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CASE REPORT

Immunotherapy and Tumor Heterogeneity in Advanced Goblet Cell Adenocarcinoma of the Appendix: A Case Report

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Goblet cell adenocarcinoma (GCA) of the appendix is a rare and aggressive malignancy, often presenting with peritoneal dissemination. Treatment typically involves surgical resection and chemotherapy, though the management of metastatic disease remains complex and with no clear guidelines. We present a case of advanced GCA with extensive peritoneal involvement in a 66-year-old woman, in which repeated molecular testing led to the identification of a clone with high microsatellite instability, guiding the successful use of immunotherapy. Additionally, molecular profiling via liquid biopsy played a key role in subsequent treatment decisions. This personalized treatment approach resulted in a survival period of 42 months, which is nearly double the median overall survival (mOS) for this condition. The patient's journey highlights the importance of personalized medicine, illustrating how continuous molecular monitoring and adaptability to emerging data can significantly improve outcomes in patients with tumors exhibiting molecular heterogeneity.

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Introduction

Goblet cell adenocarcinoma (GCA) of the appendix is a rare malignancy characterized by features of both adenocarcinoma and neuroendocrine tumors. Up to 80% of GCA cases manifest with peritoneal dissemination,

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with a high tropism for the ovaries; however, hematogenous metastases are a very rare occurrence.[1] Management of GCA often involves a combination of surgical resection and chemotherapy, with right hemicolectomy recommended for localized tumors, even in the presence of negative margins. The management of metastatic GCA is more challenging due to the absence of clear guidelines. Prognosis is closely linked to tumor grading and TNM staging, with markedly different survival outcomes depending on these factors. In advanced stages, particularly stage IV, survival rates decline significantly. The 5-year overall survival (OS) for stages I, II, III, and IV has been estimated at 91.1%–100%, 67.0%–90.5%, 36.0%–57.0% and 4.2%–18.9%, respectively,[1] and median overall survival (mOS) is 22.4 months in all stage IV cases.[2] Another factor that has been identified as an independent prognostic factor is the grade of differentiation. In poorly differentiated cases (group C),[3] 3-year OS is only 17%.

In this report, we discuss a complex case of GCA that underwent multiple molecular tests, ultimately leading to the identification of a tumor population with high microsatellite instability (MSI-high). This finding allowed for the administration of immune checkpoint inhibitor therapy, which resulted in a marked clinical benefit. Additionally, this case provides insights into tumor heterogeneity, as different subpopulations with distinct mutation profiles were identified.

Case presentation

We present the case of a 66-year-old woman, non-smoker, with previous medical history of gastroesophageal reflux and depression. Her surgical history included conization at the age of 27 and hemorrhoidectomy. The patient was initially followed at another institution where she presented with abdominal pain in December 2019.

An abdominal computed tomography (CT) scan revealed a large mass (14 cm) in the left iliac fossa, initially suspected to be ovarian cancer. The carbohydrate antigen 125 (CA-125) level was elevated at 159 kU/I. Following negative gastroscopy and colonoscopy findings, CT-guided biopsy of the lesion was performed indicating a carcinoma with mucinous features.

The patient underwent extensive surgery, which included total hysterectomy, salpingo-oophorectomy, omentectomy, peritonectomy and partial deperitonealization of the mesentery. During the procedure, a mass involving the appendix and the ileocecal valve was discovered, leading to the decision to perform a right hemicolectomy. Histological examination identified high-grade goblet cell adenocarcinoma of the appendix (group C according to Tang[3]) with extensive peritoneal carcinomatosis and left ovarian metastasis. The Ki-67 proliferation index was up to 70% in some regions. The peritoneal lavage was negative for malignant cells. The resection was deemed complete (R0). The final staging, according to the Union for International Cancer

Control (UICC) TNM classification for colorectal cancer (8th edition),[4] was pT4a, pN1b (2/21), M1c, with lymphovascular and perineural invasion, stage IV.

Molecular analysis via the FoundationOne CDx test (analysis of 309 genes with full coding exonic regions for the detection of substitutions, insertion-deletions (indels), and copy-number alterations (CNAs) as well as 36 intronic regions for the detection of gene rearrangements)[5] on the initial tissue sample revealed *NRAS* (p.Q61R) and *TP53* (p.R273C) mutations; allele frequency was not reported. However, the tumor content was only 20%, making the tumor mutational burden (TMB) and microsatellite status unevaluable.

The patient was initially treated with adjuvant carboplatin and etoposide, which were poorly tolerated, resulting in the completion of only three cycles. She was then referred to our institution for further care. Subsequent imaging with fluorodeoxyglucose (FDG)-positron emission tomography (PET)-CT showed no evidence of disease.

After multidisciplinary discussion within our institution and our reference center for peritoneal surgery, we proposed completion of adjuvant chemotherapy with the folinic acid, fluorouracil and oxaliplatin (FOLFOX) regimen for four cycles, followed by a second-look surgery and potential pressurized intraperitoneal aerosol chemotherapy (PIPAC).

Despite a negative CT scan and magnetic resonance imaging (MRI), exploratory laparotomy revealed multiple peritoneal nodules, defining a peritoneal carcinomatosis index (PCI) of 6. Nonetheless PIPAC was not delivered as per decision of the expert surgeon. The resection was complete (R0). Histological analysis confirmed metastases consistent with the known GCA, with mismatch repair (MMR) protein expression conserved, classifying the tumor as microsatellite stable (MSS). The patient completed adjuvant chemotherapy with FOLFOX for five more cycles, with suboptimal tolerance (nausea, fatigue). From November 2020 the patient was followed with abdominal magnetic resonance imaging (MRI) and thoracic CT every three months.

In August 2021, MRI indicated peritoneal progression with new, non-resectable nodules. The tumor markers CA-125 and carcinoembryonic antigen (CEA) were negative. The patient received second-line chemotherapy with folinic acid, fluorouracil and irinotecan (FOLFIRI) plus bevacizumab, analogous to the standard second-line treatment for colorectal carcinoma (CRC). The treatment was complicated by a pulmonary embolism, leading to interruption of bevacizumab. A near-complete radiological response was observed.

In March 2022, during a multidisciplinary discussion, cytoreductive surgery with potential hyperthermic intraperitoneal chemotherapy (HIPEC) was proposed to consolidate the achieved response, although the timing of PIPAC

or HIPEC remains controversial and not studied prospectively.[6] However, only adhesiolysis and resection of the intestinal stenosis were performed due to the intraoperative finding of extensive peritoneal carcinomatosis (PCI 34), which was not detectable on preoperative imaging.

Subsequent next-generation sequencing (NGS) testing (xGen® Lockdown® IDT Large Custom Cancer Panel IPA) on the newly resected tumor specimen, with 80–90% tumor content, confirmed the *NRAS* mutation (p.Q61R with 70% allele frequency) and identified a distinct *TP53* mutation (p.R175H with 73% allele frequency). The analysis also revealed high TMB (18.9 mut/MB) and MMR deficiency, with loss of MSH2 and MSH6 expression and *MSH2* gene deletion, classifying the tumor as MSI-high. These results differed from the initial report of MMR proficiency.

Based on these findings, treatment with pembrolizumab was initiated,[7] resulting within three months in marked clinical benefit with resolution of abdominal pain and constipation, paralleled by a complete radiological response, confirmed with a CT. However, treatment was discontinued after seven months due to severe cutaneous toxicity – immunomediated lichenoid dermatitis grade 3 – requiring both topical and systemic steroid treatment.

Six months after the suspension of pembrolizumab, imaging revealed tumor relapse in the peritoneum, vagina and lymph nodes (CT and FDG-PET-CT). Transvaginal biopsy confirmed metastasis of the known GCA. Molecular analyses using NGS (Oncomine Comprehensive Assay v3 panel) did not identify any clinically relevant mutations (i.e., *NRAS* and *TP53* full-length genes appeared wild-type), and the tumor was again classified as MSS with normal expression of MMR proteins. Notably, the cellularity of the tumor sample was 30%.

Based on the absence of any *RAS* mutation, treatment with an anti-EGFR antibody (panitumumab) could be considered.[8]

The disappearance of a previously detected *RAS* mutation, a phenomenon known as neo-RAS wild type, is well described in the literature; estimates of its frequency in CRC vary ranging from 5.5 to 78%.[9] This ample variability could be explained by differences in clinical parameters (disease biology, selective pressure by exposure to chemotherapy and targeted agents) and in the sensitivity of the testing method employed. Taking into account tumor heterogeneity, we opted to perform liquid biopsy in order to obtain a better overall representation of the tumor subpopulations.

Cell-free DNA (cfDNA) was analyzed using NGS Ion Torrent technology and an Oncomine TM Colon cfDNA Assay panel was performed. Three mutations were detected: *NRAS* (p.Q61R), *TP53* (p.R175H), *TP53* (p.C277Y). Notably, the first two mutations were identical to those described previously and were present at a frequency of approximately 3%. The last

mutation was rarer, with an allelic frequency of 0.5% and it was impossible to determine whether it originated from a tumoral subclone or clonal hematopoiesis.[10] The persistence of the *NRAS* mutation excluded the use of panitumumab and we proposed a third-line treatment with trifluridin/tipiracil (TAS 102).

Despite mixed responses to subsequent treatments, including fruquintinib (a new oral selective inhibitor of vascular endothelial growth factor receptors), the patient's condition deteriorated due to tumor progression, and she passed away in July 2024.

Discussion

GCA is a rare entity, first described in 1969 as a tumor that shares features of carcinoid and carcinoma.[11] In the following years many denominations and classifications were introduced (e.g. goblet cell carcinoid, mucinous carcinoid tumor, mixed carcinoid-adenocarcinoma, etc.) underlining the disputes whether to attribute the tumor under the "carcinoid" or "carcinomatous" family. It was only in 2019 that the World Health Organization (WHO) Classification of Tumors proposed the name of Goblet Cell Adenocarcinoma (carcinoma being accepted) and established that the entity should not be classified as a neuroendocrine tumor.[12] This reclassification underlines the importance of accurate terminology, as misclassification can lead to inappropriate treatment strategies. Due to the rarity of the disease and diagnostic challenges, there are no clear consensus or guidelines, and it is recommended to follow the colon cancer guidelines in the management of GCA.[6]

In the diagnostic workup, octreotide single-photon emission computed tomography (SPECT) or ^{68}Ga-DOTATATE-PET-CT are not routinely recommended and FDG-PET-CT is preferred. The mucinous component, however, might be poorly visualized by FDG-PET-CT as well; therefore MRI may be a more suitable method.[13] In our case, we initially performed an FDG-PET-CT scan according to ENETS Consensus guidelines.[6,13] However, subsequent imaging was conducted with CT or MRI due to the limited evidence supporting the use of PET-CT for monitoring disease extension. Despite this, we encountered discrepancies between the imaging results and the actual extent of the disease, which was later confirmed through laparoscopy.

Elevated tumor markers, such as CEA, CA 19-9 or CA-125, are reported in some studies; however, there is no definite role for them in diagnosis or follow up. Additionally, serum chromogranin A and neuron-specific enolase (NSE) have no value for the detection and monitoring of GCA.[6]

The mutational landscape of GCA differs from that of CRC[14] and may contribute to explain the different clinical behavior and response to treatments. The use of chemotherapy regimens similar to those indicated for CRC, such as FOLFOX and FOLFIRI, is common, although responses are often suboptimal.

The prevalence of *RAS, APC* and *TP53* mutations tends to be lower in GCA than in appendiceal colorectal-type adenocarcinoma and is usually linked with poor differentiation.[15-17]

In our case, the tumor harbored an *NRAS* mutation at diagnosis that was confirmed in subsequent analyses. Liu et al.[18] described *NRAS* in only one case of GCA (4%), while in CRC the prevalence of this mutation is around 9%[19] and its prognostic value is controversial. According to some sources, *NRAS* mutations may be a predictor of poor prognosis in CRC patients.[20] On the contrary, its role in GCA remains unknown.

Appendiceal carcinomas in general have a low prevalence of MSI (3%) as compared to right colon adenocarcinomas (20%).[21] To our knowledge, no case of high-level microsatellite instability was yet described in goblet cell carcinomas originating from the appendix specifically. Indeed, according to data published in the literature [Taggart et al 2013, Jesinghaus et al in 2017, Dimmler et al in 2014, as well as by M. Johncilla et al in 2018 and H. Arai et al in 2020] – a total of 131 patients with GCA were tested and no case of MSI was detected.[15,16,21-23] MSI has been reported once in GCA of the right colon; the authors did not specify though whether the lesion was of appendiceal origin.[24] Some studies included an evaluation of other immunotherapy-related biomarkers such as TMB or programmed death ligand 1 (PD-L1).[23] Low rates of MSI, PD-L1 and TMB suggest that GCA is an immunologically "cold" tumor.

In general, in solid tumors, MSI-high is considered an early event in carcinogenesis and is maintained throughout the entire course of the disease; however, intra- and inter-tumoral heterogeneity as detected in our case has previously been described, although literature data are lacking concerning the evolution of MSI status over time and under selective pressure from treatments.[25] A direct role of chemotherapy and/or immunotherapy in shaping tumor characteristics through Darwinian-like selection cannot be excluded.

Indeed, our case is an example of tumor heterogeneity as proven by the different results of repeated testing for MSI, *NRAS* and *TP53* mutations.

The absence of *NRAS* mutation in the vaginal metastasis, while the same mutation remained detectable in a liquid biopsy collected at the same time point, highlights the presence of spatial heterogeneity, where distinct tumor regions can harbor different genetic profiles.

On the other hand, temporal heterogeneity – intended as the modification of the molecular profile over time – remains more challenging to assess. For instance, the identification of two distinct *TP53* mutations at different time points does not necessarily imply that these mutations evolved sequentially or independently. It remains possible that both mutations were already present at the time of diagnosis, but only one was detected in the initial tissue biopsy due to sampling bias. This is a well-known limitation of tissue biopsies, where a small amount of tissue obtained may not fully represent the genetic diversity of the tumor.

Our molecular characterization highlights the potential role of liquid biopsies in revealing molecular heterogeneity, which was indeed clinically significant in our case, as it guided the treatment choice by identifying the persistence of the *NRAS* (p.Q61R) pathogenic mutation.

As the clinical implications of molecular markers continue to evolve, the need for diagnostic precision becomes increasingly important. The question of whether discrepancies in mutations observed in different tissue biopsies should be verified through liquid biopsy, and what role liquid biopsy should play in detecting tumor heterogeneity and guiding treatment decisions remains under active discussion. However, despite its potential, there is currently insufficient evidence to base treatment decisions solely on liquid biopsy results in place of histological analysis, outside of specific circumstances.

Conclusion

This case report highlights the complexities of treating advanced GCA of the appendix, particularly in the context of extensive peritoneal involvement. The patient's treatment journey underscores the importance of personalized medicine, with molecular profiling guiding the use of targeted therapies and immunotherapy. Despite the aggressive nature of the disease and the challenges posed by tumor heterogeneity, the strategic use of molecular testing and a multidisciplinary approach contributed to prolonged survival and improved quality of life.

Future research should focus on optimizing treatment strategies for GCA, including the potential benefits of novel therapeutic approaches such as immunotherapy. The importance of multidisciplinary collaboration, involving surgeons, radiologists, pathologists, molecular biologists, and geneticists, cannot be overstated in the management of this rare and complex tumor.

Ethics approval and consent to participate

Written consent for the further use of patient data was obtained.

Consent for publication

Consent for publication was obtained.

Availability of data and materials

All patient data that support this case report are included in the anonymized form in the published article.

Conflict of interest

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

All authors contributed to and approved the final manuscript.

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