Gynecologic Oncology 153 (2019) 471–478

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Gynecologic Oncology Tumor Board Presentation

Diagnosis and management of a recurrent polymerase-epsilon (*POLE*)-mutated endometrial cancer

Jennifer Taylor Veneris [a,h,*], Elizabeth K. Lee [b], Emily A. Goebel [c], Marisa R. Nucci [c,h], Neal Lindeman [d,h], Neil S. Horowitz [e,h], Larissa Lee [f,h], Chandrajit P. Raut [g,h], David Crotzer [i], Ursula Matulonis [a,h], Panagiotis A. Konstantinopoulos [a,h,1], Susana Campos [a,h,1]

[a] Division of Gynecologic Oncology, Dana-Farber Cancer Institute, Boston, MA, United States of America
[b] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, United States of America
[c] Division of Women's and Perinatal Pathology, Department of Pathology, Brigham and Women's Hospital, Boston, MA, United States of America
[d] Department of Pathology, Brigham and Women's Hospital, Boston, MA, United States of America
[e] Gynecologic Oncology, Brigham and Women's Hospital and Dana-Farber Cancer Institute, Boston, MA, United States of America
[f] Radiation Oncology, Brigham and Women's Hospital, Boston, MA, United States of America
[g] Division of Surgical Oncology, Department of Surgery, Brigham and Women's Hospital and Dana-Farber Cancer Institute, Boston, MA, United States
[h] Harvard Medical School, Boston, MA, United States of America
[i] Gynecologic Oncology, Nebraska Methodist Hospital, Omaha, NE, United States of America

HIGHLIGHTS

- *POLE* mutations occur in 7–12% of endometrial cancers and are identified by molecular analysis.
- *POLE*-mutated endometrial cancers have high tumor mutation burden, tumor neoantigen production, and tumor infiltrating T cells.
- This case illustrates a marked response to immune checkpoint inhibition in a POLE-mutated endometrial cancer.

ARTICLE INFO

Available online 29 March 2019

ABSTRACT

Polymerase-epsilon (*POLE*)-mutated carcinomas are a rare, but well-known subtype of endometrial cancer. While typically associated with good prognosis, recurrences are documented. Here we present a case of recurrent *POLE*-mutated endometrial cancer, discuss pathologic features, current methods of molecular classification, and explore therapeutic implications for the *POLE*-mutation phenotype.

1. Introduction

A 49-year-old premenopausal woman initially presented to her gynecologist's office with intermenstrual bleeding.

An endometrial biopsy was done which demonstrated a mixed high grade-adenocarcinoma consistent with a diagnosis of endometrial cancer. She underwent complete surgical staging including hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and bilateral pelvic and para-aortic lymphadenectomy.

Initial pathology review from her staging surgery demonstrated a primary uterine tumor, characterized as a high-grade mixed carcinoma with serous, clear cell, and endometrioid components. Pathology was also reviewed after referral to our center. Internal pathology review reported the primary tumor specimen most consistent with a grade 2 endometrioid adenocarcinoma, supported by the presence of wild type p53 staining pattern (Fig. 1). The tumor invaded 75% of the myometrial thickness. Cervical stromal invasion and lymphovascular invasion were present. The tumor was metastatic to the right ovary, omentum, and pelvic lymph nodes contained micrometastases. At the conclusion of the case there was no evidence of gross residual disease. Microsatellite testing by polymerase chain reaction (PCR) did not demonstrate instability, and expression of mismatch repair (MMR) proteins was intact by immunohistochemistry (IHC). A diagnosis was made of International Federation of Gynecology and Obstetrics (FIGO) IVB endometrioid endometrial carcinoma.

She was treated for advanced stage endometrial cancer with adjuvant carboplatin and paclitaxel, for a total of six cycles.

Approximately two years later, the patient began experiencing progressive nausea and emesis. Radiographic imaging with PET/CT

* Corresponding author at: Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, United States of America.
E-mail address: Jennifer_Veneris@dfci.harvard.edu (J.T. Veneris).
[1] Co-last authors.

https://doi.org/10.1016/j.ygyno.2019.03.247
0090-8258/

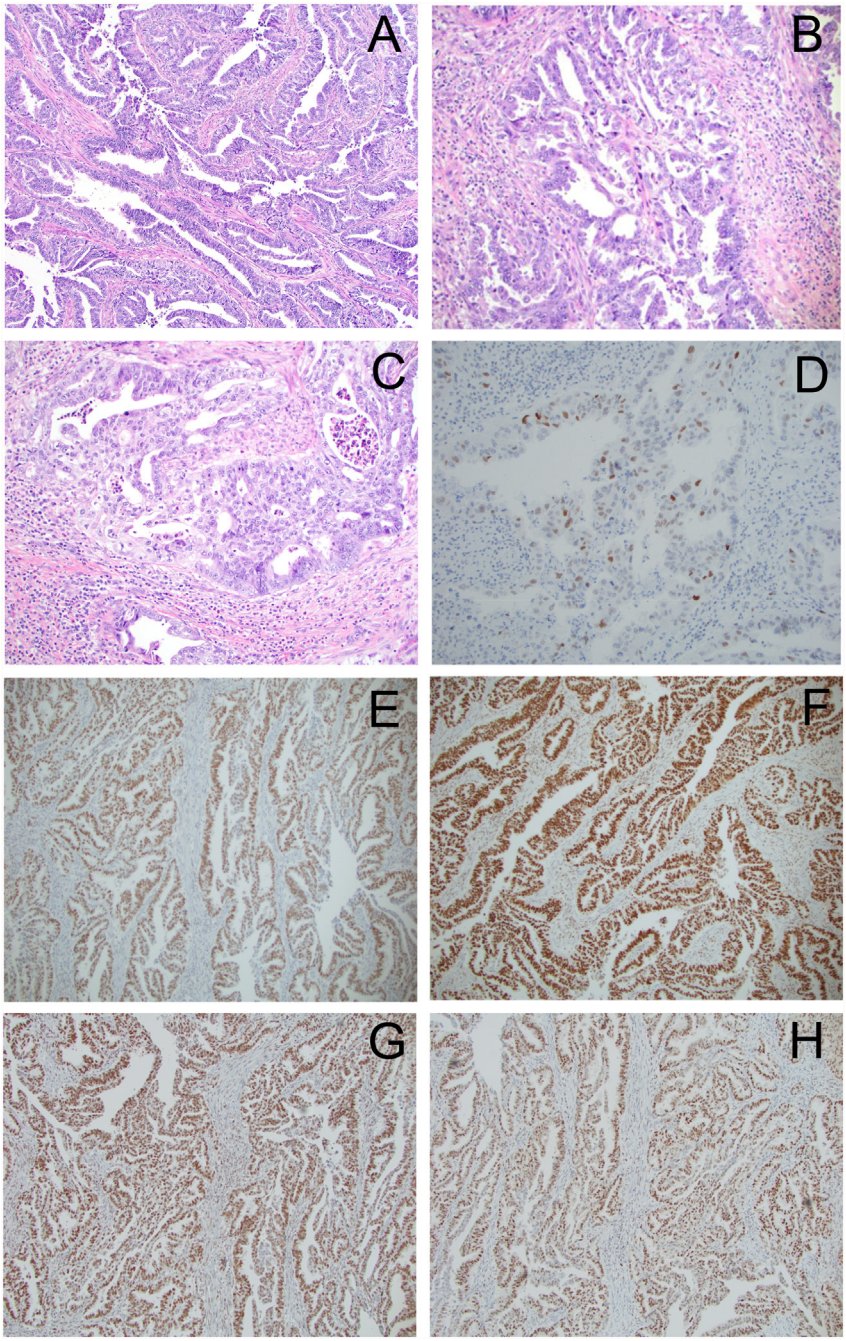

Fig. 1. Primary endometrioid endometrial adenocarcinoma, A. low power magnification showing glandular architecture (H&E, 100×); B. area resembling serous morphology with surrounding lymphocytes in the stroma (H&E, 200×); C. area with typical endometrioid morphology (H&E, 200×); D. immunohistochemical staining of p53 showing patchy, weak, heterogeneous staining, consistent with a wildtype pattern (200×); E–H: immunohistochemical staining of mismatch repair proteins showing retention of nuclear staining, E. MLH1, F. PMS2, G. MSH2, H. MSH6 (all 100×).

demonstrated a large heterogeneous abdominal mass along the medial aspect of the stomach measuring 8.6 × 8.9 cm and a hypermetabolic lymph node in the left aspect of the retroperitoneum concerning for neoplastic disease. There was no other evidence of cancer. A biopsy of the abdominal mass was performed and demonstrated metastatic adenocarcinoma with morphology consistent with recurrent endometrial cancer.

The patient was then re-treated with intravenous carboplatin and paclitaxel chemotherapy. Her symptoms improved after three cycles and radiographic imaging demonstrated a decrease in size in the mass along the lesser curvature of the stomach to 3.2 cm. She received an additional three cycles of chemotherapy (total of six). Repeat CT scan approximately one month following completion of the chemotherapy regimen showed progression of her cancer with an 8 cm heterogeneous mass located in the lesser sac which invaded the body of the stomach and the left lateral segment of the liver. There was also compression and narrowing of the distal splenic vein.

At this time, she presented to our institution for therapeutic recommendations. She became increasingly symptomatic from the upper abdominal mass and underwent an exploratory laparotomy, resection of recurrent multifocal endometrial carcinoma, en bloc distal gastrectomy, subtotal distal pancreatectomy, splenectomy, transverse colectomy with primary anastomosis, resection of the left lateral segment of liver, Billroth II gastrojejunostomy, partial omentectomy and liver biopsy. At the conclusion of the case there was no evidence of gross

residual disease remaining. Pathology from the surgery demonstrated recurrent metastatic endometrial adenocarcinoma invading the omentum, stomach and pancreas. There was normal MMR protein expression by IHC in the recurrent specimen (Fig. 2).

Next generation sequencing using Dana-Farber Cancer Institute in-house OncoPanel test [1] was performed on the primary tumor specimen and the recurrent specimen. The primary tumor demonstrated an ultramutated phenotype with tumor mutation burden of 231 mutations/megabase in the primary specimen, and a *POLE* exon 13 hotspot mutation with a *POLE* mutational signature, consistent with a *POLE*-mutated endometrial cancer. The recurrent specimen obtained from the gastric mass demonstrated a further increase in tumor mutation burden to 305 mutations/megabase, persistence of the *POLE* exon 13 hotspot mutation, and *POLE* mutational signature. The results of the histologic and molecular pathology findings are summarized in Table 1.

2. Clinical and epidemiology characteristics of *POLE*-mutated endometrial cancers

Historically, endometrial cancers have been categorized into two groups based on their clinicopathologic features. Type I encompasses endometrioid tumors, usually favorable in prognosis and associated with hormone receptor positivity and estrogen excess. Type II predominantly includes serous carcinoma, as well as clear cell carcinomas, carcinosarcomas, and other histologies. This group is associated with a poorer prognosis, generally occurs in older women, is not associated

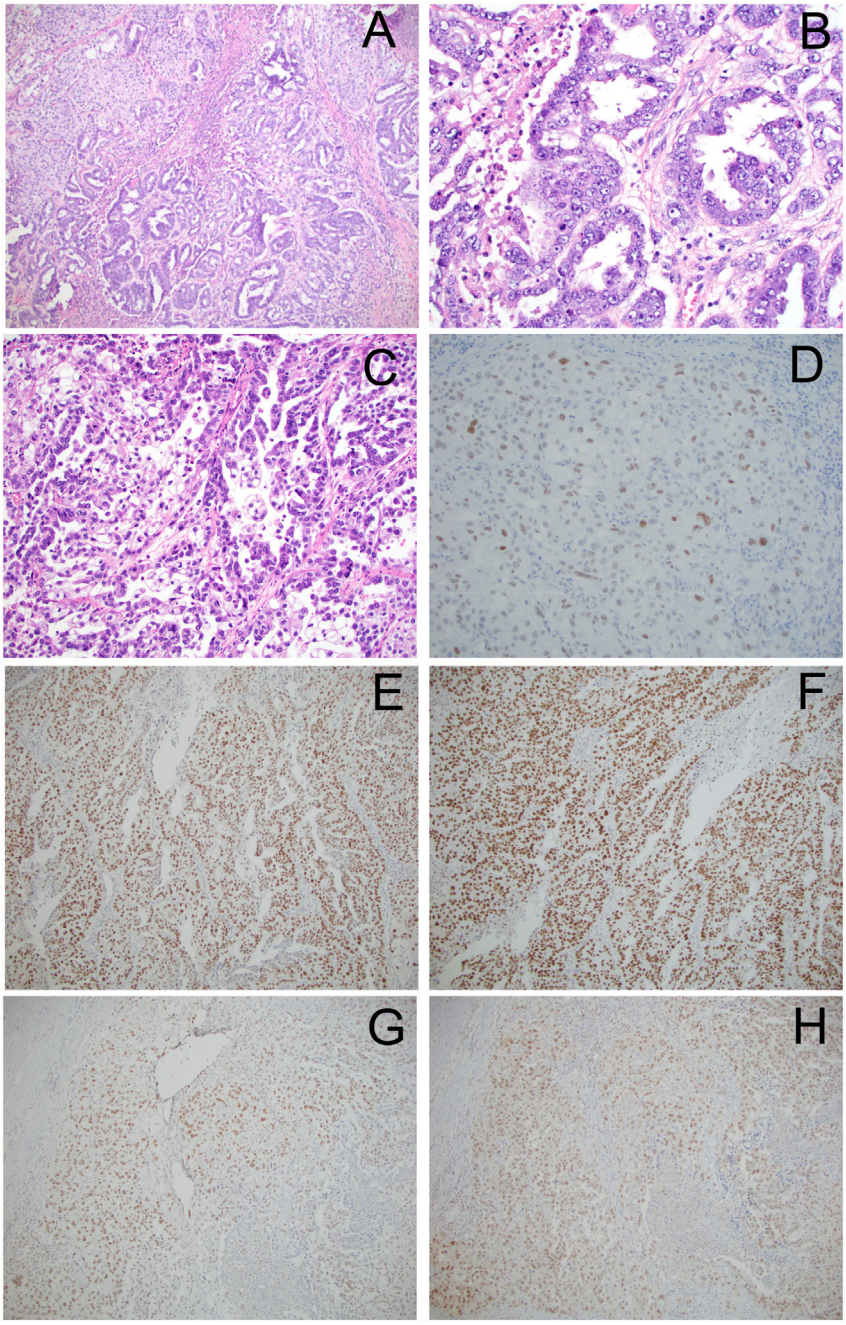

Fig. 2. Recurrent metastatic endometrial adenocarcinoma from the abdominal mass adjacent to the stomach, A. low power magnification showing a heterogeneous tumor with glandular and solid architecture (H&E, 100×), B. high grade cytomorphology of the glandular component (H&E, 400×); C. area resembling clear cell morphology (H&E, 200×); D. immunohistochemical staining of p53 in the tumor showing patchy, weak, heterogeneous staining, consistent with a wildtype pattern (200×); E–H: immunohistochemical staining of mismatch repair proteins showing retention of nuclear staining, E. MLH1, F. PMS2, G. MSH2, H. MSH6 (all 100×).

Table 1
Histologic and pathologic characteristics.

Pathological features	Primary tumor	Recurrence
Histology	Grade 2 endometrioid carcinoma	Metastatic high grade Mullerian carcinoma
Other features	Centered in lower endometrial cavity and lower uterine segment Deep myometrial and cervical stroma involvement LVSI+	
MSI/MMR (IHC)	MMR intact (MLH1, MSH2, MSH6, PMS2)	MMR intact (MLH1, MSH2, MSH6, PMS2)
MSI (PCR)	No MSI by PCR (0/5 loci)	n/d
Hormone status (IHC)	ER 18% (1+) PR 17% (1+)	n/d
P53 (IHC)	Wild type	Wild type
TMB (NGS)	231.165 (98th %ile of endometrial, 100th %ile for all DFCI OncoPanel cases)	305.685 (98th %ile endometrial, 100th %ile for all DFCI OncoPanel cases)
Mutational signature (NGS)	POLE signature MMR deficiency signature	POLE signature MMR deficiency signature
Selected variants (NGS)	*POLE* c.1231G>T (V411L), exon 13 hotspot mutation *POLE* c.6391C>T (p.R2131C), VUS *POLE* c.5378+2T>G, VUS *IDH1* c.394C>T (p.R132C) *MSH6* c.3202C>T (R1068*) *PIK3CA* c.263G>A (p.R88Q)	*POLE* c.1231G>T (V411L), exon 13 hotspot mutation *POLE* c.6391C>T (p.R2131C), VUS *POLE* c.5378+2T>G, VUS *IDH1* c.394C>T (p.R132C) *MSH6* c.3202C>T (R1068*) *PIK3CA* c.263G>A (p.R88Q)

with estrogen excess, and is considered high-risk for recurrence. In current guidelines from the National Comprehensive Cancer Network (NCCN v2.2018), this histologic grouping serves as a branchpoint in choosing appropriate frontline therapy.

In 2013, a large scale analysis by the Cancer Genome Atlas (TCGA) project, proposed a new genomic/molecular classification for endometrial cancer. Multiplatform genomic analysis of endometrial and serous endometrial cancers identified four distinct clusters based on nucleotide substitution frequencies and patterns: *POLE*-mutated (ultramutated), microsatellite instability-high (MSI-high, hypermutated), copy-number (CN) low (endometrioid-like), and CN high (serous-like) [2]. In the *POLE*-mutated subgroups, hotspot *POLE* mutations were identified at Pro286Arg (p.P286R) and Val411Leu (p.V411L), which lie in the exonuclease domain. The MSI-high group consisted of tumors with MMR deficiency, which can develop as a result of somatic *MLH1* promoter hypermethylation or inherited mutations in MMR proteins (i.e. Lynch syndrome). The CN-low (endometrioid) group demonstrated a high frequency of *CTNNB1* mutations (52%) and *PTEN* mutations, as well as increased progesterone receptor expression suggesting a basis for hormone responsiveness. The CN-high (serous-like) group harbored greater frequencies of mutations in *CCNE1*, *PIK3CA*, *MYC*, *CDKN2A*, and *TP53*, reflecting cell cycle dysregulation. These four genomic groups were shown to have prognostic significance, wherein *POLE*-mutated tumors were associated with significantly improved progression free survival (PFS), and CN-high tumors were associated with worse PFS. Notably, this seminal study did not include or address the hierarchy of genomic parameters in tumors that harbor more than one genomic alteration (e.g. concurrent *POLE* mutation and *TP53* mutation). Additionally, all cancers were from initial diagnosis. Subsequent studies have found a *POLE* mutation frequency of 5–12% in endometrial cancers, and have generally recapitulated the favorable prognosis associated with this genomic alteration [3–7].

3. Polymerase-epsilon (POLE) mechanism of action

The *POLE* gene encodes the major catalytic subunit of DNA polymerase-ε, which, with polymerase-δ, synthesizes the leading and lagging strands during DNA replication in eukaryotes [8]. Polymerase-ε is thought to function in leading strand synthesis, though there is conflicting data as to whether polymerase-δ synthesizes both leading and lagging strands and polymerase-ε serves only in repair and proofreading [9,10]. What is agreed upon is that mutations in the exonuclease domain of polymerase-ε may compromise the 3′-to-5′ proofreading function, leading to loss of replication fidelity, development of genomic instability, and consequently an ultra-mutated phenotype. The effects of individual mutations on exonuclease activity are likely to be distinct [11]. Certain mutations in exon 13, which harbors the exonuclease domain, can generate more than 8000 neoepitopes [12], fostering and resulting in a highly immunogenic microenvironment as reflected by increased numbers of peri-tumoral and tumor-infiltrating lymphocytes (TILs) and concomitant elevated PD-1 or PD-L1 expression [13]. *POLE*-mutated tumors demonstrate a high frequency of C-to-A transversions [2]. In addition to endometrial cancers, somatic *POLE* mutations have also been found in colorectal cancers, and rarely in breast, pancreatic, gastric, bladder, and CNS tumors [14].

Several hypotheses have been proposed to explain why *POLE*-mutated endometrial cancers are prognostically favorable. As will be discussed in subsequent sections, a leading hypothesis is that the extremely high mutational burden and neoepitope landscape may generate a robust immune response. A less favored hypothesis is that the inherently defective DNA repair resulting from *POLE* mutations may render tumor cells more susceptible to standardly used chemotherapy, though in vitro data from patient-derived cell lines harboring *POLE* mutations actually demonstrated platinum resistance rather than susceptibility [13]. Another less favored hypothesis is that the numerous mutations may prove overall deleterious to the tumor cell, rendering it less capable of proliferation or metastasis, however *POLE*-mutated tumors have presented as widespread disease and can recur [15].

4. Pathology

Figs. 1 and 2 demonstrate histologic features of the primary tumor and the tumor recurrence.

At initial diagnosis, the patient had a primary uterine grade 2 endometrioid adenocarcinoma, with an increase from grade 1 to 2 based on cytologic atypia. The majority of the tumor was compromised of glandular areas with features resembling serous histology including irregular luminal borders, cell tufting and high nuclear grade (Fig. 1, A and B). IHC staining for p53 showed a wildtype pattern, supporting endometrioid type (Fig. 1, D). A minor component resembled a more classical endometrioid morphology with cribriform glands and smooth luminal borders with squamous differentiation (Fig. 1, C). There were scattered lymphocytes within the tumor. Tumor infiltrating lymphocytes and peritumoral lymphocytes were not brisk (Fig. 1, B and C). IHC staining of the tumor showed retention of nuclear expression of MMR proteins MLH1, PMS2, MSH2, and MSH6 (Fig. 1, E–H).

In the recurrent specimen, the metastatic endometrial carcinoma was identified at all metastatic sites, involving the liver, stomach, pancreas, ligament of Treitz, and one lymph node. The recurrent carcinoma was heterogeneous in appearance with glandular and solid architecture (Fig. 2, A). Some areas resembled more classical endometrioid adenocarcinoma, while others had high-grade cytomorphology (Fig. 2, B) and clear cell morphology (Fig. 2, C). IHC staining of the tumor showed a p53 wildtype staining pattern (Fig. 2, D) and retention of nuclear expression of MMR proteins (Fig. 2, E–H).

These complex histologic features and discordance between outside and internal pathology review highlight the difficulty in identifying *POLE* mutated cases on a purely histologic basis. High-grade endometrioid and serous carcinomas may be particularly difficult to distinguish histopathologically, and significant interobserver variability in diagnosis has been observed [16].

5. Molecular features of *POLE*-mutated tumors and mutational signature analysis

Next generation sequencing assays have become increasingly valuable in the era of molecular medicine, due in part, to the ability to identify genomic alterations beyond small insertions and deletions. In addition to somatic mutations, large structural rearrangements - which generate novel fusion proteins and copy number changes (i.e. gene amplifications or deletions) - can also be identified. Tumor mutational burden, i.e. the number of non-synonymous somatic mutations that occur per megabase of exonic sequence data across all the targeted genes on the panel, can also be calculated.

The OncoPanel assay developed at Dana-Farber Cancer Institute is a targeted next generation sequencing assay [1,17]. In addition to detecting somatic mutations, the OncoPanel assay also detects copy number variation, identifies structural DNA rearrangements, and performs mutational signature analysis. The current version of the OncoPanel test, and the version used for this patient's specimens, surveys exonic sequences in 447 genes implicated in cancer biology and 191 regions across 60 genes for rearrangement detection.

The mutational signature analysis is a unique aspect of this panel. The method of mutational signature analysis was initially developed for whole exome sequencing (WES) and has recently adapted for target sequencing panels. By analyzing the nucleotide substitutions across all sequenced exomes with pattern recognition software, samples can be classified by the pattern of the mutational changes [18,19]. Mutational signatures that can be detected with this approach include patterns of DNA damage associated with UV light exposure, tobacco exposure, alkylating agent exposure, dysregulation of apolipoprotein B mRNA editing enzyme (APOBEC), MMR deficiency, and impaired DNA *POLE* function. Each carcinogen or DNA repair defect leaves a characteristic pattern in the mode of alterations in the DNA. For example, MMR deficiency signature is detected by determining the number of small insertions and deletion events occurring in regions with microsatellite repeats within exonic sequence data across all genes on the panel [20]. The *POLE* signature on the other hand exhibits a strand bias for C-to-A mutations at TpCpT locations and T-to-G mutations in TpTpT genomic context. Currently 30 mutational signatures have been characterized; a list and descriptions can be obtained from the Catologue of Somatic Mutations in Cancer (COSMIC) database. A specimen is considered to harbor a mutational signature if the signature is detected, therefore it is possible for a specimen to have more than one (or none) signatures attributed.

POLE-mutated endometrial cancer has typically been associated with microsatellite stable phenotype, although occasional examples of co-occurrence of MMR deficiency and *POLE*-mutations have been reported. The presence of MMR deficiency, or MSI, is detected clinically by three methods: MMR protein IHC to detect loss of MMR protein expression, PCR at a panel of microsatellite loci in the genome to detect repetitions, or by observation of a MMR mutational signature in next generation sequencing tests. None of these methods is specific for the mechanism that resulted in MMR deficiency. If MMR deficiency is detected by any of these methods, additional testing for inherited Lynch syndrome is recommended.

Several mechanisms have been proposed for incidences of co-occurrence of MMR deficiency and *POLE*-mutated phenotypes. Individual *POLE* mutations may induce specific genomic mutation patterns. A recent report has characterized mutational signatures of 232 endometrial cancers and 57 carcinosarcomas with WES from the TCGA cases [11]. Among the 17 endometrial cancer patients in TCGA with *POLE* exonuclease domain mutations, 13 (76%) harbored a dominant COSMIC signature 10, associated with high numbers of somatic mutations (i.e. ultramutators) and *POLE* mutations. Of the remaining four cases, one had a dominant aging signature (COSMIC signature 1), one had a dominant MMR deficiency signature (COSMIC signature 6), and two had dominant COSMIC signature 14 which has also been associated with high somatic mutation burden [18], suggesting that specific *POLE* mutations may have specific influences on genomic integrity. The majority of cases were also classified as microsatellite stable (76%), and *POLE*-mutated endometrial cancers with a dominant *POLE* signature 10 were less likely to be classified as MSI-high compared to those with an alternate mutational signature (8% versus 75%). Another mechanism for co-occurrence of MMR deficiency and *POLE*-mutation has also been described, in which MSI is thought to occur from somatic inactivation of an MMR gene, consequent to a truncal *POLE* mutation [21–23]. Thus the type and interpretation of molecular testing impacts correct identification of *POLE*-mutated tumors.

In the case reported here, OncoPanel testing detected a *POLE* signature in the primary tumor, consistent with the ultra-mutated phenotype, and *POLE* exon 13 hotspot mutation (p.V411L). In addition, a mutational signature of MSI was also detected. MMR status by both IHC and PCR on the other hand demonstrated a microsatellite stable (MSS) pattern, suggesting that the MSI mutational signature here may be a consequence of the high mutational frequency and not true evidence of MSI. Interestingly, these molecular and histologic characteristics were retained in the metastatic specimen, where a *POLE* mutational signature was again identified in the recurrence, along with persistence of the *POLE* p.V411L mutation, the MSI mutational signature and retained expression of MMR proteins (Table 1). These findings are consistent with a microsatellite stable and *POLE*-mutated endometrial cancer.

6. Algorithms for molecular evaluation of endometrial cancer

Due to interobserver discordance and lack of specific histologic features, molecular characterization of endometrial cancer is necessary to identify *POLE*-mutated cases. This testing, however is not yet standard of care.

The identification of four genomically-distinct groups of endometrial carcinomas in the landmark TCGA study raises the question of how best to incorporate molecular markers into clinical practice. Several groups have since attempted to reproduce these subgroups using more clinically accessible techniques for molecular characterization [24–29]. Like the TCGA, these methods attempt to classify endometrial cancers into mutually exclusive categories based on molecular features. These algorithms largely differ in terms of the sequence and specific assays used for molecular classification. For example, in the transPORTEC analysis, p53 mutation status was assessed by p53 IHC, followed by *TP53* sequencing in cases with indeterminant IHC, followed by testing for MMR deficiency by MSI analysis, and then sequencing of *POLE* at exons 9 and 13 [24]. In contrast, the decision tree for the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) study differs in determining MMR IHC first, followed by *POLE* sequencing, and then p53 IHC [28]. Bosse et al. applied genomic subgroup classifications to high-grade (grade 3) endometrioid endometrial carcinomas [25]. *POLE* mutation status by Sanger sequencing of exons 9–14, MMR deficiency by IHC, and p53 mutation by IHC were used to classify four subgroups. Cases which could not be assigned due to ambiguity of a test or the presence of more than one classifier were excluded. Notably, grade 3 endometrioid endometrial carcinomas fell across all four prognostically-correlated molecular subgroups, further supporting that molecular alterations cannot be precisely predicted from histology alone.

While these molecular algorithms contribute to moving diagnosis beyond histology and have demonstrated prognostic significance, several issues remain unaddressed: how to appropriately sequence the order of classification, how to classify tumors that harbor multiple genomic alterations (e.g. concurrent *POLE* and *TP53* mutations, or concurrent MSI and *TP53*, both of which have been seen), and how to incorporate these molecular characterizations into clinical practice.

7. Management of *POLE*-mutated endometrial cancers and role for immunotherapy

The treatment implications of a *POLE*-mutation or ultramutated tumor have not yet been defined. Although there is evidence that these tumors are associated with early stage and low rate of recurrence, is this because they are often found early, or because they respond well to the adjuvant radiation regimens prescribed for high risk, early stage endometrial cancer? Without additional prospective data incorporating molecular classifications, it is not known if these tumors should be treated differently in the adjuvant setting, and can have adjuvant treatment safely withheld, as is the case for early stage MSI-H colorectal cancers [30].

Despite the generally favorable prognosis of *POLE*-mutated tumors, recurrences have been reported. The optimal treatment in the advanced and recurrent setting has not yet been established. Evidence is accumulating, however, that these tumors illicit an immune response and may be candidates for immune checkpoint inhibition. *POLE*-mutated cancers have been associated with high tumor mutation burden, neoantigen load, and the presence of tumor-infiltrating T-cells, which have been proposed the potential mechanisms for the favorable prognosis, and perhaps the basis of reported responses to immune checkpoint inhibition [12,31]. A report by van Gool et al. described the analysis of 150 endometrial cancers, and demonstrated that *POLE*-mutant endometrial cancers had increased lymphocytic infiltrate in comparison to *POLE*-wild type/MSI-high and *POLE*-wild type/MSS subgroups [31]. Expression of CD8 on tumor infiltrating lymphocytes was increased in *POLE*-mutant cases, and 60% of *POLE*-mutant cases had above average number of CD8+ T cells per high powered field. Analysis of the TCGA endometrial cancer cases further demonstrated increased expression of immune-activating cytokines in *POLE*-mutant cases compared to MSI and MSS cases, including expression of genes encoding CD8, IFNγ, Tbet, perforin and granzymes. Furthermore, *POLE*-mutated endometrial cancers demonstrated increased production of tumor neoantigens, suggesting a potential mechanism of immune system activation. Our group previously described in silico analysis of TCGA endometrial cancer cases using neoantigen prediction software. This work demonstrated that *POLE*-mutated endometrial cancers have 15-fold higher median neoantigen load per tumor compared to MSI cases, and 118-fold higher neoantigen load compared to MSS cases [12]. These findings support a model in which *POLE* mutation leads to genomic instability, which results in high mutational burden and production of abundant tumor neoantigens, which in turn leads to cytotoxic immune cell infiltration and activation.

The robust immune cell infiltration and cytokine gene expression signature suggest immune system activation in *POLE*-mutated endometrial cancers. Markers of immune evasion and exhaustion are also more abundant, however. Expression of LAG-3, TIM-3, PD-1, PD-L1 and CTLA4 genes is increased in *POLE*-mutant tumors compared to MSI and MSS cancers [31]. This is also supported by IHC data, which demonstrate increased intraepithelial and peritumoral PD-1 expression *POLE*-mutant and MSI tumors relative to MSS tumors [12]. *POLE*-mutated endometrial cancers are also significantly more immunogenic. In cell culture model systems, dendritic cells pulsed with tumor extracts from *POLE*-mutant tumors promoted abundant CD4+ and CD8+ T cell proliferation in comparison to extracts from wild type endometrial cancers [32].

There is active interest in the evaluating the use of PD-L1 expression as an additional biomarker for response to immune checkpoint inhibition in a variety of solid tumors, including endometrial cancer. Fig. 3 demonstrates PD-L1 expression in this patient's recurrent specimen. PD-L1 was negative in the tumor cells and positive in 10% of immune cells in the tumor microenvironment. This is consistent with prior reports in endometrial cancer demonstrating increased expression of PD-L1 in the stroma of sporadic MSI-high endometrial cancers [33], and in *POLE*-mutated cancers [12] compared to microsatellite stable (MSS) cases, and a generally low expression of PD-L1 in the tumor cells in all groups [12,33].

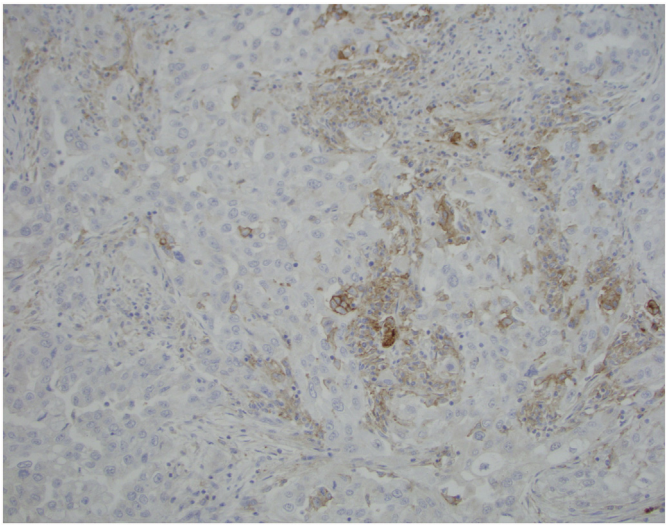

Fig. 3. PD-L1 immunohistochemical staining of the metastatic recurrent endometrial adenocarcinoma showing positivity in some of the infiltrating immune cells (10%) and negativity in the tumor cells (200×).

Several clinical trials have investigated the role of immune checkpoint inhibitors in endometrial cancers. In an unselected endometrial cancer population, the anti-PD-1 agent atezolizumab demonstrated a trend toward an association of stromal PD-L1 expression and response. Two of two patients in an endometrial cancer cohort who had a partial response to atezolizumab had tumors that exhibited PD-L1 expression on greater than 5% of immune cells [34]. In the endometrial cancer cohort of KEYNOTE-028, patients with PD-L1 positive endometrial cancers were enrolled, where PD-L1 positive was defined as staining in at least 1% of tumor and associated inflammatory cells or positive staining in the stroma. In this PD-L1 positive population, overall response rate was 13% [35]. These studies were not selected for MSI or *POLE* mutation status. Studies are ongoing to more clearly define the relationship between MSI and *POLE* status and response to immune checkpoint inhibition. An ongoing study of avelumab in a cohort of *POLE* and MSI mutated cancers and a second cohort of MSS endometrial cancer has shown preliminary response rates of 37.5% in the MSI/*POLE* cohort but only 5% in the MSS cohort [36]. Several cases of *POLE*-mutated cancers responding to immune checkpoint inhibition have also been reported in the literature [15,35,37].

Immune checkpoint inhibition may offer possibility for extreme and durable responses, however the appropriate timing for their incorporation into the treatment of these cancers is an open question.

8. Case resolution

Less than three months following secondary cytoreductive surgery, the patient experienced widespread multifocal recurrence of her cancer. Because next generation sequencing testing demonstrated *POLE* ultramutator phenotype, she was started on systemic treatment with the immune checkpoint inhibitor, pembrolizumab. She received three cycles, and underwent restaging imaging. Computed tomography imaging of the chest, abdomen and pelvis demonstrated a dramatic response, with a reduction in size of all lesions (Fig. 4). Multiple hepatic metastases have decreased in size with the largest 1.6 cm from 2.3 cm, perigastric mass now 1.9 cm from 7.4 cm, and decreased size of mesenteric nodules and small retroperitoneal lymph nodes. She has now received six cycles and continues on pembrolizumab. This robust response supports the hypothesis that somatic mutations in the *POLE* exonuclease domain generate genome-wide instability resulting in increased presentation of neoantigens, thus priming the tumor immune microenvironment to the effects of immune checkpoint inhibition. Furthermore,

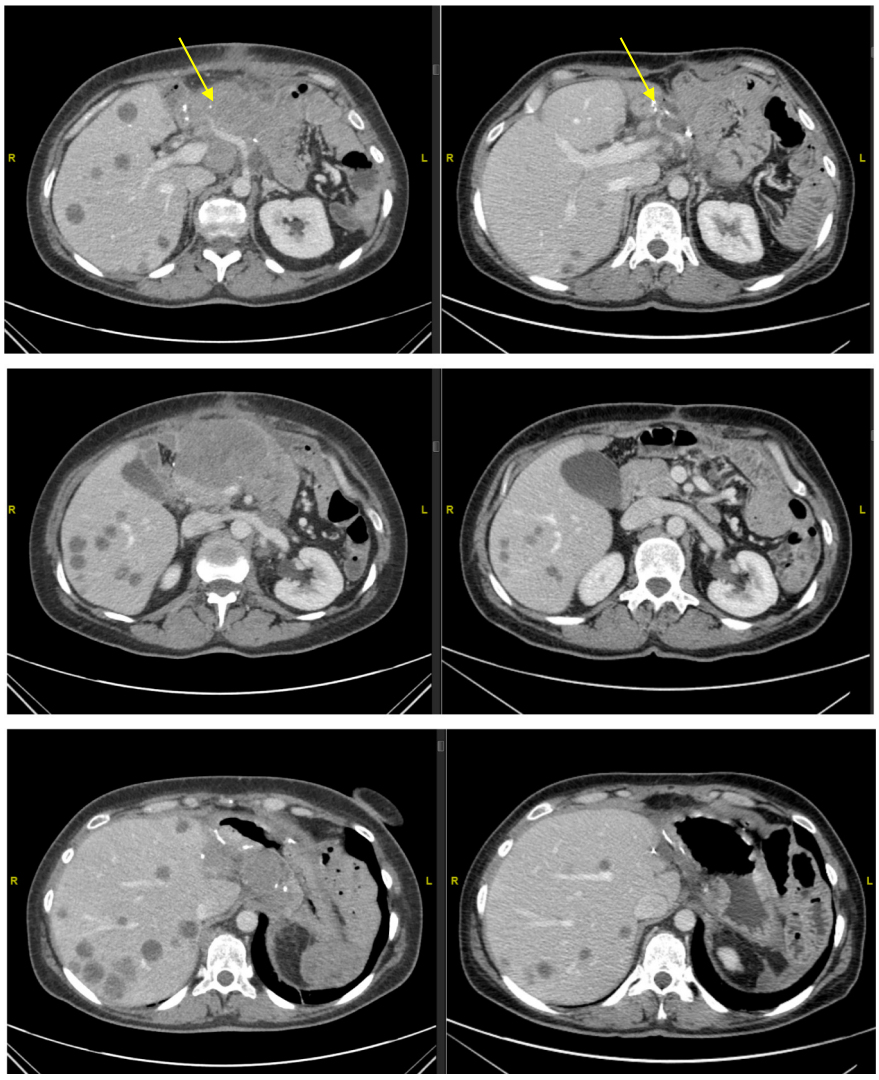

Fig. 4. Computed tomography (CT) scan of the chest, abdomen and pelvis at the time of second recurrence (left) and following three cycles of therapy with immune checkpoint inhibitor (right). The top panels demonstrate a large 7.4 cm mass in the central abdomen surrounding the superior mesenteric artery (yellow arrow), with marked shrinkage following immune checkpoint inhibition. The middle panels demonstrate a cross section of this mass at the level of the left renal vein with several hepatic metastases, all reduced in size following immune checkpoint inhibition. The inferior panels demonstrate a cross section from the level of the right and middle hepatic veins, which depicts several hepatic metastases, reduced in size in number following immune checkpoint inhibition.

these findings support the use of high tumor mutation burden, *POLE* mutation, and genomic *POLE* mutational signature as tools to predict response to immune checkpoint inhibitors, beyond MSI status. These findings support the use of immune checkpoint inhibitors in the treatment of patients with recurrent *POLE*-mutated endometrial cancer.

Author contributions

Conception and design: Jennifer Taylor Veneris, Panagiotis A. Konstantinopoulos, Susana Campos, Ursula Matulonis. Collection and assembly of data: Jennifer Taylor Veneris, Elizabeth K. Lee, Emily A. Goebel. Data analysis and interpretation: Jennifer Taylor Veneris, Neal Lindeman, Marisa R. Nucci, Neil S. Horowitz, Larissa Lee, Chandrajit Raut, David Crotzer, Ursula Matulonis. Manuscript writing: all authors. All authors have approved the final article.

Conflict of interest statement

Dr. Veneris reports spouse employment at Takeda. Dr. L. Lee reports non-financial support from AstraZeneca, personal fees from AstraZeneca, grants from Koch Institute at Massachusetts Institute of Technology and Dana-Farber Cancer Institute, outside the submitted work. Dr. Crotzer reports personal fees from AstraZeneca and Tesaro, outside the submitted work. Dr. Matulonis reports personal fees from AstraZeneca, Myriad Genetics, Clovis, Merck, Eli Lilly, Mersana, Geneos, Fuji Film, Ceruleean, Immunogen, and other from 2X Oncology, outside the submitted work. Dr. Konstantinopoulos reports other from AstraZeneca, Pfizer, Merck, and Vertex, outside the submitted work. Other authors have nothing to disclose.

Acknowledgement

The authors wish to acknowledge funding from the Breast Cancer Research Foundation (UAM, PAK).

References

[1] N. Wagle, et al., High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing, Cancer Discov 2 (1) (2012) 82–93.
[2] N. Cancer Genome Atlas Research, et al., Integrated genomic characterization of endometrial carcinoma, Nature 497 (7447) (2013) 67–73.
[3] D.N. Church, et al., Prognostic significance of POLE proofreading mutations in endometrial cancer, J. Natl. Cancer Inst. 107 (1) (2015) 402.
[4] J.M. Piulats, et al., Molecular approaches for classifying endometrial carcinoma, Gynecol. Oncol. 145 (1) (2017) 200–207.
[5] B. Meng, et al., POLE exonuclease domain mutation predicts long progression-free survival in grade 3 endometrioid carcinoma of the endometrium, Gynecol. Oncol. 134 (1) (2014) 15–19.

[6] E. Stelloo, et al., Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts, Clin. Cancer Res. 22 (16) (2016) 4215–4224.
[7] M.K. McConechy, et al., Endometrial carcinomas with POLE exonuclease domain mutations have a favorable prognosis, Clin. Cancer Res. 22 (12) (2016) 2865–2873.
[8] S.A. Lujan, J.S. Williams, T.A. Kunkel, DNA polymerases divide the labor of genome replication, Trends Cell Biol. 26 (9) (2016) 640–654.
[9] R.E. Johnson, et al., A major role of DNA polymerase delta in replication of both the leading and lagging DNA strands, Mol. Cell 59 (2) (2015) 163–175.
[10] E. Rayner, et al., A panoply of errors: polymerase proofreading domain mutations in cancer, Nat. Rev. Cancer 16 (2) (2016) 71–81.
[11] C.W. Ashley, et al., Analysis of mutational signatures in primary and metastatic endometrial cancer reveals distinct patterns of DNA repair defects and shifts during tumor progression, Gynecol. Oncol. 152 (1) (2019) 11–19.
[12] B.E. Howitt, et al., Association of polymerase e-mutated and microsatellite-instable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1, JAMA Oncol 1 (9) (2015) 1319–1323.
[13] S. Bellone, et al., Polymerase epsilon (POLE) ultra-mutation in uterine tumors correlates with T lymphocyte infiltration and increased resistance to platinum-based chemotherapy in vitro, Gynecol. Oncol. 144 (1) (2017) 146–152.
[14] E. Shinbrot, et al., Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication, Genome Res. 24 (11) (2014) 1740–1750.
[15] A.D. Santin, et al., Regression of chemotherapy-resistant polymerase epsilon (POLE) ultra-mutated and MSH6 hyper-mutated endometrial tumors with nivolumab, Clin. Cancer Res. 22 (23) (2016) 5682–5687.
[16] C.B. Gilks, E. Oliva, R.A. Soslow, Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma, Am. J. Surg. Pathol. 37 (6) (2013) 874–881.
[17] E.P. Garcia, et al., Validation of OncoPanel: a targeted next-generation sequencing assay for the detection of somatic variants in cancer, Arch Pathol Lab Med 141 (6) (2017) 751–758.
[18] L.B. Alexandrov, et al., Signatures of mutational processes in human cancer, Nature 500 (7463) (2013) 415–421.
[19] A. Zehir, et al., Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients, Nat. Med. 23 (6) (2017) 703–713.
[20] J.A. Nowak, et al., Detection of mismatch repair deficiency and microsatellite instability in colorectal adenocarcinoma by targeted next-generation sequencing, J Mol Diagn 19 (1) (2017) 84–91.
[21] P.A. Konstantinopoulos, U.A. Matulonis, POLE mutations as an alternative pathway for microsatellite instability in endometrial cancer: implications for Lynch syndrome testing, Cancer 121 (3) (2015) 331–334.
[22] C.C. Billingsley, et al., Polymerase varepsilon (POLE) mutations in endometrial cancer: clinical outcomes and implications for Lynch syndrome testing, Cancer 121 (3) (2015) 386–394.
[23] Campbell, B.B., et al., Comprehensive analysis of hypermutation in human cancer. Cell, 2017. 171(5): p. 1042–1056 e10.
[24] E. Stelloo, et al., Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative, Mod. Pathol. 28 (6) (2015) 836–844.
[25] T. Bosse, et al., Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups, Am. J. Surg. Pathol. 42 (5) (2018) 561–568.
[26] C.M. Cosgrove, et al., An NRG Oncology/GOG study of molecular classification for risk prediction in endometrioid endometrial cancer, Gynecol. Oncol. 148 (1) (2018) 174–180.
[27] R. Murali, et al., Evolving roles of histologic evaluation and molecular/genomic profiling in the management of endometrial cancer, J. Natl. Compr. Cancer Netw. 16 (2) (2018) 201–209.
[28] S. Kommoss, et al., Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series, Ann. Oncol. 29 (5) (2018) 1180–1188.
[29] A. Talhouk, et al., Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer, Cancer 123 (5) (2017) 802–813.
[30] D.J. Sargent, et al., Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer, J. Clin. Oncol. 28 (20) (2010) 3219–3226.
[31] I.C. van Gool, et al., POLE proofreading mutations elicit an antitumor immune response in endometrial cancer, Clin. Cancer Res. 21 (14) (2015) 3347–3355.
[32] S. Bellone, et al., Polymerase epsilon (POLE) ultra-mutated tumors induce robust tumor-specific CD4+ T cell responses in endometrial cancer patients, Gynecol. Oncol. 138 (1) (2015) 11–17.
[33] J.B. Pakish, et al., Immune microenvironment in microsatellite-instable endometrial cancers: hereditary or sporadic origin matters, Clin. Cancer Res. 23 (15) (2017) 4473–4481.
[34] F. Fleming Gini, et al., Clinical activity, safety and biomarker results from a phase Ia study of atezolizumab (atezo) in advanced/recurrent endometrial cancer (rEC), J. Clin. Oncol. 35 (15) (2017) 5585.
[35] P.A. Ott, et al., Safety and antitumor activity of pembrolizumab in advanced programmed death ligand 1-positive endometrial cancer: results from the KEYNOTE-028 study, J. Clin. Oncol. 35 (22) (2017) 2535–2541.
[36] P.A. Konstantinopoulos, et al., Phase II, Two-stage Study of Avelumab in Patients With Microsatelitte Stable (MSS), Microsatelitte Instable (MSI) and Polymerase Epsilon (POLE) Mutated Recurrent or Persistent Endometrial Cancer, in Society for Gynecologic Oncology, 2018.
[37] J.M. Mehnert, et al., Immune activation and response to pembrolizumab in POLE-mutant endometrial cancer, J. Clin. Invest. 126 (6) (2016) 2334–2340.