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Digging into phenotype change in mismatch repair deficient endometrial carcinoma and treatment with immune checkpoint inhibition, a case report

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1. Introduction

Endometrial carcinoma (EC) is the second most common gynaecologic cancer worldwide, when both developed and developing countries are considered (Sung et al., 2021). In addition, EC represents the solid tumour with the highest prevalence of deficient mismatch repair (dMMR), and subsequent high levels of microsatellite instability (MSI-H), according to the 2013 *The Cancer Genome Atlas* (TCGA) research network. Mismatch repair deficiency occurs in up to 31.4 % of EC, being more common in the endometrioid subtype (Bonneville et al., 2017). All patients with EC should undergo MSI/MMR testing as per guideline-based recommendations (Colombo et al., 2016), not only to identify genetic predisposition in the context of Lynch syndrome but also as a prognostic and predictive biomarker (León-Castillo et al., 2020; Le et al., 2017; Dudley et al., 2016). In fact, the dMMR status may be used to select patients that benefit from immune checkpoint blockade inhibitor (ICI) monotherapy.

Treatment options in recurrent EC are limited, and response rates to single agent chemotherapy are poor. Three landmark studies using ICI proved clinical efficacy in this setting. In patients with MSI/dMMR EC, both pembrolizumab and dostarlimab are valid treatment options after progression to first line chemotherapy (Marabelle et al., 2020; Oaknin et al., 2020). The phase II KEYNOTE-158 study (NCT02628067) evaluated pembrolizumab in dMMR non-colorectal carcinoma, including a large cohort of EC patients and demonstrating an objective responsive rate (ORR) of 57.1 % (Marabelle et al., 2020). Similarly, the phase I/II GARNET trial (NCT02715284) explored the role of dostarlimab in the same setting (Oaknin et al., 2020). The study showed an ORR of 45.5 % (Tinker, 2022). The phase III KEYNOTE-775 trial (NCT03517449) included both proficient MMR (pMMR) (n = 411) and dMMR (n = 65) patients with EC, who were randomly assigned to receive pembrolizumab and lenvatinib or single agent chemotherapy (Makker et al., 2022). The study showed a significant longer progression-free survival (PFS) and overall survival (OS) favouring the pembrolizumab and lenvatinib arm in the intention to treat population.

Here, we describe a case report of a patient with recurrent endometrioid EC initially classified as pMMR which proved to be a false negative and review the literature on the issue.

1.1. Informed consent statement

A written informed consent was obtained from the patient for publication of this case report and accompanying images.

2. Case report

A 60-year-old female with a prior medical history of hypertension was diagnosed with locally advanced grade 1 endometrioid endometrial

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adenocarcinoma in January 2021. She underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy, with no visible residual disease. Pathological examination of the surgical specimen confirmed a grade 1 endometrioid adenocarcinoma with total uterine wall invasion and negative margins. Parametria, vagina and ovaries were negative for malignancy; however, two lymph nodes (LN) were positive for malignancy (right common iliac and right retrocural LN). The final diagnosis was a FIGO stage IIIC1 (pT2N1) endometrioid EC. Immunohistochemistry (IHC) of the surgical tumour sample revealed intact expression of MMR proteins, including MSH2, MSH6, PMS2 and MLH1; positivity for PAX8, hormone receptors (HR), p53 wild-type pattern, negativity for p16 and WT1 and a proliferative index Ki67 of 60 %.

The patient received adjuvant treatment, consisting of four cycles of carboplatin AUC 5 and paclitaxel 175 mg/m^2 as well as external beam radiotherapy. At completion of the treatment, during the brachytherapy planification, a local recurrence at the vagina was suspected. Several biopsies were performed, that were negative for malignancy.

Three months after the completion of adjuvant therapy, an extensive pelvic recurrence was confirmed (Figs. 1 and 2), with extension to the right perirenal fascia. Pembrolizumab and lenvatinib combination was requested as compassionate access medication but it was denied. Subsenquently, the patient requested a second opinion at our institution. As a complication of such local relapse, she presented with a vagino-rectal fistula and bilateral hydronephrosis that required percutaneous nephrostomy placement. Clinically, the patient presented symptoms of vaginal bleeding and pelvic pain, experiencing a deterioration in her performing status (2 points in the Eastern Cooperative Oncologic Group scale). Surgical salvage or local radiotherapy were dismissed. A vaginal biopsy was repeated, which was compatible with recurrent grade 2 endometrioid carcinoma. The IHC of the pathology sample at recurrence showed HR expression and p53 wild type pattern, with a lack of expression of two MMR proteins (MLH-1 and PMS-2), with MLH1 promoter hypermethylation (Fig. 3). The *POLE* mutational status was negative.

With the diagnosis of recurrent EC, not amenable to local therapy, and a suspected switch in the MMR protein status, ICI monotherapy based on dostarlimab was initiated as part of an expanded access program. The patient exhibited a rapid response to treatment both clinical and radiological. After six cycles of dostarlimab the physical examination showed a complete response (Fig. 1) with radiological response

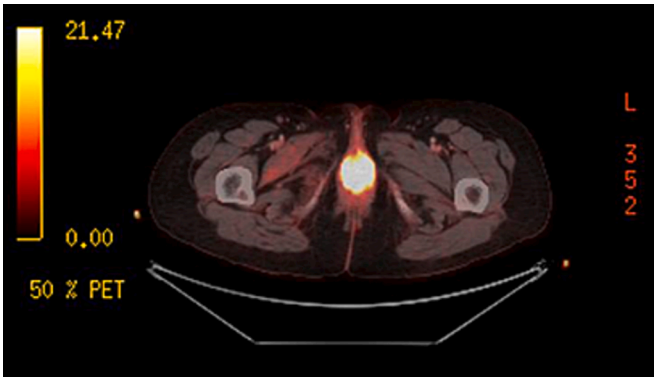

Fig. 2. FDG-PET/CT showing local recurrence (on the left) and pathologic right perirenal adenopathies (on the right).

enduring 18 months after initiation of therapy.

During treatment course, the initial surgical sample was requested for review at our centre as an internal quality control and given the MMR status discordance between the former and the relapse sample. The initial sample also showed dMMR concluding that it was a false negative case (Fig. 4).

3. Discussion

Immune checkpoint blockade inhibitors have transformed the treatment landscape for multiple solid and hematologic malignancies, including EC. There is strong evidence showing that immune cells and cytokines in EC tissue stimulate endogenous anti-tumour immune response (Cao et al., 2021). Moreover, immunotherapy has proved efficacy not only in DNA repair-deficient tumours but also in EC with intact DNA repair pathways (Tinker, 2022; Makker et al., 2022; Mittica et al., 2017). Hence, the large number of clinical trials examining the efficacy of ICI in monotherapy or combination with other agents, especially in the metastatic scenario.

The 2013 TCGA molecular classification provided a better understanding of the biology of EC, but also detected clinical actionability of several biomarkers that proved utility on daily practice (Colombo et al., 2016). Four genomic subtypes were defined: POLE/ultramutated (7 %),

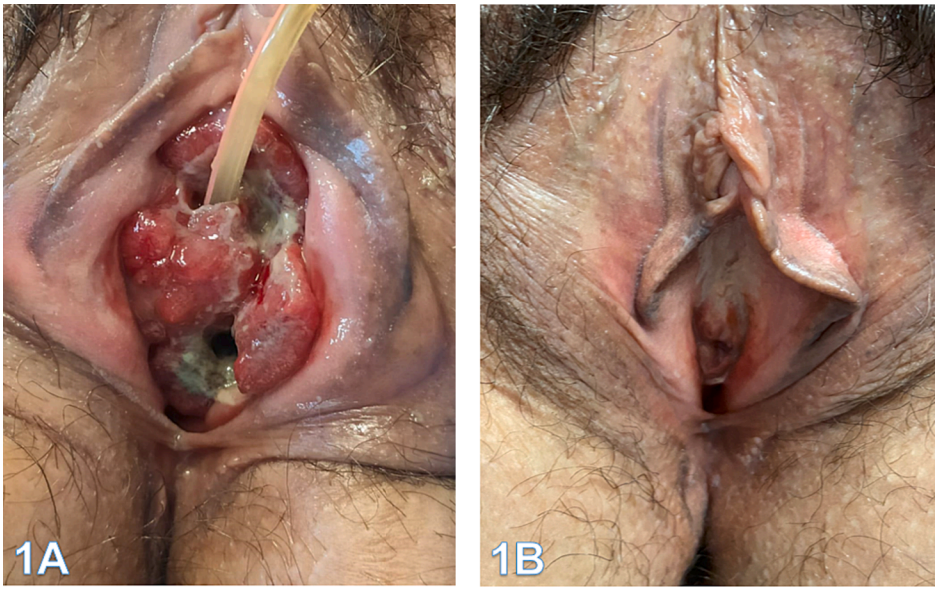

Fig. 1. Vaginal recurrence with mass prolapse.
Complete clinical response after treatment initiation.

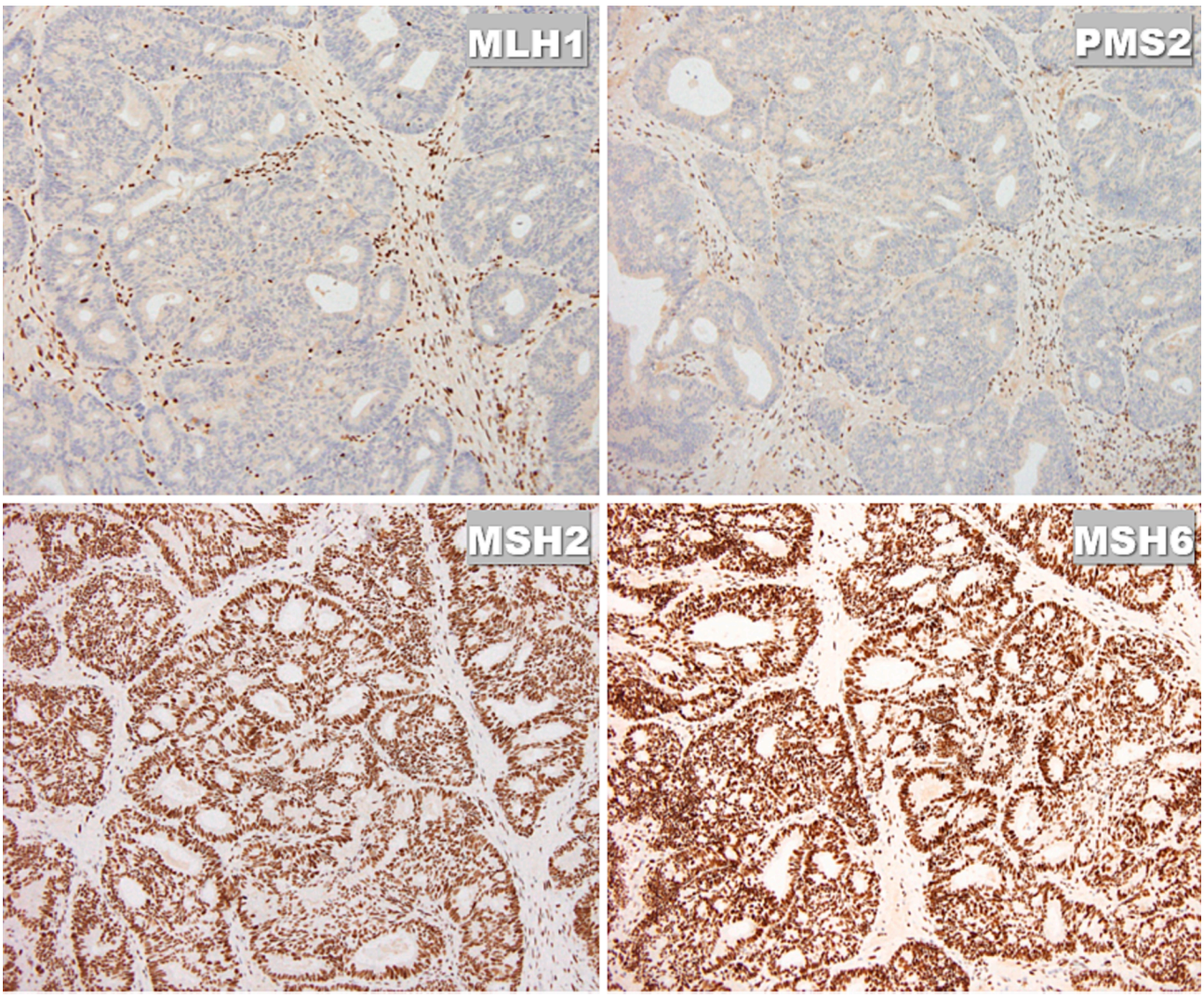

Fig. 3. Vaginal recurrence specimen. Immunohistochemical staining showing null-phenotype for the four main proteins involved in mismatch repair (MLH1, PMS2, MSH2, MSH6).

MSI/hypermutated (28 %), copy-number low (39 %), and copy-number high (26 %). These four categories have distinct clinical, pathologic and molecular features. Among them, it is known that POLE ultramutated and MSI-hypermutated are more immunogenic as they express a high number of neo-antigens and an elevated amount of tumour infiltrating lymphocytes (TILs) providing the rationale for a potential activity of ICI (Mittica et al., 2017). According to the new 2023 FIGO staging and ESMO Clinical Practice guidelines, the performance of complete molecular classification is encouraged in all cases of EC for prognostic risk-group stratification and as potential influencing factors of adjuvant or systemic treatment decisions (Berek et al., 2023; Oaknin et al., 2022). However, not all laboratories are currently able to carry out this molecular classification. Several studies have demonstrated that surrogate markers such as IHC techniques can be utilized for a TCGA-inspired molecular classification in routine surgical pathology, without the need for extensive sequencing.

Placing the focus on MMR, it is important to highlight its clinical relevance, as it is considered both a prognostic and predictive biomarker (Marabelle et al., 2020). MMR expression in EC expands beyond Lynch syndrome as it has role as a predictive marker for ICI response. Immunohistochemical assessment of MMR proteins is used as a reliable surrogate of MSI, corresponding to the hypermutated subtype which is known to be responsive to ICI (Buza et al., 2016). Well-established IHC staining for MMR proteins is an affordable technique, recommended as standard practice highlighting the continued importance of IHC techniques.

The case illustrates the importance of pathological review. Despite IHC being the reference technique for MMR protein assessment, false negative results may occur in up to 8 % of the cases due to fixation artefacts or unawareness of unusual staining patterns (Buza et al., 2016). While some tumours show uniform and widespread loss of MMR protein expression, cases with subclonal loss of MMR protein expression can be observed. Such cases present with two populations of tumour cells; one with retained expression, and another with abrupt and complete regional loss of MMR protein expression. Studies identifying the frequency of such staining patterns in large patient series are sparse. Secondly, MMR expression may change over the course of the disease, with potential discordancy in the MMR status between the primary tumour and tissue at recurrence. Retesting for MMR at relapse has not been considered standard practice; however, recent research shows that clonal evolution can lead to a change in MMR phenotype. Spinosa et al reported that retesting for dMMR at recurrence should be considered in EC patients (Spinosa et al., 2022). By studying matched specimens, both at diagnosis and relapse, they observed three patients had a dMMR tumour at recurrence, not reported previously (9 %, 3/29). Further research will be needed to assess the frequency of the MMR phenotype change in the disease evolution and its potential therapeutic implications.

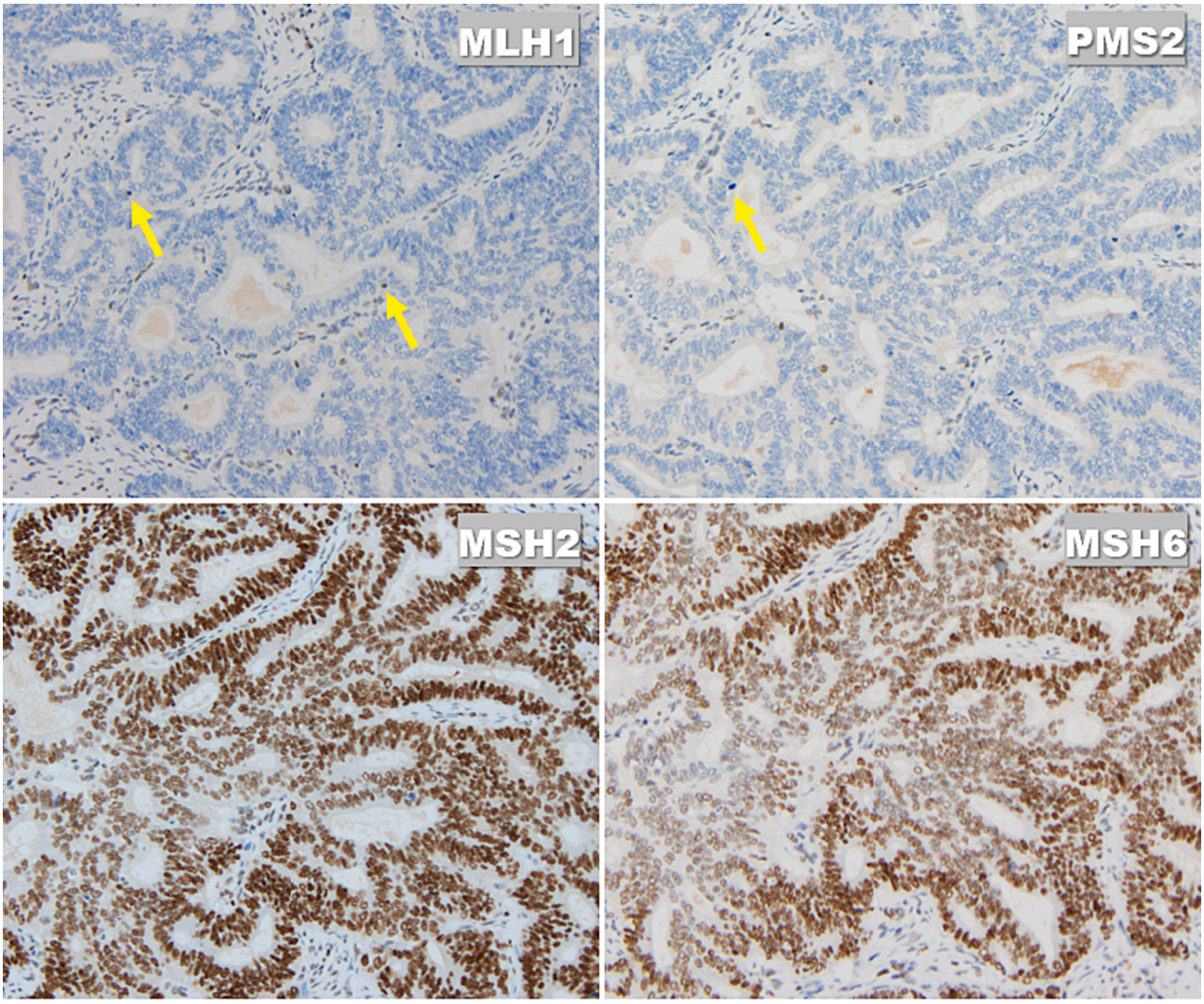

Fig. 4. Initial surgical sample. Immunohistochemical staining shows a loss of homogenous MMR protein expression. Yellow arrows show positive MMR protein staining in isolated stromal and immune cells, intermingled with tumour cells, which may raise doubts.

The second key question is whether mechanisms underlying dMMR alter responses to ICIs. EC with dMMR could be classified in three subgroups: Lynch syndrome (germline mutations in MMR genes), Lynch-like cases (somatic mutations in MMR genes), and sporadic cases with MLH1 promoter hypermethylation (MLH1-PHM). The latter, which also corresponds to the case of our patient, may predict long-term poorer prognosis as it is likely to influence tumour differentiation and progression (Kaneko et al., 2021). The impact of the molecular pathway underlying the dMMR phenotype has been explored in small studies. Bellone et al developed a phase 2 trial (NCT02899793) evaluating pembrolizumab for recurrent Lynch-like and sporadic EC with MSI. They observed a response advantage for Lynch-like tumours vs sporadic MSI EC (ORR 100 % vs 44 %) (Bellone et al., 2022). Similarly, a retrospective study showed a correlation between MLH1-PHM and poor response to pembrolizumab in recurrent EC (Borden, 2022). In contrast, in a post-hoc analysis assessing ORR by MMR status of the GARNET trial, the underlying mechanism of dMMR did not influence response to dostarlimab (Tinker et al., 2022).

At the time of choosing the therapeutic strategies, it is important to note the differences in the toxicity profile of drugs. High discontinuation rates and treatment emergent grade $\geq$3 adverse events have been reported in patients who receive ICI and tyrosine kinase inhibition combination, with 33 % of discontinuations with pembrolizumab and/or lenvatinib (Makker et al., 2022). The toxicity profile of single agent ICI is more manageable. Studies assessing single agent pembrolizumab and dostarlimab in EC, showed discontinuation rates of 9.4 % and 8.5 %, respectively (Marabelle et al., 2020; Oaknin et al., 2020). At such, molecularly driven selection of treatment with single agent ICI is generally considered in dMMR EC.

In conclusion, EC has a relatively high proportion of dMMR tumours providing rationale for molecularly selected therapy. Contemporary therapeutic strategies with ICI monotherapy provide durable responses with a manageable adverse event profile in dMMR EC. Further research assessing dMMR phenotypes and the role of retesting at relapse is needed to continue paving the treatment pathway.

CRediT authorship contribution statement

Macarena Rey-Cárdenas: Writing – original draft, Writing – review & editing, Resources. **Lucia Parrilla-Rubio:** Resources. **Luis Manso:** Validation. **Rodrigo Sanchez-Bayona:** Validation. **Carmen Alvarez-Conejo:** Validation. **Ainhoa Madariaga:** Conceptualization, Supervision, Validation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Rey-Cárdenas M. has received travel grants from Pfizer, Pierre Fabre, Ipsen, BMS, AstraZeneca, MSD, Roche, Kyowa Kirin and Accord. Parrilla-Rubio L and Alvarez-Conejo C. have no conflicts of interest. Manso L. reports honoraria and advisory/consultancy, travel/accommodation/expenses, and speaker's bureau from GSK GlaxoSmithKline, outside the submitted work. Sanchez-Bayona R. has received travel grants from Pfizer, Astra Zeneca, and Novartis, and honoraria for speaker or advisory board participation from Novartis, Lilly, Astra Zeneca, Daiichi Sankyo, Roche, Glaxo Smith Kline, Clovis Oncology, Seagen, and Accord. Non-financial interests: European Society of Medical Oncology Young Oncologists Committee member, Spanish Society of Medical Oncology – Scientific Secretary. Madariaga A. has received honoraria from Astra Zeneca, GlaxoSmithKline, MSD, Clovis and PharmaMar.].

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