 (48.7)
Intestinal	19 (24.3)
Mixed	4 (5.1)
Unknown	17 (21.8)
Clinical stage at diagnosis, n (%)	
I	7 (10.5)
II	9 (13.4)
III	29 (43.3)
IV	22 (32.4)
ECOG-PS at diagnosis, n (%)	
0	34 (43.6)
1	37 (47.4)
2	3 (3.9)
3	4 (5.1)
Surgery, n (%)	58 (74.4)
Adjuvant treatment (n = 36)	36 (62.1)
Gemcitabine	14 (38.9)
Capecitabine	8 (22.2)
CTRT	5 (13.9)
Gemcitabine + CTRT	3 (8.3)
Gemcitabine–capecitabine	3 (8.3)
FOLFIRINOX	1 (2.8)
5-FU	1 (2.8)
Unknown	1 (2.8)
Metastatic at any moment (n = 54), n (%)	54 (69.2)
Liver metastasis	38 (70.4)
First-line treatment at metastatic stage (n = 54), n (%)	
Cisplatin–gemcitabine	23 (42.6)
Other gemcitabine-based	10 (18.5)
FOLFOX	8 (14.8)
FOLFIRINOX	7 (13.0)
Other	5 (6.0)
BSC alone	1 (1.9)

5-FU, 5-fluorouracil; BSC, best supportive care; CTRT, chemoradiotherapy; ECOG-PS, Eastern Cooperative Oncology Group performance status scale.

patients who underwent surgical resection, 62.1% received various schemes of adjuvant chemotherapy, with a median DFS of 18.3 months (95% CI 12.0 months-not reached). Moreover, we analyzed DFS according to histological subtype and compared patients who received adjuvant chemotherapy versus those who did not; however, we found a trend in favor of adjuvant chemotherapy, but not

statistically significant ($P = 0.21$ and $P = 0.84$, respectively) (Figure 2D-F).

Genomic landscape and targetable alterations in AC. We carried out targeted NGS in 75 AC (96.2%) patients (see 'Materials and methods' section, Figure 1). Key results were validated in the BMC cohort, comprising 160 AC patients.

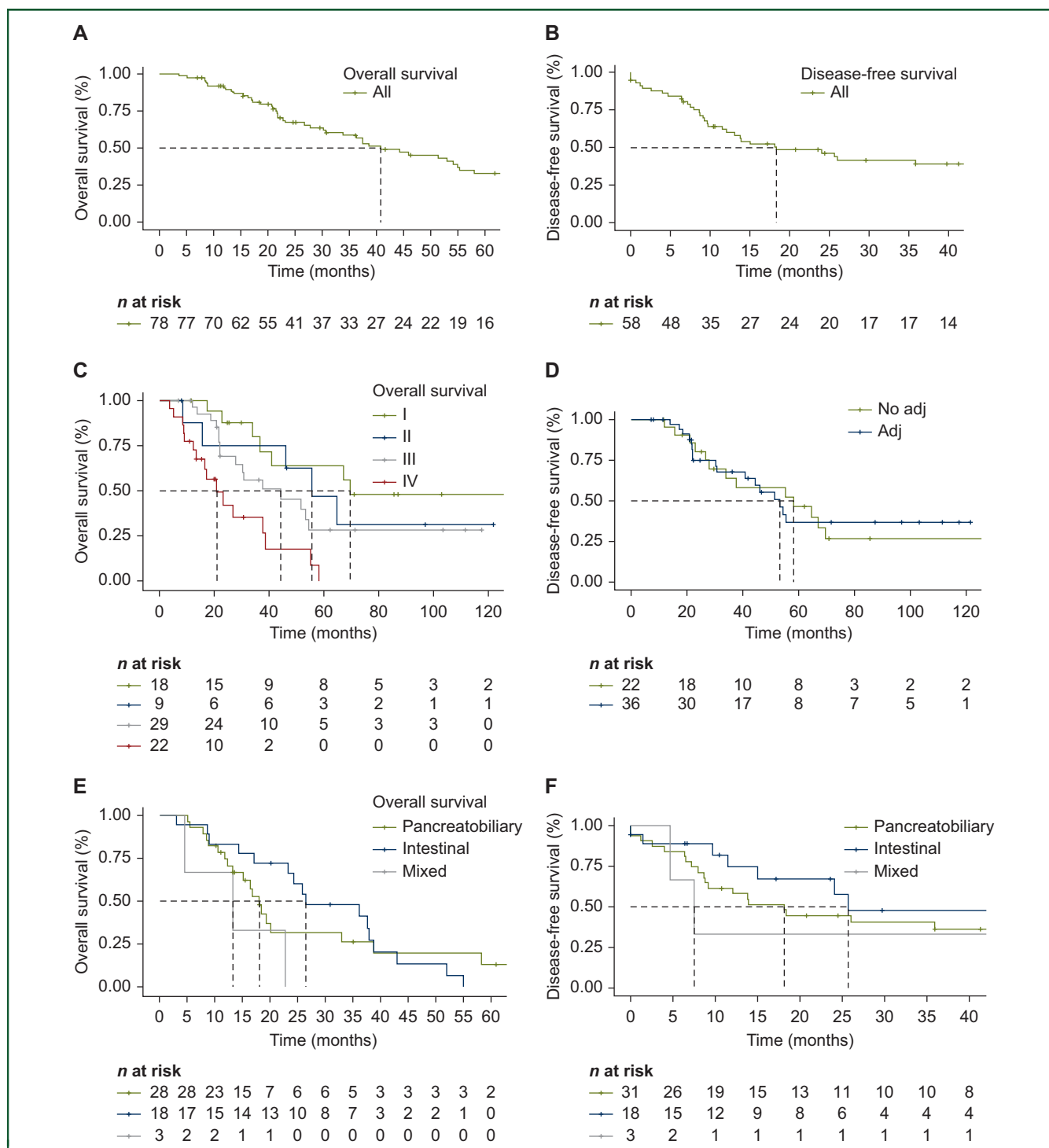


Figure 2. Survival outcomes of the VHIO AC cohort. (A) Kaplan–Meier OS curve in the overall population. (B) Kaplan–Meier DFS curves in the overall population. (C) Kaplan–Meier OS curves in the overall population comparing stage I, II, III, and IV. (D) Kaplan–Meier DFS curves in the overall population comparing patients who received adjuvant chemotherapy with those who did not. (E) Kaplan–Meier OS and (F) DFS curves in the overall population comparing patients with PB, INT, and MIX subtypes.

AC, ampullary carcinoma; DFS, disease-free survival; INT, intestinal; MIX, mixed; OS, overall survival; PB, pancreatobiliary; VHIO, Vall d'Hebron Institute of Oncology.

We could observe that the altered pathways vary between the histological subtypes, highlighting the enrichment of TGF- β ($P = 0.032$) pathway alteration in the INT subtype and a trend on WNT pathway enrichment in the INT subtype ($P = 0.09$) (Figure 3).

We found that the most common driver events in our cohort were *TP53* (45.3%), *KRAS* (42.7%), *ERBB2* (16%), *PIK3CA* (16%), and *SMAD4* (12%) (Figure 4A). The prevalence of *KRAS*^{MUT} was 42.7% in the VHIO cohort and 53.8% in the BCM cohort (Figure 4A, specific mutations in Supplementary Figure S1, available at <https://doi.org/10.1016/j.esmoop.2025.104480>). The most common *KRAS* variant was *KRAS* G12D (Supplementary Figure S1, available at <https://doi.org/10.1016/j.esmoop.2025.104480>).

To determine whether *KRAS*^{WT} samples were enriched in potentially targetable alterations, we classified molecular alterations following the ESCAT guidelines and focused on the I-IIIa categories (Supplementary Table S3, available at <https://doi.org/10.1016/j.esmoop.2025.104480>). In both the VHIO and BMC cohorts, there was a higher prevalence of ESCAT I-IIIa alterations in *KRAS*^{WT} (37.2% and 23.0%, respectively) when compared with *KRAS*^{MUT} (9.4% and 9.3%, respectively), showing statistically significant differences in both cohorts ($P = 0.018$ and $P = 0.013$, respectively) (Figure 4B and D). In the VHIO cohort, the *KRAS*^{WT} subpopulation harbors 18.6% of ESCAT IC alterations and 18.6% of ESCAT IIIa (Figure 4D). Of note, the most frequent actionable alterations were *ERBB2* amplifications, *ERBB2* mutations, DNA damage response gene mutations (*PALB2*, *BRCA2*, *ATM*, and *BRCA1*), and microsatellite instability (MSI) status (Figure 4E).

Correlation between clinical outcome and genomic alterations. Considering that *KRAS* and *TP53* are the two most frequent alterations in our cohort, we next assessed the survival outcomes considering the main genomic features observed in AC. In our cohort, patients with tumors with

KRAS mutations have similar OS to *KRAS*^{WT}, even when we compared *KRAS* G12D with other *KRAS* mutations (Supplementary Figure S2A and B, available at <https://doi.org/10.1016/j.esmoop.2025.104480>). Focusing on *TP53*, we do not observe significant differences in OS (Figure 5A). Nevertheless, in the metastatic cohort, we observed an ORR of 45% in tumors with *TP53*^{MUT} and 25% in tumors with *TP53*^{WT} ($P = 0.16$), which was not translated into a benefit in OS (Figure 5A and B).

Next, we assessed the influence of ESCAT alterations on patient OS. Among 51 patients with metastatic disease who underwent NGS analysis, those with tumors carrying genomic alterations categorized as ESCAT I-IIIa achieved a median OS of 25.8 months, compared with 18.3 months in patients with other alterations (hazard ratio 0.52, 95% CI 0.25-1.08, $P = 0.08$) (Figure 5C and D).

Interestingly, we identified six patients who received alteration-matched targeted therapies during their disease course (Figure 5E). Four patients had an amplification in *ERBB2*: two were treated with zanidatamab, and two with trastuzumab deruxtecan (T-DXd). One patient with *ERBB2* S310F mutation received T-DXd, and another patient with MSI status was treated with pembrolizumab. Notably, three patients received targeted therapy in the third line and three in the fourth line. Two patients were not assessable for ORR as they received only a single dose due to concurrent sepsis and subsequent clinical deterioration. However, the remaining patients demonstrated notable clinical benefit. Of particular interest, one patient with MSI-high status treated with fourth-line pembrolizumab achieved a PFS of over 13 months, while another patient with *ERBB2* amplification, treated with fourth-line zanidatamab, achieved a complete response sustained for >15 months and remains on treatment.

DISCUSSION

In this retrospective cohort study, we investigated the clinical and molecular characteristics of AC patients diagnosed at VHIO over a 15-year period. Our findings elucidate several pivotal aspects of AC management and provide valuable insights into potential targeted treatment strategies for this heterogeneous malignancy.

Firstly, our analysis of clinical characteristics revealed notable patterns in AC presentation, classification, and disease evolution. The overall cohort demonstrated a median OS of 40.5 months and a recurrence rate of 66%, with substantial heterogeneity in systemic treatment approaches and emphasizing the pressing need for advancements in therapeutic strategies for advanced-stage AC. Furthermore, histological subtyping based on IHC analysis revealed distinct subtypes, including INT, PB, and MIX. Although we did not observe significant differences in OS among these histological subtypes, further research is warranted to explore subtype-specific treatment responses and prognostic implications.

Secondly, our genomic profiling highlighted the importance of *KRAS* alterations as a driver with potential

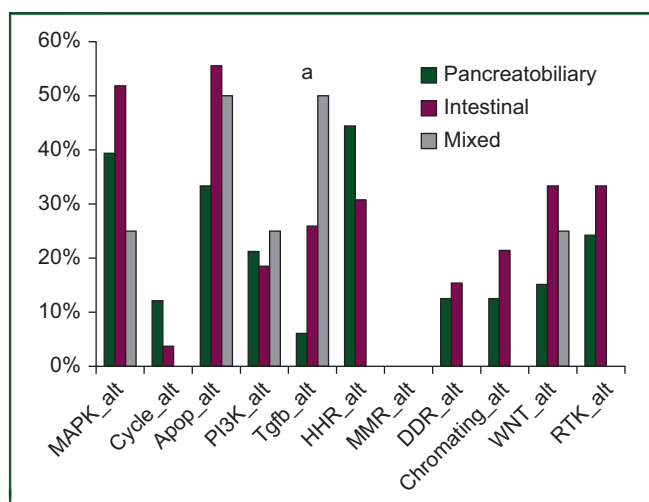


Figure 3. Oncogenic pathway detected with the NGS panels and comparison between PB, INT, and MIX subtypes.

INT, intestinal; MIX, mixed; NGS, next-generation sequencing; PB, pancreatobiliary.

^aStatistically significant.

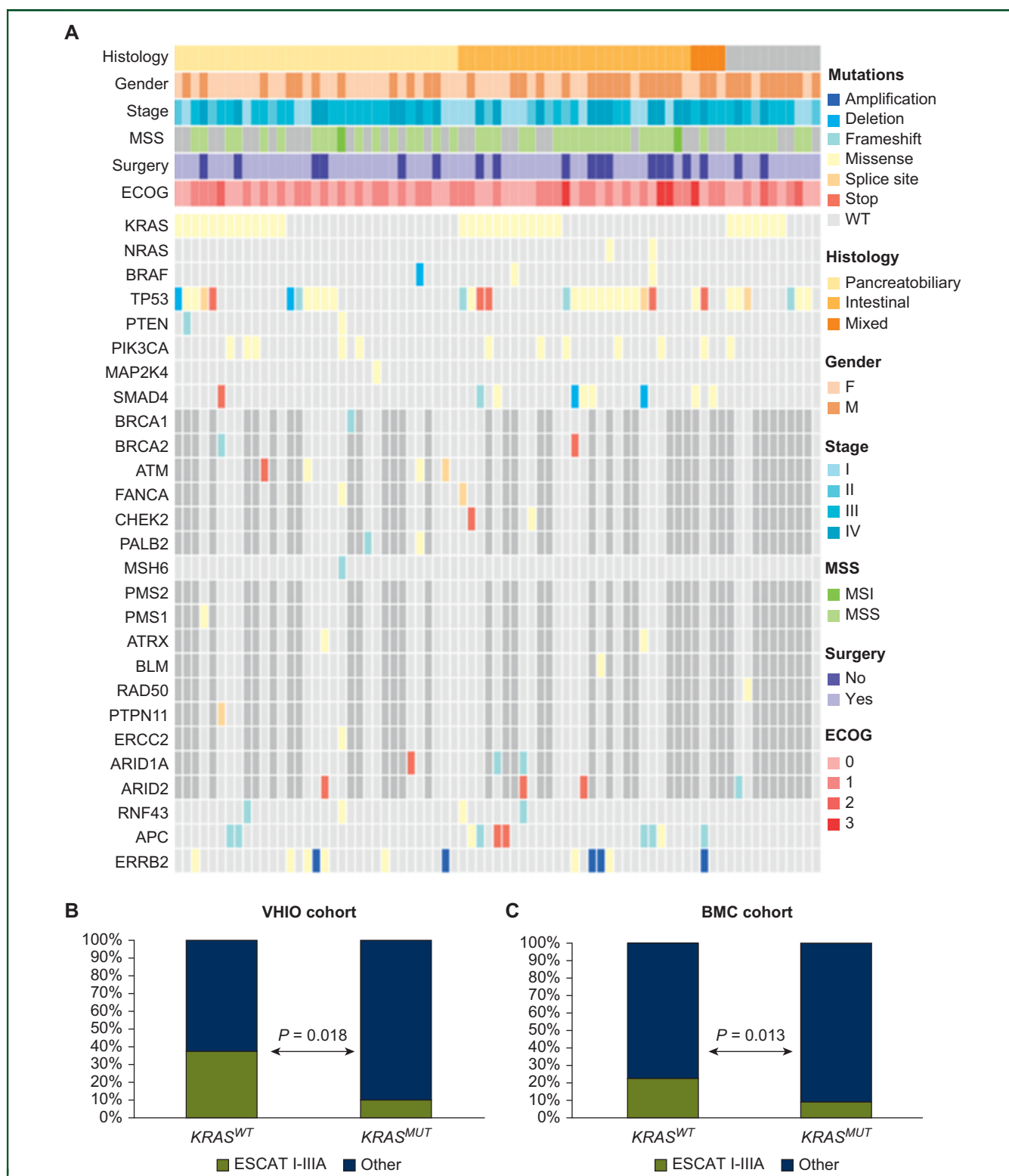


Figure 4. Genomic landscape and targetable alterations in AC. (A) OncoPrint showing the frequency of the most prevalent gene mutations in AC. (B, C) Prevalence of targetable alterations depending on $KRAS$ mutation status in both the VHIO and BMC cohorts. (D) Prevalence of potentially targetable alterations following ESCAT criteria in $KRAS^{MUT}$ and $KRAS^{WT}$. (E) Distribution of cases classified according to the highest-ranking ESCAT alteration in AC.

AC, ampullary carcinoma; BCM, Baylor College of Medicine; ECOG, Eastern Cooperative Oncology Group; ESCAT, ESMO Scale for Clinical Actionability of Molecular Targets; MSI, microsatellite instability; MSS, microsatellite stability; VHIO, Vall d'Hebron Institute of Oncology.

^aOne patient presented with $ERBB2^{MUT}$ and $BRCA2$.

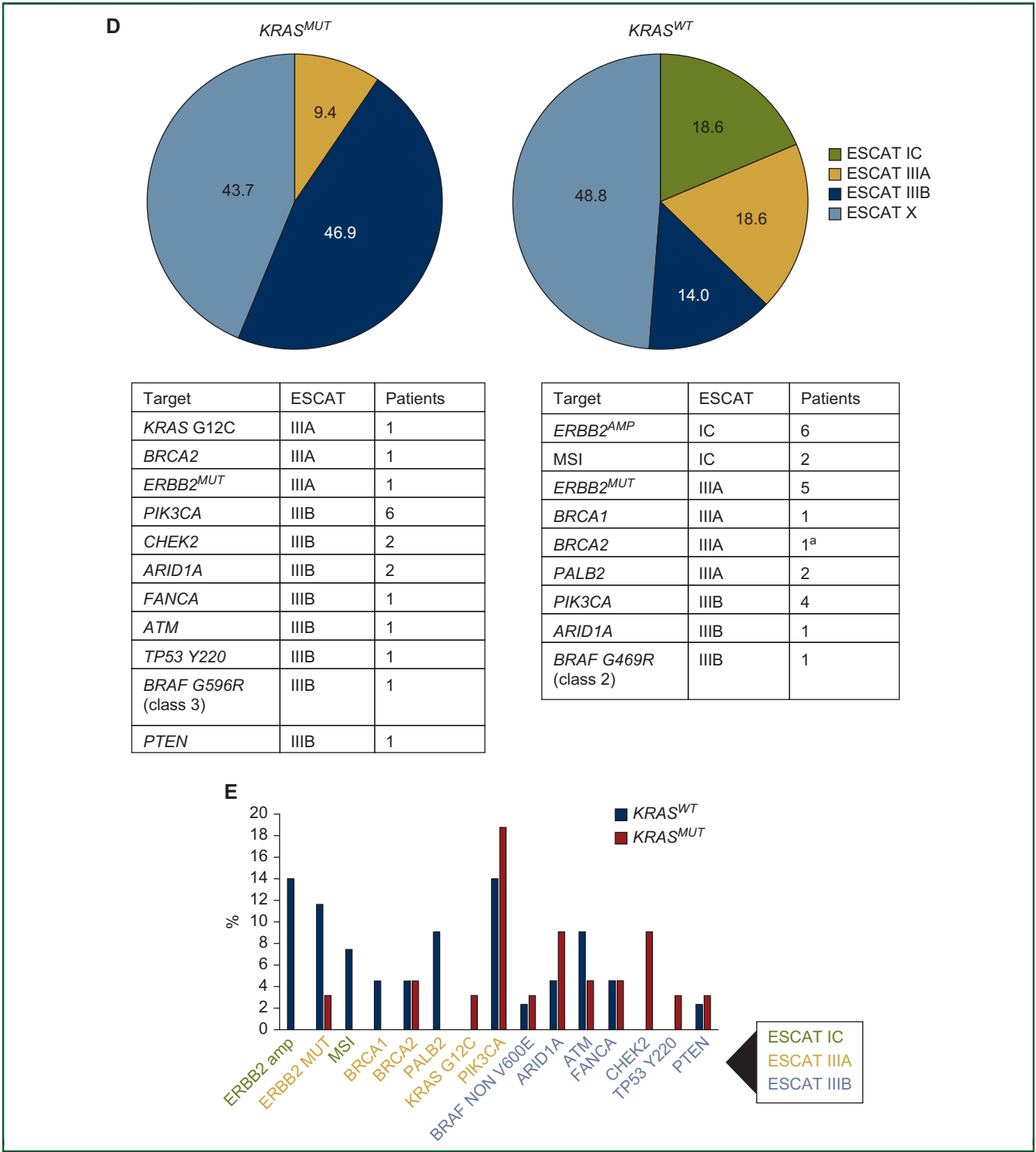


Figure 4. Continued.

implications for targeted therapy approaches. Notably, *KRAS*^{WT} tumors exhibited a higher prevalence of potentially targetable alterations according to the ESCAT guidelines. This suggests that a subset of patients could benefit from personalized treatment strategies.

Our analysis also highlighted the potential utility of targeted therapies in AC management. Patients with *ERBB2* amplifications, which are often linked to human epidermal growth factor receptor 2 (HER2) overexpression,

demonstrated promising responses to *ERBB2*-targeted agents such as zanidatamab and T-DXd, underscoring the importance of molecular profiling in guiding treatment decisions. Additionally, immune checkpoint inhibitors such as pembrolizumab showed efficacy in a patient with MSI-high, suggesting a potential role for immunotherapy in selected subgroups of AC patients.

It is crucial to consider the growing array of agnostic targeted therapies recently approved by the United States

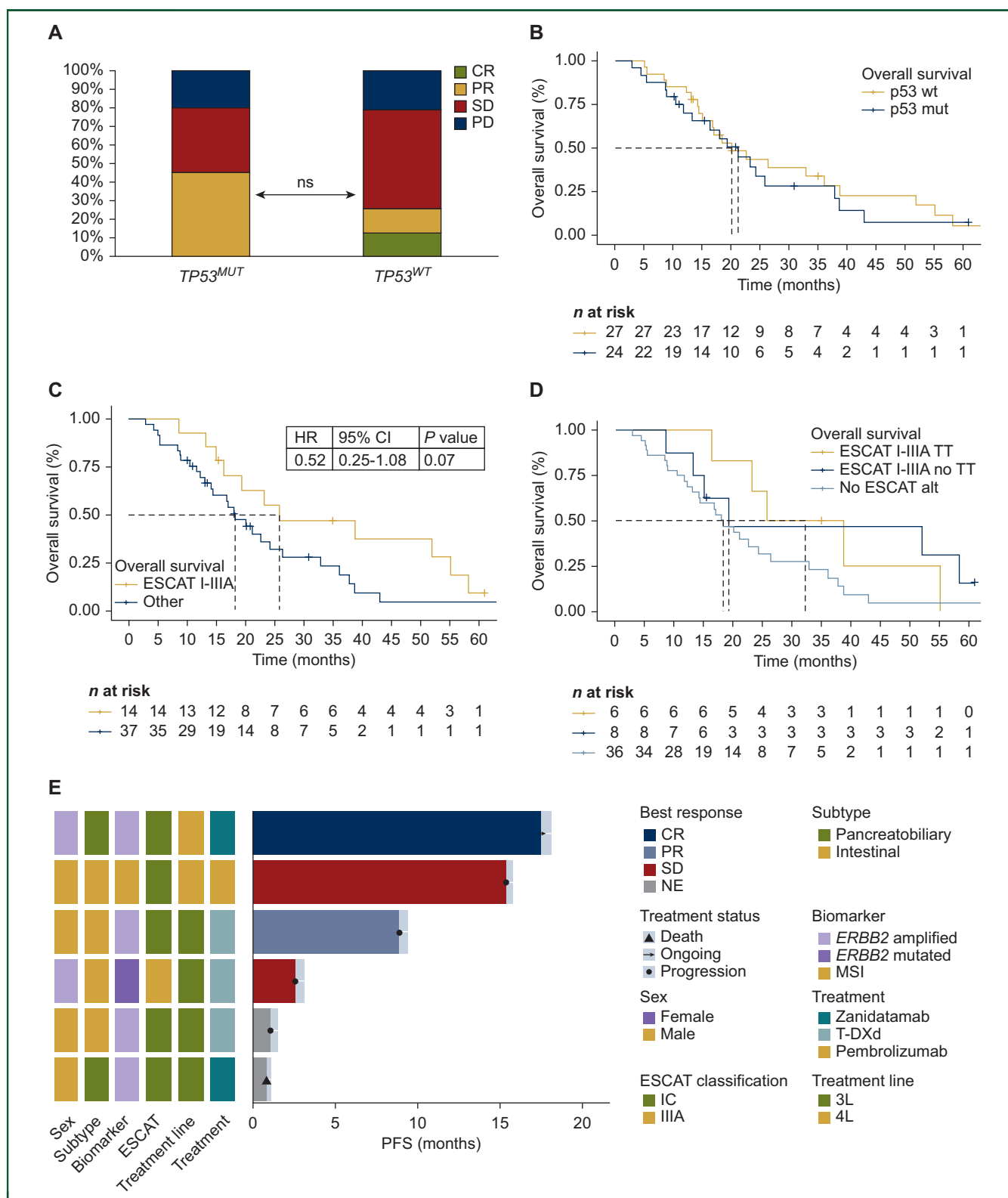


Figure 5. Survival outcomes and treatment response in metastatic AC. (A) BOR using RECIST 1.1 in patients with AC with $TP53^{MUT}$ versus $TP53^{WT}$. (B) Kaplan-Meier OS curves in the metastatic population comparing $TP53^{MUT}$ versus $TP53^{WT}$. (C) Kaplan-Meier OS curves in the metastatic population comparing patients with ESCAT I-IIIa alterations versus others. (D) Kaplan-Meier OS curves in the metastatic population comparing patients with ESCAT I-IIIa alterations treated with targeted therapy versus ESCAT I-IIIa alterations non-treated with targeted therapy versus others. (E) Swimmer's plot of the six patients treated with targeted therapies. 3L, third line; 4L, fourth line; AC, ampullary carcinoma; BOR, best overall response; CI, confidence interval; CR, complete response; HR, hazard ratio; NE, not evaluable; ns, not significant; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; T-DXd, trastuzumab deruxtecan; TT, targeted therapy.

Food and Drug Administration, such as immunotherapy for MSI status and inhibitors targeting HER2 overexpression, *NTRK* fusions, *RET* fusions, and *BRAF* V600E mutations. In our cohort, we identified patients with *ERBB2* amplifications and mutations and MSI status who benefited from targeted therapy. However, most genomic tests in our study were unable to detect *RET* and *NTRK* fusions, indicating that the frequency and therapeutic benefit of these matched treatments in AC remain inadequately characterized.²⁸⁻³⁵ We were unable to evaluate the efficacy of epidermal growth factor receptor inhibitors in our cohort because these therapies are not approved for the treatment of AC in Spain, despite their established efficacy in colorectal cancer, which often serves as a treatment model for INT-type AC.⁷ Other trials showed promising activity of tyrosine kinase inhibitors or antibodies against HER2 in *ERBB2* mutations/amplifications, in patients with related tumors such as biliary tract cancers or colorectal cancers.³⁶⁻³⁸

Despite these promising findings, several limitations of our study must be acknowledged. Firstly, the retrospective design inherently introduces biases and restricts control over data collection and interpretation, potentially impacting the study's internal validity. Secondly, the limited sample size may constrain the generalizability of our results, especially in subgroup analyses where statistical power is further reduced. Thirdly, heterogeneity in NGS testing among patients introduces variability in the genomic data, which could influence the interpretation of molecular findings. These limitations are largely attributable to the low incidence of this tumor type and the lack of standardized protocols in clinical practice guidelines, both of which complicate the establishment of consistent treatment approaches and uniform data collection.

Future research should focus on prospective studies with larger cohorts and standardized NGS testing protocols to further validate our findings and better understand the clinical implications of molecular subtypes and targeted therapies in AC. Additionally, exploring the mechanistic basis of resistance to targeted therapies and the potential role of combination therapies could further enhance treatment outcomes for AC patients.

Conclusion

In conclusion, our study provides valuable insights into the clinical and molecular landscape of AC, highlighting the potential for targeted therapies in this heterogeneous disease entity. Further prospective studies with larger cohorts and integrated multi-omic analyses are warranted to validate our findings and inform personalized treatment strategies for AC patients.

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DISCLOSURE

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DATA SHARING

Data from the VHIO cohort are not publicly available due to patient privacy but are available on reasonable request from the corresponding author. The validation data were obtained from the Baylor College of Medicine²⁰ and are available at cBioPortal (<https://www.cbioportal.org/>).

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