Clinical Cancer Research

Regression of Chemotherapy-Resistant Polymerase ε (POLE) Ultra-Mutated and MSH6 Hyper-Mutated Endometrial Tumors with Nivolumab

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Abstract

Purpose: The management of endometrial carcinoma no longer amenable to treatment with surgery or radiotherapy has not improved significantly with modern chemotherapy. Alternative therapeutic options are desperately needed.

Experimental Design: We describe 2 heavily pretreated patients with recurrent disease refractory to surgery, radiotherapy, and chemotherapy who were treated with the anti–PD-1 immune checkpoint inhibitor nivolumab.

Results: Patient #1 harbored an ultra-mutated tumor (mutation load/MB = 117.3, total mutations = 4,660) driven by mutation in the exonuclease domain of the DNA polymerase ε gene. Patient #2 harbored a hyper-mutated tumor (mutation load/MB = 33.5, total mutations = 1,037) due to a germinal MSH6 gene mutation. Both patients demonstrated a remarkable clinical response to the anti–PD-1 immune checkpoint inhibitor nivolumab. Patients' clinical responses remain unchanged at the time of the writing of this report, with no grade 3 or higher side effects reported to date.

Conclusions: Anti–PD-1 inhibitors represent a novel treatment option for recurrent/metastatic, ultra/hyper-mutated human tumors refractory to salvage treatment. *Clin Cancer Res; 22(23); 5682–7. ©2016 AACR.*

See related commentary by Piulats and Matias-Guiu, p. 5623

Introduction

Recent next-generation sequencing (NGS) studies from The Cancer Genome Atlas (TCGA) network and our group identified a subgroup of patients with ultra- and hyper-mutated endometrial cancer harboring large numbers of mutations secondary to DNA polymerase ε gene mutations and/or deficiency in mismatch repair (MMR) gene functions (1, 2). The high mutation numbers and heavy infiltration of tumor-infiltrating lymphocytes (TIL; ref. 3) support the potential high immunogenicity of these tumors commonly arising in the endometrium and gastrointestinal tract, and provided a rationale for testing the activity of immune checkpoint inhibitors as a novel form of immunotherapy. We describe 2 patients with recurrent ultra- and hyper-mutated endometrial cancers refractory to conventional treatments who demonstrated remarkable clinical responses to nivolumab.

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Materials and Methods

Immunohistochemistry

Briefly, for immunohistochemistry (IHC), 4 μm sections were cut from the formalin-fixed paraffin-embedded (FFPE) blocks of both cancer patients and stained with the following antibodies according to the manufacturers' instructions: p53 (clone DO7, 1:6,000; Dako), CD8 (clone 144B, ready to use; Dako), and PD-L1 (clone E1L3N, 1:200; Cell Signaling Technology). For antigen retrieval, the sections were pretreated at low pH for PD-L1 and CD8 and at high pH for p53.

Whole-exome sequencing

Briefly, DNA was extracted from FFPE samples using a the BiOstic FFPE Tissue DNA Isolation Kit (MO BIO Laboratories #12250-50) with a modified protocol. Genomic DNA was captured on the NimbleGen 2.1M human-exome array and subjected to 74-base paired-end reads on the Illumina HiSeq 2000 instrument, as described (2). Sequence reads were mapped to the reference genome (hg19) using the ELAND program. Reads outside the targeted sequences were discarded, and statistics on coverage were collected from the remaining reads using in-house Perl scripts, as previously described (2).

Results

Patient #1 is a 57-year-old woman with widespread abdominal carcinomatosis secondary to recurrent endometrial cancer. In 2007, she underwent surgical staging for a mixed clear cell and endometrioid (CC/EAC) endometrial cancer, stage IIIA (Fig. 1).

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Translational Relevance

Patients with endometrial cancer have extremely limited therapeutic options when the disease becomes resistant to platinum and taxane chemotherapy. Recent next-generation sequencing studies in patients with recurrent endometrial cancer have demonstrated that about 25% of the patients with recurrent endometrial cancer harbor an ultra- and hyper-mutated endometrial tumor harboring large numbers of somatic mutations attributable to mutations in DNA polymerase epsilon and/or DNA mismatch repair genes. Our report represents the first demonstration of the clinical activity of an immune checkpoint inhibitor in POLE ultra-mutated endometrial cancer patients with recurrent/chemotherapy-resistant disease. These results suggest that anti–PD-1 may prove efficacious in other patients with POLE ultra-mutated microsatellite instability stable and MMR gene–deficient, MSI-H endometrial cancer with recurrent disease refractory to standard salvage treatment.

Postoperatively, she received cisplatin/adriamycin/paclitaxel for 7 cycles and vaginal cuff radiation. The patient did well until March 2012, when CT imaging revealed widespread metastatic lesions in the abdominal cavity. She was placed into a clinical trial with cediranib from April to December 2012 and, subsequently, on Megace (megestrol acetate; Bristol-Myers Squibb) from January 2013 to April 2013. Because of disease progression, in June 2013, the patient underwent an additional laparotomy with tumor debulking, omentectomy, and resection and re-anastomosis of the transverse colon. She was thereafter placed on 6 additional cycles of carboplatin/paclitaxel. Disease progression was confirmed in December 2014 by CT-guided biopsies. She was, therefore, placed on bevacizumab single-agent therapy until February 2015, when she was referred to our institution for additional treatment options due to disease progression, proteinuria, and hypertension. She was started on dose-dense paclitaxel protein-bound particles (100 mg/m^2 weekly). A CT scan obtained in September 2015 after 3 cycles of chemotherapy demonstrated interval increase in her retroperitoneal lymphadenopathy measuring up to 2 cm and pelvic and abdominal carcinomatosis with masses up to 8.1 cm in diameter (Fig. 2). NGS testing [Foundation Medicine (FM)] and microsatellite instability (MSI) results became available at this time and demonstrated an MSI-stable tumor with a POLE (P286R) exonuclease mutation and an ultra-mutated phenotype (i.e., mutations in 87 of 315 cancer gene tested at FM). Whole-exome sequencing (WES) performed at the Center for Genome Analysis at Yale University School of Medicine confirmed the tumor to be ultra-mutated (i.e., total number of mutations = 4,660) and the typical signature of POLE-mutated tumors (i.e., high number of C > A transversion; Fig. 3). CD8 T-cell infiltration and PD-L1 expression on the pretreatment biopsy were also evaluated. A moderate amount of peri- and intratumoral lymphocytic infiltrate was seen on hematoxylin–eosin (H&E) stain and CD8 immunostain. Approximately 5% of the tumor cells demonstrated weak membranous PD-L1 expression by immunohistochemistry, whereas the peri- and intratumoral lymphocytes were PD-L1 negative (Fig. 1). In search of a last attempt to inhibit tumor progression, on the basis of the encouraging report presented at the 2015 American Society of Clinical Oncology Annual Meeting by Le and colleagues (4) demonstrating a clinical response by anti–PD-1 immune checkpoint inhibitors in 2 MSI-H hyper-mutated endometrial cancer patients enrolled in NCT01876511, and after extensive counseling and informed consent, the patient was started on nivolumab single-agent therapy (3 mg/kg biweekly) 6 weeks after her last chemotherapy

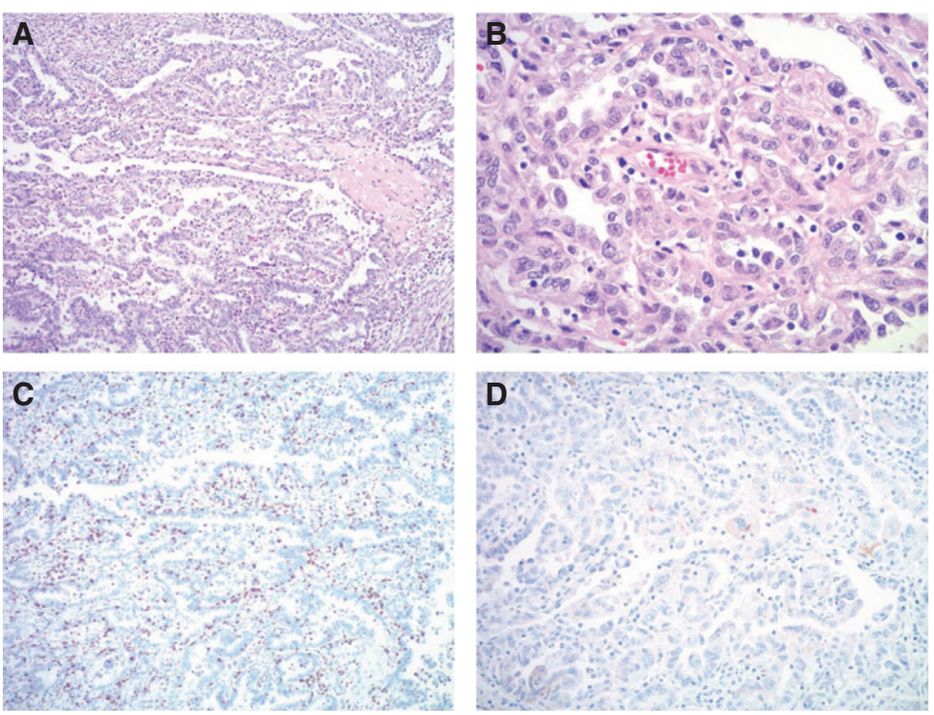


Figure 1.
Microscopic images of the endometrial carcinoma in patient #1. The tumor shows predominantly a papillary and glandular architecture (**A**), with high nuclear grade, and clear to pale eosinophilic cytoplasm (**B**), consistent with clear cell carcinoma component. **C,** Moderate peri- and intratumoral lymphocytic infiltrate is highlighted by CD8 immunostain. **D,** PD-L1 immunostain shows focal, weak staining predominantly located in the tumor cells. (**A,** original magnification, 100x, H&E stain; **B,** original magnification, 400x, H&E stain; **C,** Original magnification, 100x; **D,** Original magnification, 200x).

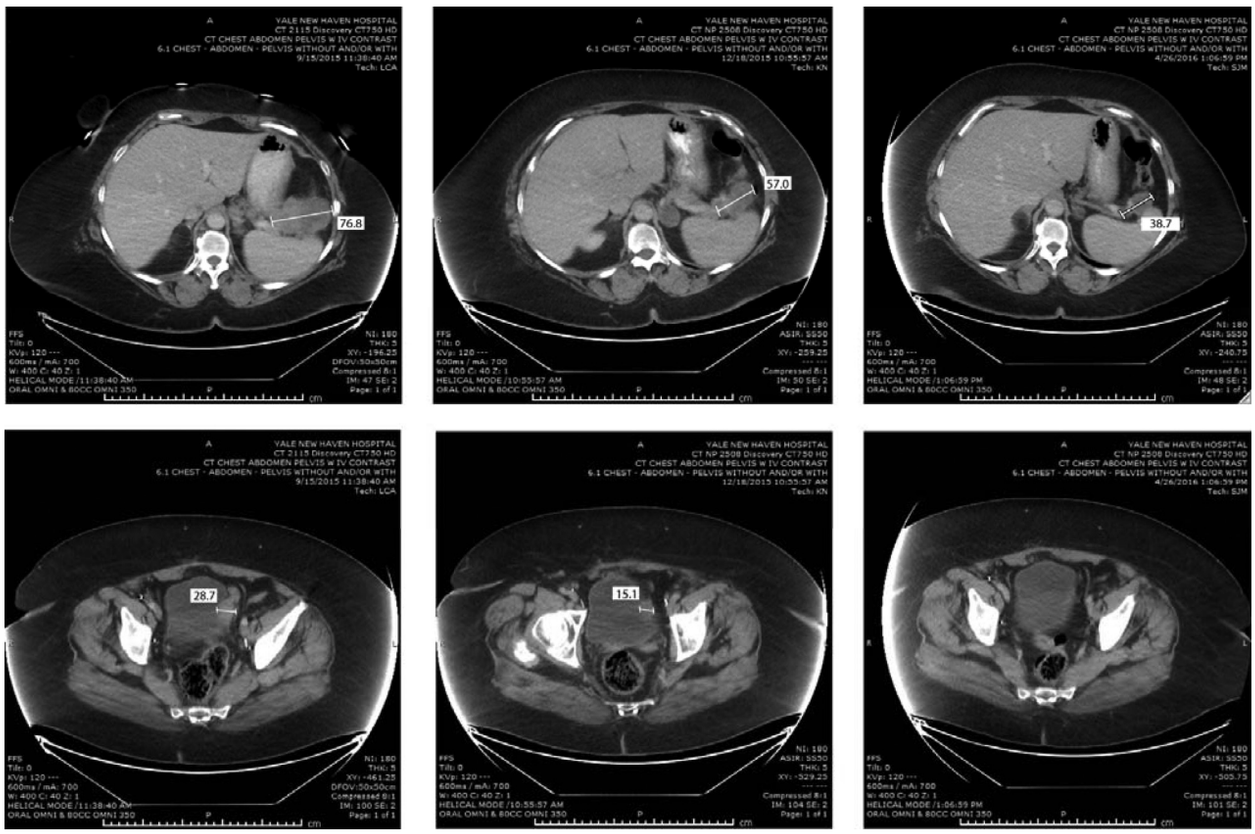


Figure 2.

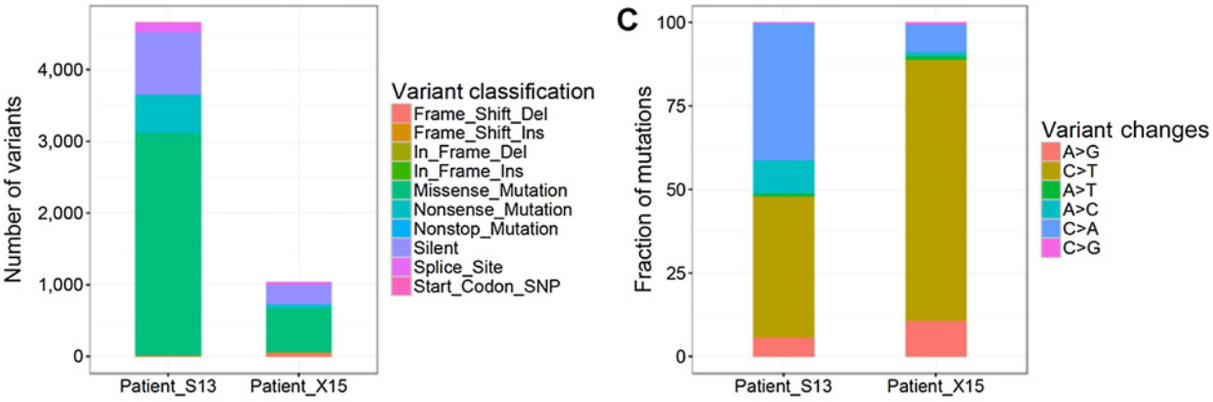
Representative CAT scans demonstrating activity (i.e., partial response) of nivolumab in patient #1 (i.e., POLE ultra-mutated). Left, pretreatment images with baseline measurements of two representative metastatic tumor deposits [i.e., mass abutting the pancreatic tail in the gastrosplenic ligament (top left) and mass involving the bladder dome wall (bottom left)]. Middle, regression of the metastatic tumor deposits, described above, 3 months after treatment initiation. Right, regression (top) and disappearance (bottom) of the metastatic tumor deposits, described above, 7 months after treatment initiation with nivolumab.

administration. CA125 tumor levels normalized within 5 weeks of immunotherapy (i.e., baseline 53 U/mL to 8 U/mL). Six weeks after initiation of nivolumab treatment, the patient was clinically improved to the point that she was able to resume and maintain all of her normal activities. CT imaging obtained 3 months (Fig. 2, middle) and 5 months thereafter (data not shown) demonstrated a remarkable response (i.e., partial response by RECIST v1.1) to the immune checkpoint inhibitor, with regression of her large metastatic pelvic and intra-abdominal tumor deposits. A CT scan obtained 7 months after treatment initiation demonstrated a sustained clinical response with continued regression of her abdominal carcinomatosis (Fig. 2, top right) and metastatic tumor deposits located in the pelvis (Fig. 2, bottom right) and retroperitoneum (data not shown). This patient's remarkable clinical response remains unchanged at the time of the writing of this report with no side effects reported to date and the patient experiencing good quality of life.

Patient #2 is a 60-year-old caucasian female with a recurrent/metastatic uterine serous carcinoma (i.e., USC, a highly aggressive variant of type II endometrial cancer; Fig. 4). Her past surgical history is significant for a robotic-assisted surgical-staging procedure performed in September 2011 for stage IIIC2 disease. She received 6 cycles of docetaxel and carboplatin (completed in February 2012), followed by brachytherapy. She was disease free until October 2013, when enlargement of multiple retroperitoneal nodes was noted. She was followed conservatively until November 2014, when she was enrolled in a phase II clinical study (MGH study 13-520) comparing two different formulations of doxorubicin. She received two administrations of doxorubicin at 60 mg/m^2 every 3 weeks. Secondary to grade 4 neutropenia and gastrointestinal symptoms, chemotherapy was discontinued in December 2014. In March 2015, she was referred to our institution for a second opinion and, in April 2015, she was started on a salvage dose-dense paclitaxel protein-bound particles chemotherapy regimen (80 mg/m^2 weekly). Unfortunately, a CT scan performed after 3 cycles of therapy demonstrated progression of her carcinomatosis, with enlarging retroperitoneal masses up to 7 cm in diameter invading the right psoas muscle (Fig. 5, left) and right-sided hydroureteronephrosis. Her CA125 level was now elevated at 222 U/mL. NGS testing by FM and MSI results revealed an MSI-high tumor secondary to an MSH6 gene mutation (i.e., mutation = F1088). Thirty-nine additional genes were found mutated out of 315 tested (i.e., hyper-mutated). WES performed at the Center for Genome Analysis at Yale University School of Medicine confirmed MSH6-related tumor with hypermutation (i.e., total number of mutations = 1,037; Fig. 3). TP53

A

	Patient S13		Patient X15	
Category	**Normal**	**Tumor**	**Normal**	**Tumor**
Number of reads per sample (M)	86.8	518.9	97.1	403.2
Mean independent reads at each targeted base (X)	58.6	225.1	66.9	191.7
Median independent reads at each targeted base (X)	52	218	59	175
Percent of targeted bases with ≧8x independent reads (%)	95.60	97.00	95.80	97.1



D

Sample	Frame_Shift _Del	Frame_Shift _Ins	In_Frame_ Del	In_Frame_ Ins	Missense_ Mutation	Nonsense_ Mutation	Nonstop_ Mutation	Silent	Splice_Site	Start_Codon _SNP	Total
Patient S13	3	10	4	0	3101	533	2	873	129	5	4660
Patient X15	43	17	2	2	619	48	3	270	32	1	1037

Figure 3.

WES results of patient #1 (i.e., POLE ultra-mutated, S13) and patient #2 (i.e., MSH6 hyper-mutated, X15). **A,** Exome sequencing quality statistics for both patients' tumor and normal DNA. **B,** Exonic mutation burden in patients S13 and X15, with different variant classifications displayed in the barplot. **C,** Distribution of six different mutation conversions in patients S13 and X15. **D,** Number and classification of the distinct variant of mutations detected in patients S13 and X15.

expression, CD8 T-cell infiltration, and PD-L1 expression on the pretreatment tumor biopsy were also evaluated. IHC for p53 showed a wild-type staining pattern (weak to moderate staining in approximately 10% of tumor cell nuclei; Fig. 4). The peritumoral lymphocytic infiltrate was moderate, whereas there were fewer CD8-positive lymphocytes within the tumor cell nests. PD-L1 immunoreactivity was observed in approximately 20% of peri- and intratumoral lymphocytes, and the tumor cells did not show significant PD-L1 expression (Fig. 4). The patient's performance status rapidly deteriorated due to tumor progression and acute development of pulmonary emboli. The patient declined additional chemotherapy after consideration of her options. However, because of early data demonstrating potential clinical activity of pembrolizumab in MSI-H tumors (4) and no good treatment options available for metastatic, chemotherapy-resistant endometrial cancer that would preserve quality of life, the patient decided to pursue an off-label trial of nivolumab (intravenous infusions, 3 mg/kg every 2 weeks). She tolerated the nivolumab treatment with no significant adverse effects other than mild fatigue. Her performance status significantly improved after only 3 nivolumab administrations. A CT scan obtained 3 months after treatment initiation revealed a significant decrease in the diameter of the large abdominal metastases (Fig. 5, middle), with continued improvement at 9 months after treatment initiation (i.e., partial response by RECIST v1.1; Fig. 5 right). The patient continues to do well at the writing of this report, with significant improvement in her performance status and a normalized CA125.

Discussion

To our knowledge, this is the first report demonstrating clinical activity of an immune checkpoint inhibitor in POLE ultra-mutated endometrial cancer patients with recurrent/chemotherapy-resistant disease. POLE ultra-mutated tumors account for 7% to 12% of all endometrial carcinomas (1, 2, 5, 6) and occur secondary to mutations in the exonuclease domain of POLE, a nuclear DNA polymerase endowed with intrinsic proofreading activity (5). Most POLE ultra-mutated cancers (including the POLE tumor of patient #1, described above) are MSI stable and cannot be recognized using clinically approved IHC and PCR-based MSI assays (1, 5, 6). In contrast, hyper-mutated endometrial tumors may occur secondary to different genetic events, such as germline mutations in one of the MMR genes (as observed in

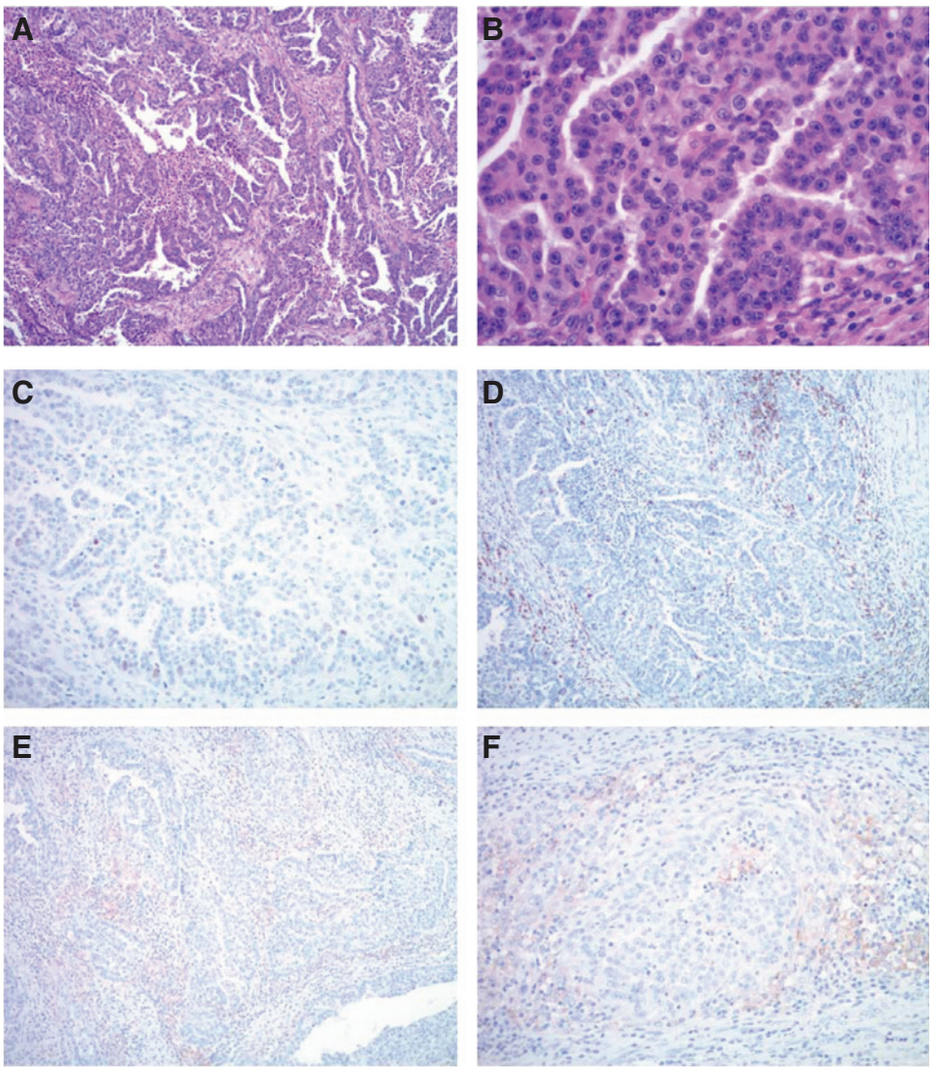


Figure 4.
Microscopic images of the endometrial carcinoma in patient #2. **A,** The tumor shows a predominant glandular architecture with irregular, slit-like spaces and focal necrosis. **B,** The nuclei are large with a high nuclear-to-cytoplasmic ratio, prominent nucleoli, and brisk mitotic activity. **C,** P53 immunostain shows a wild-type staining pattern with weak to moderate nuclear positivity in approximately 10% of tumor cells. **D,** The lymphocytic infiltrate is predominantly peritumoral; less frequent intratumoral lymphocytes are also highlighted by CD8 immunostain. **E** and **F,** PD-L1 immunostain shows diffuse, weak to moderate staining predominantly in peritumoral lymphocytes. (**A,** original magnification, 100x, H&E stain; **B,** original magnification, 400×, H&E stain; C, original magnification, 200×; **D,** original magnification, 100×; **E,** original magnification, 100×; **F,** original magnification, 200×).

patients with Lynch syndrome, including patient #2), or because of epigenetic silencing of the promoters of both alleles of the MLH1 gene (1). Moreover, another group of tumors with the MSI phenotype (also referred as "Lynch-like" tumors) exist that exhibit loss of expression of one or more of the MMR genes secondary to somatic mutations (1, 6). Recent reports analyzing the genetic landscape of endometrial cancers by WES in 232 patients with treatment-naïve endometrial cancer (i.e., TCGA; ref. 1) and 243 patients with recurrent endometrial cancer enrolled in GOG-86P (7) have demonstrated that the overall frequency of ultra- and hyper-mutated tumors may range from 35% (i.e., 7% POLE, 28% MSI; ref. 1) to 26% (i.e., 2% POLE, 24% MSI; ref. 7), respectively. Taken together, these data suggest that up to 26% of patients who develop recurrent endometrial cancer may harbor tumors with either POLE or MMR gene defects.

Although it is currently not understood why patients with ultra/hyper-mutated phenotype may have improved outcomes when compared with the other endometrial cancer groups (1, 2, 5), it is possible that the large number of somatic mutations present in these tumors may render these cancers highly immunogenic for the host due to the large number of mutated epitopes. Consistent with this view, POLE-mutated and MMR gene–defective endometrial cancers are characterized by a high rate of infiltration of T lymphocytes (3). In agreement with these results, our group recently demonstrated that POLE ultra-mutated endometrial tumors, unlike copy number–low and copy number high/serous-like tumors, may trigger activation of both the T-helper arm and the cytotoxic arms of the immune system (8). Anti–PD-1 targeting agents might, therefore, represent a novel therapeutic approach in patients with POLE ultra-mutated MSI stable and MMR gene–deficient, MSI-H endometrial cancer with recurrent disease refractory to standard salvage treatment. Accordingly, a recent report published in the *New England Journal of Medicine* (4) demonstrated that patients with MMR-deficient cancers, including gastrointestinal malignancies and endometrial cancers, had a significantly better clinical response to PD-1 blockade by pembrolizumab than those whose cancers did not have MMR deficiencies. Importantly, in this report, 2 patients treated with pembrolizumab harbored MSI-high endometrial cancers, and both patients experienced a clinical response (i.e., a complete

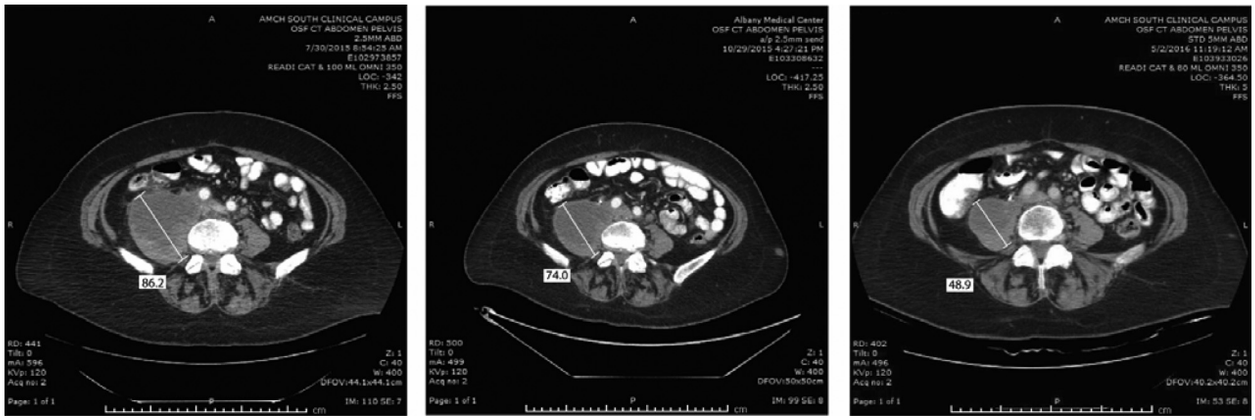


Figure 5.

Representative CAT scans demonstrating activity (i.e., partial response) of nivolumab in patient #2 (i.e., MSH6 hyper-mutated). Left, pretreatment image with baseline measurement of a representative metastatic tumor deposit (i.e., right common iliac mass invading the psoas muscle and obstructing the right ureter). Middle, regression of the metastatic tumor deposit, described above, 3 months after treatment initiation. Right, continued regression (right) of the metastatic tumor deposit 9 months after treatment initiation with nivolumab.

and a partial response, respectively; ref. 4). Our current results using nivolumab in POLE ultra-mutated MSI stable and MSH6 hyper-mutated MSI-H endometrial cancer patients are consistent with these data and further support evidence of noteworthy clinical activity of anti–PD-1 immune checkpoint inhibitors against ultra/hyper-mutated, recurrent/metastatic endometrial cancers resistant to chemotherapy. On the basis of these encouraging results, an investigator-initiated phase II study using pembrolizumab in POLE ultra-mutated and MMR gene–defective hyper-mutated endometrial cancer patients with recurrent disease identified by concurrent assessment of tumor mutation burden and MSI status (9) is pending opening at Yale University.

Disclosure of Potential Conflicts of Interest

J. Schlessinger reports receiving a commercial research grant from a Yale-Gilead Collaboration and is a consultant/advisory board member for Kolltan. R.P. Lifton is an employee of Roche. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.D. Santin, J. Choi, P.E. Schwartz, J. Schlessinger, R.P. Lifton
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References

1. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. Cancer Genome Atlas Res Netw Nat 2013;497:67–73.
2. Zhao S, Choi M, Overton JD, Bellone S, Roque DM, Cocco E, et al. Landscape of somatic single-nucleotide and copy-number mutations in uterine serous carcinoma. Proc Natl Acad Sci U S A 2013;110:2916–21.
3. Howitt BE, Shukla SA, Sholl LM, Ritterhouse SA, Watkins JC, Rodig S, 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 2015;1: 1319–23.
4. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015; 372:26;2509–20.
5. Rayner E, van Gool IC, Palles C, Kearsey SE, Bosse T, Tomlinson I, et al. A panoply of errors: Polymerase proofreading domain mutations in cancer. Nat Rev Cancer 2016;16:71–81.
6. Konstantinopoulos PA, Matulonis UA. POLE mutations as an alternative pathway for microsatellite instability in endometrial cancer: Implications for lynch syndrome testing. Cancer 2015;121:331–4.
7. Aghajanian C, Filiaci VL, Dizon DS, Carlson J, Powell MA, Alvarez-Secorde A, et al. A randomized phase II study of paclitaxel/carboplatin/bevacizumab, paclitaxel/carboplatin/temsirolimus and ixabepilone/carboplatin/bevacizumab as initial therapy for measurable stage III or IVA, stage IVB or recurrent endometrial cancer, GOG-86P. J Clin Oncol 33, 2015 (suppl; abstr 5500).
8. Bellone S, Centritto F, Black J, Schwab C, English D, Cocco E, et al. Polymerase ε (POLE) ultra-mutated tumors induce robust tumor-specific CD4+ T cell responses in endometrial cancer patients. Gynecol Oncol 2015;138:11–7.
9. Santin AD, Moore KN, Gunderson C, Gowen K, Fabrizio D, Frampton G.M, et al. Immunotherapy (IO) versus targeted therapy triage in endometrial adenocarcinoma (EA) by concurrent assessment of tumor mutation burden (TMB), microsatellite instability (MSI) status, and targetable genomic alterations (GA). J Clin Oncol 34, 2016 (suppl; abstr 5591).