

Characteristics of microsatellite instability-high gynecologic cancer and efficacy of pembrolizumab: a single-institution experience

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Objective: The aim of this study was to examine the frequency and characteristics of patients with gynecologic cancer with microsatellite instability (MSI)-high, as well as the efficacy of pembrolizumab in these patients, by reviewing the experience of a single institution that performs MSI testing. **Methods:** We retrospectively investigated the frequency and characteristics of MSI-high in patients with advanced or recurrent gynecologic cancer who underwent MSI testing at Toho University Omori Medical Center. As MSI testing, the distribution of microsatellite length at five microsatellite markers (BAT25, BAT26, NR21, NR24, and MONO27) was analyzed by multiplex polymerase chain reaction. We considered to be MSI-high if two or more microsatellite markers were positive. Among patients with MSI-high, we investigated the clinical course of those who received pembrolizumab. **Results:** MSI testing was performed on 51 patients, four of whom (7.8%) showed MSI-high. Two of the four patients had recurrent endometrial cancer (poorly differentiated endometrioid in one and moderate differentiated endometrioid + clear cell + serous in the other), one had advanced endometrial cancer (stage IVB, poorly differentiated endometrioid), and one had advanced ovarian cancer (stage IIIC, clear cell). No MSI-high was found in the other gynecological cancers. The rate of MSI-high was 15.0% (3/20) for endometrial cancer and 6.7% (1/15) for ovarian cancer. Of the four patients with MSI-high, two received pembrolizumab monotherapy. Both of these patients responded well and have maintained the response for more than 12 months. **Discussion:** This is the first real-world study investigating MSI-high in Japanese women with gynecologic cancer. MSI testing should be performed aggressively in clinical practice to identify patients with advanced and recurrent gynecologic cancers who can benefit from pembrolizumab.

Keywords

MSI-high; Endometrial cancer; Ovarian cancer; Gynecologic cancer; Pembrolizumab

1. Introduction

Microsatellite instability (MSI) is an abnormality that occurs in the microsatellite region of the genome and is caused by a dysfunction of the mismatch repair (MMR) system. Deficiencies in MMR that lead to MSI are known to be frequently found in several types of human cancers [1]. When MMR function is lost, unrepaired DNA errors can accumu-

late and become cancerous. These cancers are called “MSI-high solid tumors” because the microsatellite region shows an unusual number of repetitions. The most common causes of MSI-high are Lynch syndrome and acquired abnormal methylation of the promoter region of *MLH1* gene [1]. MSI-high solid tumors are thought to have more somatic gene mutations than normal cells [2, 3]. As a result, the expression of tumor-specific antigen is increased, which is thought to make the cells more susceptible to recognition by T cells. Thus, the amount of MSI is known to predict the therapeutic effects of immune checkpoint inhibitors including pembrolizumab [4]. Pembrolizumab was approved in Japan in December 2018 as a treatment for solid tumors with MSI-high among advanced or recurrent cancers that have worsened after standard chemotherapy or are difficult to treat with standard therapy. This was the first time in Japan that a drug approval was based solely on a biomarker, regardless of the type of cancer. Since then, pembrolizumab monotherapy has been widely used for MSI-high solid tumors in Japan.

MSI-high was found to be more common in gastrointestinal and gynecologic malignancies, such as colon cancer and endometrial cancer [5, 6]. However, these findings were based on analyses of patients in Western countries and used samples collected for research purposes. To date, no real-world data from clinical practice are available for Japanese patients. Therefore, the objective of this study was to examine the frequency and characteristics of MSI-high in Japanese patients with gynecologic cancer, as well as the use of pembrolizumab in these patients, by reviewing the experience at a single institution where MSI testing was available.

2. Patients and methods

The study retrospectively evaluated patients with advanced or recurrent gynecologic cancer who were treated and underwent MSI testing at the Department of Obstetrics and Gynecology of Toho University Omori Medical Center (Tokyo, Japan) between February 2019 and February 2021. Since February 2019, we perform MSI testing as a routine clinical practice. The eligibility criteria for MSI testing were

Table 1. Clinical characteristics of 51 patients with MSI testing.

		All	Advanced	Recurrent
		(n = 51)	(n = 21)	(n = 30)
Age	Median (range)	59 (32–81)	57 (34–80)	63 (32–81)
Cancer type	Endometrial	20	7	13
	Ovarian	15	9	6
	Cervical	8	2	6
	Uterine sarcoma	8	3	5
FIGO Stage	I–II	15	0	15
	III–IV	36	21	15
Number of prior chemotherapy regimen	0	5	3	2
	1	30	18	12
	2	10	0	10
	≥3	6	0	6
MSI status	MSI-high	4	2	2
	Negative	47	19	28

FIGO, International Federation of Gynecology and Obstetrics; MSI, microsatellite instability; Negative, MSI-low and MSI stable.

as follows: (1) recurrent cancer after standard treatment or cancer that was refractory to standard treatment, (2) incurable advanced cancer with distant metastasis, or (3) rare cancer for which no standard treatment existed, (4) major organs still functioning, (5) expected survival period of 3 months or more, and (6) patients who underwent MSI testing as a screening for Lynch syndrome were excluded.

For the MSI test, 5- μ m slices were cut from a formalin-fixed, paraffin-embedded materials of primary tumor obtained at surgery or biopsy. A specimen containing 50% or more of tumor tissue on one slice was then submitted to a commercial laboratory (SRL, Inc., Tokyo, Japan), where the specimen was microdissected and DNA was extracted by routine methods. During MSI testing, the distribution of microsatellite length at five microsatellite markers (BAT25, BAT26, NR21, NR24, and MONO27) was analyzed by multiplex polymerase chain reaction with the Promega MSI Analysis Kit (FALCO Biosystems Ltd., Kyoto, Japan). MSI was considered to be high (MSI-high) if two or more microsatellite markers were positive and negative if one marker (MSI-low) or no markers (MSI-stable) were positive [7–9].

In addition, we retrospectively reviewed the patients' medical records to extract the following information: age, histologic type, International Federation of Gynecology and Obstetrics (FIGO) stage, number of prior chemotherapy regimens at the time of MSI testing, and administration of pembrolizumab. Moreover, among patients who received pembrolizumab we investigated the details of the clinical course.

All patients received written information about this study and informed consent was obtained in the form of opt-out on our website. They also approved the use of their data and publication of the case details and accompanying images. This study was conducted in compliance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Toho University Omori Medical Center (approval number: M20174).

3. Results

MSI testing was performed on 51 patients (Table 1), four of whom (7.8%) were MSI-high. The median age of the whole group of patients was 59 years (range, 32–81 years). The types of cancer were as follows: endometrial cancer, 20 patients; ovarian cancer, 15 patients; cervical cancer, eight patients; and uterine sarcoma, eight patients. Among the patients with endometrial cancer, five had stage I/II and 15, stage III/IV; the histological type was endometrioid carcinoma in 14 patients (grade 1 in two, grade 2 in six, and grade 3 in six, serous carcinoma in one patient, mucinous carcinoma in one patient, and mixed carcinoma in four patients), and three patients (15.0%) had MSI-high. Among the patients with ovarian cancer, four had stage I/II and 11 had stage III/IV; the histological type was serous carcinoma in six patients, endometrioid carcinoma in one patient, clear cell carcinoma in five patients, mucinous carcinoma in two patients, and another type in one patient, and one patient (6.7%) had MSI-high. Among the patients with cervical cancer, three had stage I/II and five had stage III/IV; the histological type was squamous cell carcinoma in six patients, mucinous carcinoma in one patient, and neuroendocrine carcinoma in one patient, and no patients were MSI-high. Among the patients with uterine sarcoma, three had stage I/II and five had stage III/IV; the histological type was leiomyosarcoma in three patients, rhabdomyosarcoma in one patient, undifferentiated uterine sarcoma in one patient, high-grade endometrial stromal sarcoma in two patients, and low-grade endometrial stromal sarcoma in one patient, and no patients were MSI-high.

The clinicopathological characteristics of the four patients with MSI-high are summarized in Table 2. Two of these patients, both of whom had endometrial cancer, were treated with pembrolizumab monotherapy (200 mg, triweekly). In both patients, the partial response was confirmed radiologically in the three cycles after the start of administration, and the response has been maintained as of May 2021. At the time of writing this manuscript, both patients have responded to the treatment for more than 12 months and no adverse events have occurred that required discontinuation of treatment. The other two patients with MSI-high did not receive pembrolizumab because standard therapy resulted in complete remission. Of the four patients, two underwent germline MMR genetic testing after counseling, but no pathogenic variants were detected. The remaining two did not wish to have germline genetic testing.

Below, we describe in detail one of the patients who was treated with pembrolizumab (Table 2, Case 3). At age 59, the postmenopausal woman (gravida 2, para 2) complained of genital bleeding that lasted for 3 months. She had a family history of osteosarcoma, gastric cancer, and pancreatic cancer in each of her three maternal aunts. She was diagnosed with endometrial cancer with multiple lymph node metastases, including left cervical lymph node metastasis. Her serum CA-125 level was 217.0 U/mL (standard cut-

Table 2. Clinicopathological characteristics of 4 patients with MSI-high.

Patient	Case 1	Case 2	Case 3	Case 4
Age	64	63	59	56
Indication	Recurrent	Recurrent	Advanced	Advanced
Cancer type	Endometrial	Endometrial	Endometrial	Ovarian
Histologic type	G3	Mixed (G2 + Clear + Serous)	G3	Clear
FIGO stage	I B	II	IV B	III C
Number of prior chemotherapy regimen	2	1	1	1
Details of prior chemotherapy	① PTX + CBDCA ② PTX + CBDCA → CBDCA	PTX + CBDCA	PTX + CBDCA	PTX + CBDCA
Pembrolizumab administration	(-)	(+)	(+)	(-)
Efficacy of pembrolizumab	(-)	Partial response	Partial response	(-)
Disease control period with pembrolizumab	(-)	13 months*	15 months*	(-)

G3, endometrioid carcinoma grade 3 (poorly differentiated); G2, endometrioid carcinoma grade 2 (moderate differentiated); Clear, clear cell carcinoma; Serous, serous carcinoma; PTX + CBDCA, paclitaxel 175 mg/m² and carboplatin (area under curve 6 mg·hr/L), triweekly; CBDCA, carboplatin (area under curve 6 mg·hr/L), triweekly. * Pembrolizumab monotherapy still continues because the response is maintained.

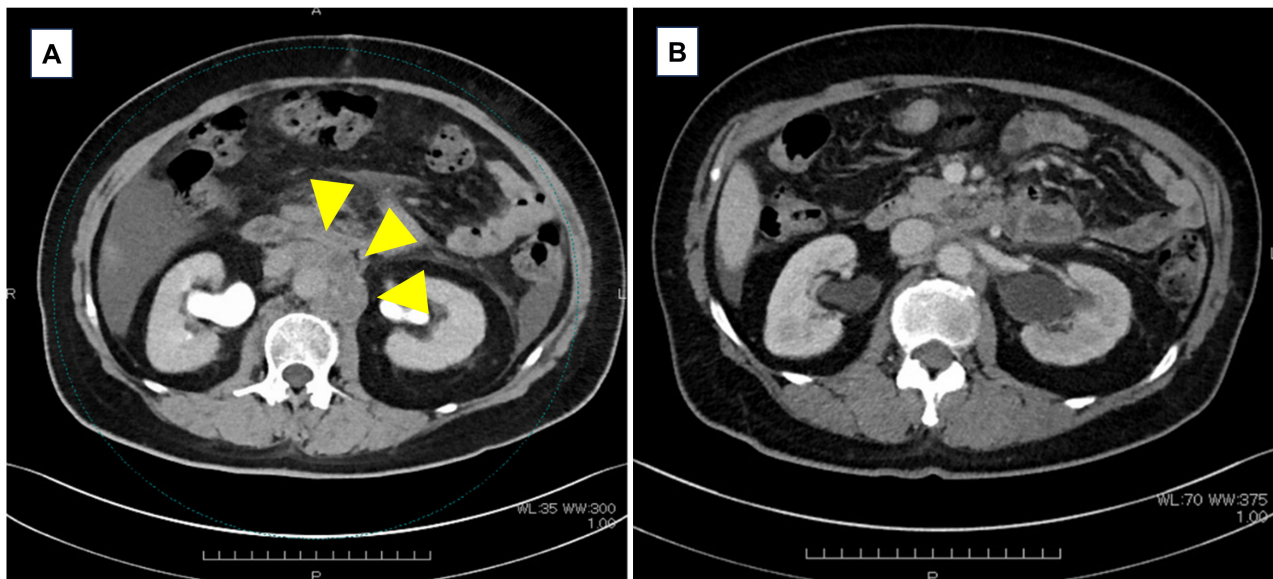


Fig. 1. Contrast-enhanced computed tomography (CT) of abdomen. (A) Before starting pembrolizumab therapy. (B) After 3 cycles of pembrolizumab therapy. Multiple para-aortic lymph nodes swelling (arrows) were markedly decreased.

off, <35 U/mL). A contrast-enhanced computed tomography (CT) scan revealed multiple venous thrombosis in her lower limbs. She underwent abdominal total hysterectomy, bilateral salpingo-oophorectomy, and biopsy of the pelvic and para-aortic lymph nodes. The postoperative diagnosis was endometrioid carcinoma grade 3, stage IVB, pT3aN1M1. On the third postoperative day, the patient developed a pulmonary embolism, which was treated successfully with anticoagulants. Then, she received postoperative chemotherapy with paclitaxel (175 mg/m²) and carboplatin (area under the curve 6 mg·hr/L), triweekly. When she had completed two cycles of postoperative chemotherapy, she was detected a new tumor in the vaginal stump and enlargement of the cervical and para-aortic lymph nodes, which together were

taken as indicating progressive disease that was resistant to standard treatment. Her MSI test result was MSI-high, so we decided to treat her with pembrolizumab monotherapy (200 mg, triweekly). After three cycles of pembrolizumab, the tumor in the vaginal stump disappeared, and a CT scan showed a marked reduction in size of the cervical and para-aortic lymph nodes (Fig. 1). The patient has now undergone up to 19 cycles of pembrolizumab monotherapy and still shows partial response without any adverse events.

4. Discussion

In this study, we examined the frequency and characteristics of MSI-high in patients with gynecologic cancer and the clinical course of patients with MSI-high who were treated

with pembrolizumab. In the past, the frequency of MSI-high among solid tumors was reported to be 3.8%. The frequency of MSI-high by gynecologic cancer type was reported to be 17% to 31% for endometrial cancer, 2.6% to 3.5% for cervical cancer, 2.5% for uterine sarcoma, and 1.3% to 1.5% for epithelial ovarian cancer [5, 6]. In our study, 51 patients with gynecologic cancer were tested for MSI, and four were found to have MSI-high. Three of these patients (15.0%) had uterine endometrial cancer, and one (6.7%) had ovarian cancer. The frequency of MSI-high in endometrial cancer found in this study is similar to that in previous studies, but the frequency in ovarian cancer is much higher. However, the reliability of our results is limited because we studied a relatively small number of patients at a single institution. The frequency of MSI-high at our institution may change in the future as an increasing number of MSI tests are performed.

In a large-scale study comparing 1024 cases of endometrioid endometrial cancer, the MSI-high group had a higher grade of cancer and more lymphovascular space invasion than the group with normal MMR [10]. Similarly, in our study the histological evaluations found that two of the three cases with MSI-high endometrial cancer were grade 3 and one was a high grade with mixed histology (clear cell and serous type). On the other hand, the above-mentioned study found that the prognosis of the MSI-high group was not significantly different from that of the MMR normal group, even though MSI-high tumors are more aggressive [10]. The authors speculated that the MSI-high group may be more sensitive to cytotoxic chemotherapy, which improved their prognosis. This issue is expected to be examined in more detail in future studies.

Pembrolizumab is a humanized monoclonal antibody against PD-1 (programmed cell death-1) that inhibits binding to PD-1 and PD-L2 (programmed cell death-ligand 2) on cancer cells, thereby allowing reactivation of T cell-mediated tumor destruction. As a result, pembrolizumab is thought to exert an antitumor effect in MSI-high solid tumors [11]. A phase II clinical trial of pembrolizumab for MSI-high solid tumors found a response rate of 57.1% and a median progression-free survival of 25.7 months for endometrial cancer. Pembrolizumab can be expected to have a high response rate and a long duration of response [12]. Two of the MSI-high patients in our institution were treated with pembrolizumab; both responded to treatment, and the treatment has continued to be effective in both for more than 12 months. This is a long duration of response for patients with recurrent endometrial cancer in clinical practice. In addition, no serious adverse events due to pembrolizumab have occurred during that time. However, pembrolizumab may have immune-related adverse events (e.g., thyroid dysfunction, pneumonitis, and type 1 diabetes), so patients must be closely monitored.

The prognosis for recurrent or advanced endometrial cancer is poor. In particular, no effective treatment options are available for patients with recurrence after standard treat-

ment. In advanced or recurrent endometrial cancer, the median progression-free survival is 13 months and the median overall survival is 37 months, even with standard treatment with paclitaxel and carboplatin [13]. However, patients confirmed to have MSI-high cancer can now receive pembrolizumab, which can be expected to have a positive therapeutic effect and is considered to be highly beneficial in patients with advanced or recurrent endometrial cancer. Currently, pembrolizumab for MSI-high solid tumors is prescribed as a single agent in Japanese health insurance practice. Furthermore, combination therapy with lenvatinib, a multi-tyrosine kinase inhibitor, and pembrolizumab is likely to be widely used for patients with advanced or recurrent endometrial cancer, regardless of MSI status, and is expected to be an even better treatment option [14].

Ovarian clear cell carcinoma is also widely known to show high resistance to chemotherapy and to have a poor prognosis. Several studies found that 14.3% of ovarian clear cell carcinomas are MSI-high [15] and that some are a highly immunogenic subtype [16]. Pembrolizumab is expected to show efficacy in this subtype and to be a beneficial addition to the treatment strategy for patients with ovarian clear cell carcinoma.

The MSI test is also used to screen for Lynch syndrome, so performing the test may increase the likelihood of identifying hereditary tumors. No patients with Lynch syndrome were found in this study, but if Lynch syndrome is suspected, careful patient management is required, including genetic testing and genetic counseling regarding germline abnormalities of MMR genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) in the family [17].

MSI can also be investigated by comprehensive genomic profiling (CGP) with next-generation sequencers (NGS). NGS can measure tumor mutation burden in addition to MSI, and can broadly identify patients with immunogenic tumor who will benefit from anti-PD-1 immunotherapy [18]. We hope that the clinical implementation of CGP with NGS will become widespread in the near future.

5. Conclusions

We retrospectively examined the frequency and characteristics of Japanese women with gynecologic cancers that were MSI-high and the clinical course of those who received pembrolizumab at our institution. This is the first real-world study investigating MSI-high in Japanese patients with gynecologic cancer. Our findings indicate that MSI testing should be performed routinely in patients with advanced and recurrent gynecologic cancers to identify those patients who may benefit from pembrolizumab. Because this study was performed in a relatively small sample, further studies with larger samples are needed to explore the significance of our findings in clinical practice.

Author contributions

TST, SK, MN and MM treated all patients, collected their data, discussed possible interpretations of the data. TST and SK co-wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All patients received written information about this study and informed consent was obtained in the form of opt-out on our website. They also approved the use of their data and publication of the case details and accompanying images. This study was conducted in compliance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Toho University Omori Medical Center (approval number: M20174).

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Conflict of interest

SK has received speaker's honoraria from Chugai Pharmaceutical, AstraZeneca, and MSD and consulting fee from Eisai. The other authors declare that they have no conflicts of interest.

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