



# A mismatch repair-deficient and HPV-negative anorectal squamous cell carcinoma

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## Abstract

Invasive primary squamous cell carcinomas involving the anorectal region are challenging to manage. Microsatellite instability has been shown to impact clinical courses and outcomes of patients affected by many types of carcinomas. To the best of our knowledge, there are no reports on microsatellite instability in anorectal squamous cell carcinomas. Here, we report a HPV-negative anorectal squamous cell carcinoma which, despite cisplatin-based chemoradiation therapy, showed progression. Interestingly, after identification of its mismatch repair-deficiency (MLH1/PMS2-absent, MSH2/MSH6-intact), pembrolizumab-based immunotherapy was initiated, leading to a marked clinical response. This unique case illustrates that microsatellite instability testing and immunotherapy targeting immune checkpoint blockade should be considered for managing anorectal squamous cell carcinomas that fail conventional chemoradiation therapies or when patients are non-surgical candidates. This report provides the first evidence of microsatellite instability in anorectal squamous cell carcinomas and supports the role for microsatellite instability testing in this cancer type to optimize patient management.

**Keywords** Squamous cell carcinoma · Anorectal · Microsatellite instability · Immune checkpoint blockade

## Introduction

Squamous cell carcinoma (SCC) of the anorectal region may be difficult to manage due to their aggressive behavior and poor response to neoadjuvant therapeutic methods [1]. Anorectal SCC is also rarer than its adenocarcinoma counterpart and little is known about the pathogenesis and risk factors of this disease, implicating distinct therapeutic approaches. However, it was found that in patients with anal SCC, tumors negative for HPV and positive for p16 expression (HPV<sup>-</sup>/p16<sup>+</sup>) usually have better prognoses than those that were negative for both HPV and p16 (HPV<sup>-</sup>/p16<sup>-</sup>) [2]. Furthermore, the latter are notorious

for not responding to the conventional chemotherapies often resulting in an unfavorable outcome. Interestingly, recent research has demonstrated that using novel immunotherapies targeting programmed cell death protein 1 (PD-1) and its ligand (PD-L1) may be a successful strategy in managing advanced anal SCCs [3, 4]. However, the prevalence of microsatellite instability (MSI) in anorectal SCCs remains unknown, and it is unclear whether determination of MMR defects could improve the care of patients burdened with anorectal SCCs.

It has been well documented that patients with MSI or MMR defect(s) face an increased risk of developing a spectrum of malignancies, such as colorectal, endometrial, gastric, and small intestinal adenocarcinomas [5]. Additionally, due to the impact of MSI and MMR status on the effectiveness of immune checkpoint inhibitors, it can have a significant influence on clinical decision making and patient management due to a variety of MSI-related neoantigens in MMR-deficient tumors, which render them susceptible to immune checkpoint blockade-mediated strategies [6]. Therefore, it is important to acknowledge that MSI/MMR defect testing can play an essential role in the initiation of novel therapeutic protocols which may significantly influence patient outcome.

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## Case report

Here, we report the novel determination of MSI anal/anorectal SCC in an 81-year-old male with a complicated history of hypertension, prostate cancer, stroke, and a 6-cm anal SCC extensively involving the lower rectum. Since he was not qualified for surgery or radiation, he had received unsuccessfully 6 cycles of cisplatin/taxol chemotherapy originally at an outside hospital. As his SCC progressed, with development of recto-urethral fistula, ulceration, stenosis of anorectal canal, and worsening rectal wall thickening, the patient was referred to our institution for further management.

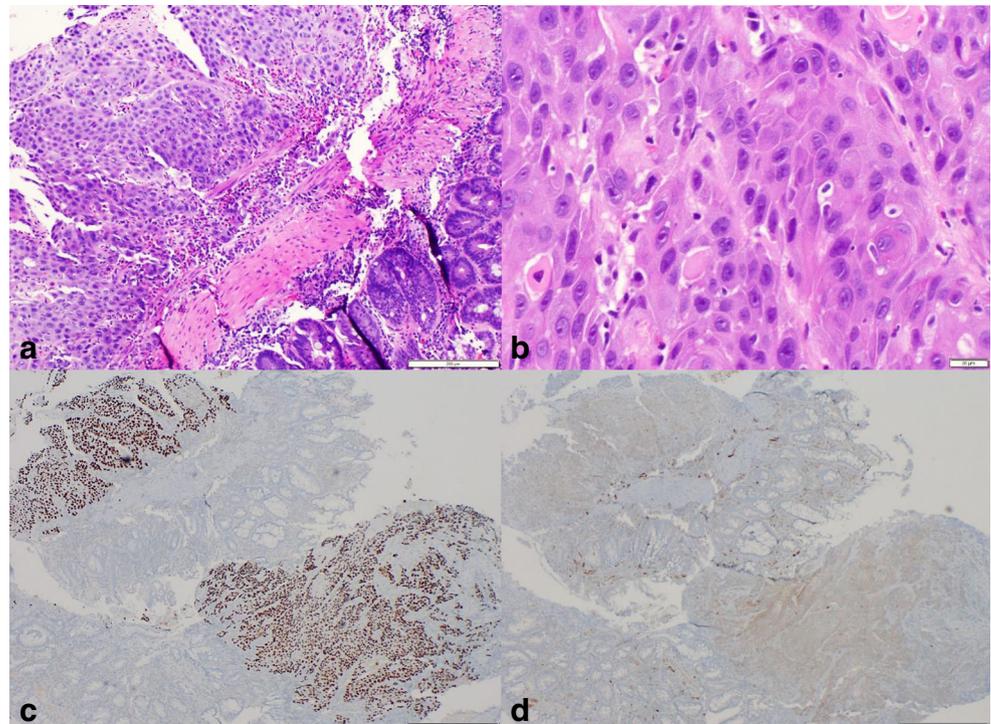
According to the clinical notes, the tumor was originally thought to be exclusively involving the anus due to its gross appearance and the patient's symptoms; however, the earlier radiologic and endoscopic studies conducted at the outside institution detected that the large mass involved the lower rectum as well. The original pretherapeutic rectal tumor biopsy was reviewed, which revealed a moderately differentiated SCC extending into and invading the rectal mucosa. Tumor cells showed abundant eosinophilic cytoplasm and large vesicular nuclei with occasional prominent nucleoli and foci of keratinization; in contrast, “basaloid” features frequently seen with HPV-related SCC were not appreciated in this tumor (Fig. 1a, b). Indeed, additional immunohistochemical studies performed at our institute showed that the tumor was P40-positive and P16- and HPV-negative (Fig. 1c, d). Given the progressive nature of his SCC,

the limited management options available, and the histomorphology of this aggressive tumor, MMR/MSI testing was ordered and demonstrated a loss of MLH1 and PMS2 expression but retained expression of MSH2 and MSH6 in tumor cell nuclei, consistent with a novel recognition of anorectal MSI SCC (Fig. 2a–d). Notably, numerous tumor infiltrating lymphocytes (TILs) were present, averaging 27 TILs per  $\times 40$  high-power field (Fig. 2e, arrows), which, upon immunostaining, were predominantly CD8-positive TILs (approximately 90%; Fig. 2f). Subsequently, pembrolizumab-based therapy was initiated replacing cisplatin/taxol protocol. Following 4 cycles of pembrolizumab-based therapy, significant tumor response was detected clinically and radiographically, evidenced by shrinkage of tumor size, opening of the rectal lumen as well as a decrease in rectal wall thickening (Fig. 3).

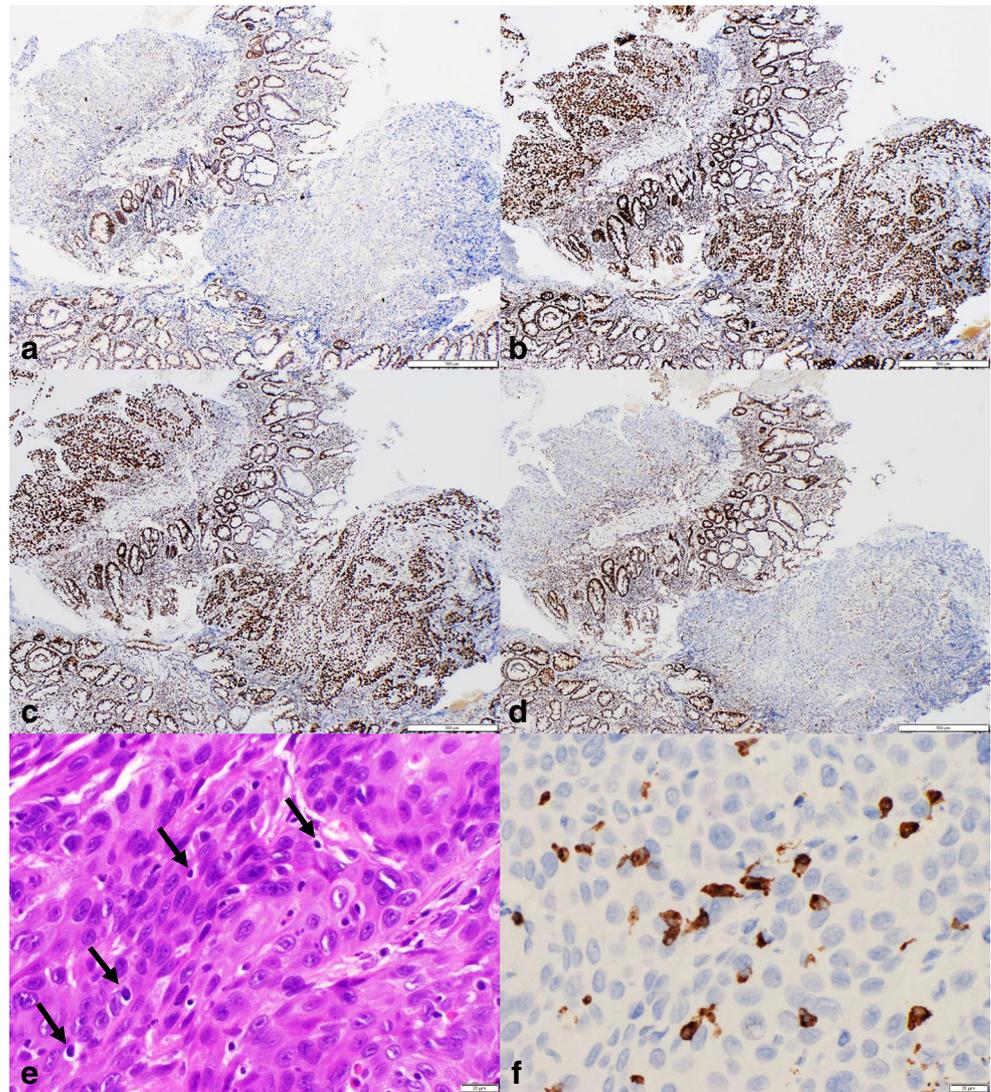
## Discussion

MSI is caused by various defects in the mismatch repair function and has been intensively studied in a variety of gastrointestinal malignancies including colorectal, ampullary, and gastric adenocarcinomas [5, 7]. These MSI/MMR-deficient tumors have a distinct clinical presentation, with certain well-recognized histopathological features. They also respond differently to conventional neoadjuvant chemotherapy and have an overall unique

**Fig. 1** Histological and immunophenotypical evaluation of a mismatch repair-deficient anorectal squamous cell carcinoma. HE sections of tumor (**a** and **b**,  $\times 100$  and  $\times 400$ , respectively), and immunohistochemistry of P40 and P16 (**c** and **d**,  $\times 40$ ) were shown



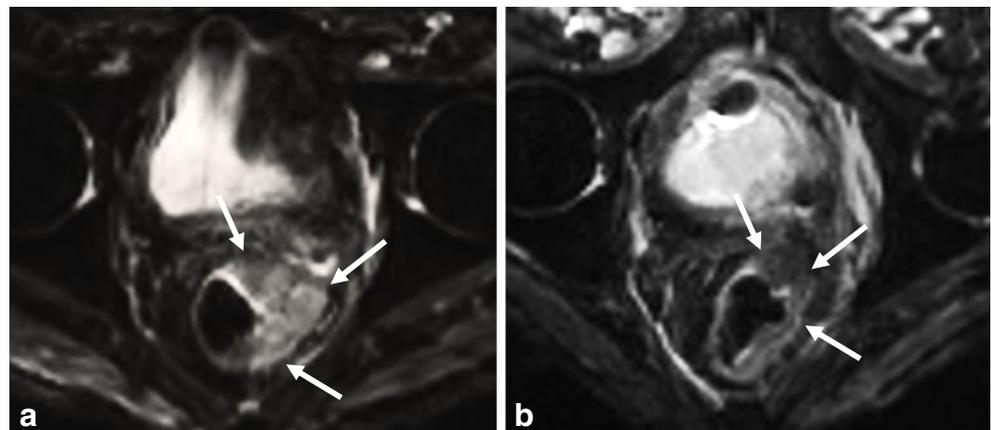
**Fig. 2** Immunophenotypical evaluation of mismatch repair proteins in this unique anorectal squamous cell carcinoma, with loss of MLH1 and PMS2 (**a** and **d**,  $\times 40$ ), and intact MSH2 and MSH6 (**b** and **c**,  $\times 40$ ). Frequent tumor infiltrating lymphocytes were observed (**e**, arrows,  $\times 400$ ), which showed predominant CD8-labeling by immunohistochemistry (**f**,  $\times 400$ )



clinical course distinct from microsatellite stable/MMR-intact tumors [6, 8–10]. Further lending support to the concept of MSI/MMR deficiency as a possible indication for immunotherapy is a recent study evaluating the

efficacy of PD-1 blockade in 12,019 MMR-defect tumors, which showed a partial response in 53% and complete responses in 21% of the cases [6]. However, as SCCs of the lower gastrointestinal tract are less frequent, it is

**Fig. 3** Radiological evaluation of a mismatch repair-deficient anorectal squamous cell carcinoma, with significant tumor shrinkage upon pembrolizumab-based immunotherapy. Imaging from before the initiation of pembrolizumab (**a**) and that following 4 cycles of pembrolizumab-based treatment (**b**) were shown. Arrows indicate tumor



unknown whether evaluation of MMR defects should be routinely performed and whether immunotherapy is a viable option.

Our review of the literature identified only one MMR-deficient anal SCC (MLH1-absent; MSH2-intact), diagnosed from recurrent anal fistula [11]. This tumor was successively resected with no post-surgical recurrence reported [11]. Currently, reports of MSI/MMR evaluation in SCC tumors are rare and are mainly conducted in the field of head and neck cases of SCC (HNSCC) [12]. A recent investigation has reported complete tumor response to a PD-L1 inhibitor in MSI HNSCC [13]. Additionally, in a series of 66 analyzed cervical SCCs, 25.8% were MMR-deficient and with elevated PD-L1 expression, reinforcing a PD-1/PD-L1 strategy in the management of patients affected by cervical SCCs [14].

Our novel case illustrates the pivotal role of MMR/MSI testing in patient management and therapeutic decision, as evidenced by the key therapeutic adjustment made with improved status in this patient with an advanced anorectal SCC. It further indicates that although MMR deficiency or MSI testing is not routinely performed in cases of gastrointestinal tract SCC, pathologists and oncologists need to consider MMR/MSI status when managing such patients, especially those with advanced tumors.

It is noteworthy that there is no consensus currently on MSI/MMR testing in gastrointestinal SCCs, let alone analyzing MSI/MMR regularly. Nonetheless, for patients with advanced SCCs, MSI/MMR analysis and potential immunotherapy should be considered, especially for those with limited therapeutic options or when conventional treatment strategies have failed. It is important to take advantage of the immunohistochemical and molecular tools to analyze MMR/MSI status and to adopt the immunotherapeutic strategies for patient management.

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**Author's contributions** Dr. Jiang conceived and designed the study, and wrote, edited, and reviewed the manuscript. Drs. Martens, Meyer, Truong, and Lauwers researched the data and edited and reviewed the manuscript. All authors gave the final approval for publication. Dr. Jiang takes full responsibility for the work, including the study design, access to data, and the decision to submit and publish the manuscript.

## Compliance with ethical standards

This is a brief report on a novel and unique case of diagnostic interest; it is not a research project; there is no Human or Animal participant(s). Informed consent was acquired although no confidential personal information was cited or disclosed in this brief report.

**Conflict of interest** The authors declare that they have no conflict of interest.

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