Received: 4 April 2020 | Revised: 18 May 2020 | Accepted: 19 May 2020

DOI: 10.1111/cas.14500

ORIGINAL ARTICLE

Cancer Science WILEY

Microsatellite instability and mismatch repair protein expressions in lymphocyte-predominant breast cancer

Yoshiya Horimoto[1,2] | May Thinzar Hlaing[1] | Harumi Saeki[2] | Shigehisa Kitano[3] | Katsuya Nakai[1] | Ritsuko Sasaki[1] | Aiko Kurisaki-Arakawa[2] | Atsushi Arakawa[2] | Naomi Otsuji[2] | Shuji Matsuoka[4] | Emi Tokuda[5] | Masami Arai[6,7] | Mitsue Saito[1]

[1]Department of Breast Oncology, Juntendo University School of Medicine, Tokyo, Japan

[2]Department of Human Pathology, Juntendo University School of Medicine, Tokyo, Japan

[3]Cancer Immunotherapy Development, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan

[4]Department of Immunological Diagnosis, Juntendo University Graduate School of Medicine, Tokyo, Japan

[5]Department of Medical Oncology, Fukushima Medical University, Fukushima, Japan

[6]Diagnostics and Therapeutics of Intractable Disease, Juntendo University Graduate School of Medicine, Tokyo, Japan

[7]Clinical Genetics, Juntendo University Graduate School of Medicine, Tokyo, Japan

Correspondence
Yoshiya Horimoto, Department of Breast Oncology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, 113-0033 Tokyo, Japan.
Email: horimoto@juntendo.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 18K16287 and 19K16780

Abstract

The frequency of microsatellite instability (MSI) is reportedly extremely low in breast cancer, despite widespread clinical expectations that many patients would be responsive to immune-checkpoint inhibitors (ICI). Considering that some triple-negative breast cancers (TNBC) responded well to ICI in a clinical trial and that a high density of tumor-infiltrating lymphocytes (TILs) is frequently observed in other cancers with high levels of microsatellite instability (MSI-H), we hypothesized that some TNBC with a high density of TILs would be MSI-H. Medullary carcinoma (MedCa) of the breast, a rare histological type, is characterized by a high density of TILs. Considering that MedCa of the colon is often MSI-H, we suspected that MedCa in breast cancer might also include MSI-H tumors. Therefore, we conducted MSI tests on such breast cancers with a high density of TILs. The MSI status of 63 TIL-high TNBC and 38 MedCa tumors, all from Asian women who had undergone curative surgery, were determined retrospectively. DNA mismatch repair (MMR) proteins and PD-L1 expression were also investigated immunohistochemically. All samples were microsatellite stable, being negative for all microsatellite markers. TIL-high TNBC with low MLH1 protein had higher levels of PD-L1 in stromal immune cells (P = .041). MedCa tumors showed significantly higher PD-L1 expression in immune cells than in TIL-high TNBC (<.001). We found that MSI-H tumors were absent in TIL-high breast cancers. Examination of MMR proteins, not a purpose of Lynch syndrome screening, may merit further studies to yield predictive information for identifying patients who are likely to benefit from ICI.

KEYWORDS

breast cancer, DNA mismatch repair protein, medullary carcinoma, microsatellite instability, PD-L1, tumor-infiltrating lymphocyte

*These authors contributed equally to this work.

1 | INTRODUCTION

1.1 | Microsatellite instability (MSI) in malignancy

MSI has long been an effective diagnostic test for Lynch syndrome (LS), one of the hereditary cancer syndromes, characterized by the frequent development of colorectal and endometrial cancers. MSI reflects mismatch repair deficiency and is evaluated by identifying mutations involving microsatellites that are located throughout much of the genome as short repeated sequences. Examination of MSI is an established laboratory test designed to detect a tumor with high levels of microsatellite instability (MSI-H), which strongly suggests LS when considered with onset age and familial history.[1,2] There are certain populations of non-LS patients who harbor MSI-H tumors, although the frequency of MSI-H varies greatly across cancers according to the affected organs. Sporadic endometrial cancer and gastrointestinal cancers have high rates of MSI-H in the range 20%-30%.[3-5] We have recently reported that MSI-H tumors were observed in approximately 10% of Japanese sporadic endometrial cancer cases.[6] Conversely, the frequency of MSI-H in breast cancer is extremely low, in the range 0%-1.5% according to several reports.[4,5]

1.2 | New treatments introduced for MSI-H tumors

One of the recent breakthroughs in cancer treatment is the development of immune-checkpoint inhibitors (ICI). Major ICI target the programmed death ligand 1 (PD-L1)/programmed cell death protein (PD-1) axis between tumor cells and tumor-infiltrating lymphocytes (TILs), to boost the functions of TILs. This allows TILs to attack cancer cells again. Since ICI was first introduced for malignant melanoma, it has shown promising effects in many patients, especially those with lung cancer, while numerous clinical trials involving a range of cancers are still ongoing.[7] Effects of ICI, in general, are likely to correspond to the tumor mutational burden (TMB).[8] MSI-H tumors may have large amounts of neo-antigens that TILs target, and such tumors are reported to express high levels of PD-L1 on their cell surfaces.[9,10] Some clinical studies have shown the significant effects of ICI on MSI-H tumors and pembrolizumab for MSI-H solid tumors approved in 2017 by the US Food and Drug Administration .

1.3 | MSI-H in breast cancer

As to MSI status in breast cancer, the frequency of MSI-H is reportedly to be extremely low, in the range 0% to 1.5%, although definitions of MSI-H might differ slightly among studies.[3-5,11] Hause et al reported that there were no MSI-H tumors among 266 breast cancers tested.[3] This low frequency of MSI-H tumors might discourage further studies that were designed to reveal the clinicopathological features of MSI-H breast cancer. DNA mismatch repair (MMR) protein assessment used for LS screening does not presently appear to be suitable for breast cancer, as MMR deficiency is relatively uncommon[12] and not consistently associated with MSI-H.[11,13] According to a recent study, there was only one MSI-H tumor among 75 breast cancers that showed MMR protein loss.[13] Hence, whether patients should be recommended to undergo MSI testing, despite the enormous clinical expectations that many breast cancer patients have for ICIs, remains undecided. To optimize clinical care and avoid unnecessary MSI testing, a screening system for MSI-H breast cancer urgently needs to be established.

Notably, patients with triple-negative breast cancers (TNBC) with TILs positive for PD-L1 showed good responses to ICI in a clinical trial[14] and these agents have been recently introduced in clinical practice. Considering that a high density of TILs is frequently observed in other MSI-H cancers,[15,16] we hypothesized that MSI-H breast cancer would be more common in patients with TN tumors that showed a high density of TILs. To date, no data have been reported for such a patient population.

Medullary carcinoma (MedCa), a rare histological type of breast cancer, is characterized by a high density of TILs. Patients with MedCa are known to have better outcomes than other breast cancer patients and the TILs might contribute to this difference. We previously revealed that TILs in MedCa are comprised of larger CD8-positive lymphocyte populations than found in common invasive breast cancers.[17] However, the factors accounting for this large proportion of TILs, including tumor antigens, remain largely unknown. MedCa of the colon is reportedly quite often MSI-H,[18] and we therefore suspected that MedCa breast cancer might also include MSI-H tumors.

For the reasons described above, we conducted MSI tests on such TIL-high breast cancers in this study. MMR protein and PD-L1 expression was also investigated immunohistochemically.

2 | MATERIALS AND METHODS

2.1 | Patients

TIL-high TNBC cohort: There were 145 patients with invasive TNBC pathologically larger than 5 mm in diameter who had undergone curative surgery without pre-operative chemotherapy at our institution during the period 2013 through 2018. There were 5 medullary carcinomas (MedCa) among the 145 TNBC and these patients were included in the MedCa cohort (described later). First, surgical specimens of these 140 tumors were examined to evaluate TILs, as described in detail below. Sixty-six tumors were judged to be TIL high. We examined these 66 tumors in the current study as TIL-high TNBC.

MedCa cohort: We collected MedCa that were more than 5 mm in diameter, regardless of intrinsic subtype, during the period 2006 through 2018. There were 42 such cases. Two patients who had received systemic chemotherapies before surgery were excluded. Among the remaining 40 patients, no formalin-fixed paraffin-embedded block was available in one case. Thus, MSI testing was conducted for 39 samples. All MedCa were judged to be TIL high.

In both cohorts, we excluded patients who had received pre-operative systemic chemotherapy, to avoid any effect of these treatments on TIL expression. Samples from 64 of the TIL-high TNBC and the 39 MedCa patients were investigated for MSI status. MSI testing could not be completed in one case in each cohort due to poor DNA quality. Consequently, MSI testing was conducted in all other TIL-high TNBC (n = 63) and MedCa (n = 38) samples. Immunohistochemistry (IHC) was conducted for all 101 tumors in total. Clinicopathological features for these patients are shown in Table 1. Participants were all Asian women, 100 were of Japanese origin, and one Chinese.

This study was carried out with approval from the ethics committee of Juntendo University (No. 17-011) and all specimens were collected after obtaining informed consent from the patients.

TABLE 1 Clinicopathological features of the 2 patient cohorts

Factors examined	TIL-high TNBC (n = 63) Values	[range]/ (rate)	MedCa (n = 38) Values	[range]/ (rate)
Age (mean)	62.0	[24-88]	59.7	[37-85]
Tumor size (mean, mm)	23.7	[5-60]	25.2	[7-80]
Lymph node involvement	15/63	(23.8%)	6/38	(15.7%)
Histology				
Invasive carcinoma of no special type	51	(81.0%)	0	(0%)
Carcinoma with apocrine differentiation	7	(11.1%)	0	(0%)
Squamous cell carcinoma	3	(4.8%)	0	(0%)
Metaplastic carcinoma with mesenchymal differentiation	2	(3.2%)	0	(0%)
Invasive lobular carcinoma	1	(1.6%)	0	(0%)
Invasive micropapillary carcinoma	1	(1.6%)	0	(0%)
Medullary carcinoma	0	(0%)	38	(100%)
High grade	37/63	(58.7%)	34/38	(89.5%)
Ki67 labeling index (mean, %)	68.8	[10-100]	64.4	[15-95]
Subtype				
TN	63	(100%)	16	(42.1%)
HER2 type	0	(0%)	10	(26.3%)
Luminal HER2-positive	0	(0%)	2	(5.3%)
Luminal HER2-negative	0	(0%)	10	(26.3%)
% stromal TILs (mean)	69.4	[50-100]	88.9	[70-100]

2.2 | Pathological assessment and TIL evaluation

Pathological examinations were carried out at Juntendo University Hospital by 2 experienced pathologists, based on the General Rules for Clinical and Pathological Recording of Breast Cancer (the 18th edition published by the Japanese Breast Cancer Society).[19] MedCa was defined as a tumor in which the medullary histological features were dominant (≥50% of the invasive carcinoma area), basically corresponding to "carcinomas with medullary features" in the 4th edition of the WHO classification of Tumors of the Breast. Tumor grade was judged based on the modified Bloom-Richardson histological grading system. For Ki67 labeling index, a hotspot was chosen under ×200 magnification and cells positive for nuclear Ki67 were evaluated semi-quantitatively. Estrogen and progesterone receptor status were assessed semi-quantitatively by IHC and reported as positive when more than 1% of the nuclei of the cancer cells showed staining. Human epidermal growth factor receptor 2 (HER2) was judged to be positive if more than 10% of tumor cells showed strong staining across the entire cell membrane, or *HER2/neu* gene amplification was confirmed by fluorescence in situ hybridization. Therefore, a TNBC was defined as tumor negative for estrogen and progesterone receptors as well as for HER2.

TIL amounts were determined using hematoxylin and eosin-stained tumor surgical sections, based on recommendations made by an International TILs Working Group.[20] Briefly, TILs in the stromal compartment (% stromal TILs), using the area of stromal tissue as a denominator, were determined semi-quantitatively in 10% increments. TILs were examined within the borders of the invasive tumor, and full assessment of average TIL numbers in the tumor area, not focusing on hotspots, was conducted. TILs were judged to be present at a high level (TIL-high) if they comprised at least 50% of the stroma.

2.3 | DNA extraction and MSI test

From paraffin blocks of surgical specimens tissue sections, 10 μm in thickness, were cut from the same areas as those used for TIL evaluation, and DNA was extracted using a QIAamp DNA FFPE Tissue kit (Qiagen Inc). Tissues were sectioned using macro-dissection to obtain a high tumor cell content. MSI testing was outsourced to TaKaRa Bio Inc. Using a Promega MSI Multiplex System, 5 spots from the DNA sequence for microsatellite markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) were amplified. Two polymorphic pentanucleotide repeat markers, PentaC and PentaD, both of which were used as quality control for sample authentication, were co-amplified. Nontumorous tissue from each patient was used as the control. A tumor was determined to be MSI-H if instability was detected in 2 or more of the 5 markers, as recommended by the revised Bethesda Guidelines.[21] Tumors with one or no unstable marker were classified as having low levels of

microsatellite instability (MSI-L) or as being microsatellite stable (MSS), respectively.

2.4 | IHC

MMR proteins (MLH1, MSH2, PMS2, and MSH6) and PD-L1 were also examined by IHC. Information on antibodies and assessment criteria are presented below. Each MMR protein expression score in the nuclei of cancer cells was determined semi-quantitatively in 10% increments and an expression exceeding 50% of the MMR protein was defined as being high. If the nucleus of a cancer cell showed any positivity, the tumor was considered to be positive for MMR. For PD-L1, membrane staining of tumor cells (TC) and stromal immune cells (IC) was determined semi-quantitatively in 10% increments, respectively. IC consists of lymphocytes, macrophages, dendritic cells, and granulocytes, as assessed based on the guidelines from Roche Diagnostics for IHC assessment (SP142). Scoring for PD-L1 was assessed as 0: <1%, 1: ≥1% to <5%, 2: ≥5% to <10%, and 3: ≥10%, based on criteria applied clinically for breast cancer,[14] ie TC0 to TC3 for TC and IC0 to IC3 for IC. Details of antibodies used are MLH1: mAb ES05 (Dako), MSH2: mAb FE11, PMS2: mAb EP51, MSH6: mAb EP49, and PD-L1: mAb SP142 (Spring Bioscience). Representative images of each protein are shown in Figure S1.

2.5 | Statistical analysis

Statistical analyses were performed using JMP 14.2 statistical software (SAS Institute, Inc). Associations between 2 parameters were evaluated using Pearson chi-square test or the logistic regression model, according to the scales of the variables examined. For patient survival, Kaplan-Meier curves were estimated and the generalized Wilcoxon test was applied for comparisons of survival distributions across the 2 patient groups. A $P < .05$ was considered to indicate a statistically significant difference.

3 | RESULTS

3.1 | MSI status of TIL-high TNBC and MedCa

All 101 samples examined were MSS (Table 2). All 5 microsatellite markers were negative, ie there were no MSI-L cases.

3.2 | MMR and PD-L1 expression in TIL-high TNBC and MedCa

For MMR protein expression, one TIL-high TNBC case showed complete loss of MLH1 and PMS2, while one MedCa case showed loss of PMS2 expression (Figure S2). Details of these 2 patients, including age, outcomes, and representative MMR images are also shown. Neither patient had a family history that suggested LS syndrome, nor has developed recurrent disease to date. Table 3 presents the details of MMR protein expression in the 2 cohorts. MLH1 protein expression was significantly lower in MedCa than in TIL-high TNBC ($P = .009$), while almost all tumors were positive for MSH6. For relationships among MMR proteins, all combinations showed statistically significant correlations in both cohorts, except the PMS2 and MSH6 pair in MedCa, which did not show any correlation (Figure S3).

Next, we examined PD-L1 expression in TC and IC (Table 4). There was no difference in TC score distribution between the 2 cohorts. However, PD-L1 expression in IC was significantly higher in MedCa than in TIL-high TNBC (<.001), with 87% of MedCa showing strong expression (IC2 or IC3). For relationships of PD-L1 expression in TC and IC, a positive correlation was observed in the TIL-high

TABLE 2 MSI status of TIL-high TNBC and MedCa

	TIL-high TNBC		MedCa	
MSI status	n	(rate)	n	(rate)
MSI-H	0	(0%)	0	(0%)
MSI-L	0	(0%)	0	(0%)
MSS	63	(100%)	38	(100%)

TABLE 3 MMR protein expression in the 2 cohorts

	TIL-high TNBC			MedCa			*P* value
	Average [range]			Average [range]			
MLH1	73.4% [0-100]	High	49	56.7% [0-100]	High	20	
		Low	14		Low	18	**.009**
MSH2	64.8% [10-100]	High	47	64.5% [10-100]	High	28	
		Low	16		Low	10	.919
PMS2	55.5% [0-100]	High	41	68.2% [0-100]	High	29	
		Low	22		Low	9	.236
MSH6	95.1% [40-100]	High	62	95.0% [70-100]	High	38	.435
		Low	1		Low	0	

TABLE 4 PD-L1 expression in tumor cells and stromal immune cells

	TIL-high TNBC		MedCa		
	n	rate	n	rate	*P* value
TC0	25	40%	6	16%	
TC1	11	17%	12	32%	.34
TC2	25	40%	20	53%	(TC0/1 vs 2/3)
TC3	2	3%	0	0%	
IC0	9	14%	2	5%	
IC1	27	43%	3	8%	<.001
IC2	21	33%	19	50%	(IC0/1 vs 2/3)
IC3	6	10%	14	37%	

TNBC (*P* = .017) (Figure S4). MedCa showed no correlation in PD-L1 between TC and IC (*P* = .537), as IC were distributed over a broad range while expression was low in TC.

Relationships between MMR and PD-L1 expression are shown in Figure 1. In the TIL-high TN cohort, there was an inverse correlation between MLH1 protein expression and PD-L1 in IC (*P* = .041). MSH2 showed similar TC and IC trends, although these did not reach statistical significance (*P* = .053 for both). No significant correlations were observed between MMR and PD-L1 expression in MedCa tumors.

3.3 | Patient outcomes according to MMR protein expression

Finally, we investigated differences in patient outcomes according to MMR protein expression as there were no MSI-H cases. With the mean 40-mo and 68-mo observation periods (ranges 1-108 and 1-152), 5 patients and 1 patient (7.9% and 2.6%) developed distant recurrent disease in the TIL-high TN and MedCa cohorts, respectively. Among them, 3 patients and 1 patient, respectively, died

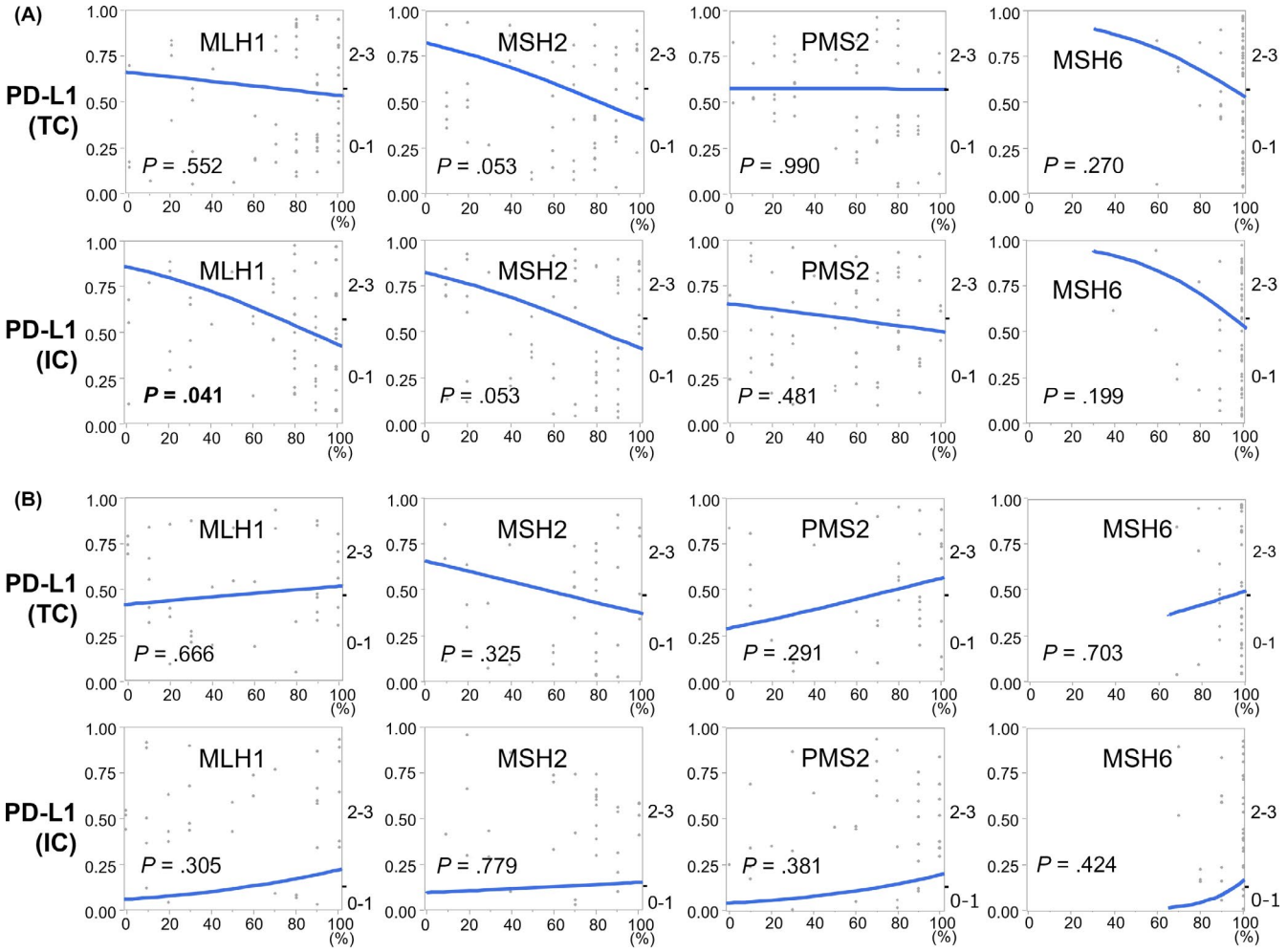

FIGURE 1 Relationships between MMR and PD-L1 expression. Relationships between MMR and PD-L1 expression in TC and IC, respectively are shown. A, TIL-high TN (n = 63). B, MedCa (n = 38). The logistic regression model was employed for evaluation of associations between these 2 parameters as the scales of MMR protein and PD-L1 expression were continuous and nominal variables, respectively

due to breast cancer. Kaplan-Meier curves of disease-free survival (DFS) according to MMR expression in the 2 cohorts are shown in Figure S5. There were no differences in DFS according to MMR protein expression in either of the 2 cohorts.

4 | DISCUSSION

To the best of our knowledge, this is the first study to focus on MSI status in breast cancer, considering the density of TILs. Also, no studies have examined MSI status in MedCa on a scale similar to that of our surgical samples.[22,23] Two large cohort studies, which examined MSI status in more than 200 TNBC cases, demonstrated very low rates of MSI-H, 0.9%-1.8%.[11,24] We found that there were no MSI-H tumors even among TIL-high tumors, which differed from our expectations.

Considering the intrinsic absence of MSI-H tumors among TIL-high TNBC, there might be no specific distribution pattern of MSI-H tumors across breast cancers. However, further studies using other criteria must be conducted before any such conclusion can be drawn. Markers other than the 5 standard MSI markers might be suitable for detecting MSI-H tumors among breast cancers.[25] MMR-deficient breast cancers might well yield such markers. More detailed information on TILs, such as T-cell subpopulations, might need to be obtained. PD-L1 expression on both TC and TILs is also of great interest. The relationship between MSI and PD-L1 has been well investigated across cancers and it is known that the correlation rates differ markedly among cancer types. For breast cancer, data are still lacking as MSI-H breast cancer is rare.[26,27] For instance, Vanderwalde and colleagues analyzed 1024 breast cancer patients using next-generation sequencing but only 6 patients were found to have MSI-H tumors (1 had a PD-L1-positive tumor).[27] Also, most previous data regarding PD-L1 expression are based only on TC. Furthermore, as an objective of analysis, TIL-low TN breast cancers and other intrinsic subtypes, such as HER2 type, might also warrant examination, which was not feasible in the current study. In terms of ethnic differences, the frequency of MSI-H breast cancer might be lower in Asian patients than in other populations, as the rate of MSI-H gastric cancer is reportedly lower in Japanese than in western populations.[28] Thus, differences among races may also have to be considered when employing clinical samples. Considering how very rare MSI-H tumors are among breast cancers, it might be necessary to employ different approaches, other than MSI status, to identify patients who would benefit from ICI treatment. As an example, evaluation of chromosome instability using next-generation sequencing has identified patients who would respond to everolimus-based treatment in a recent clinical trial, although it must be kept in mind that this drug is different from ICI.[29]

MMR protein assessment has been regarded as not being suitable for MSI screening in breast cancer[11-13] and the significance of MMR proteins has not yet been well investigated in microsatellite-stable breast cancers. Fusco and colleagues recently examined 444 surgical breast cancer specimens comprising all subtypes and revealed that MMR expression might be a prognostic factor, based on patients with MMR-deficient ER-negative tumors who had better outcomes.[13] In the current study, sample size was small, mainly due to the exclusion of patients who had received neo-adjuvant chemotherapy for more advanced disease, and the observation period was relatively short. Hence, our data could not be compared with that of previous reports in terms of patient outcomes. Nevertheless, we revealed an inverse correlation between MLH1 and PD-L1 in IC in TIL-high TNBC and, to the best of our knowledge, this is the first report to describe such findings. Similar trends among other MMRs were also observed (Figure 1), though none reached the level of statistical significance. MMRs are reportedly known to correlate inversely with TMB across solid tumors. For instance, in MSI-H gastrointestinal cancers, *MSH2* and/or *MSH6* changes were associated with higher TMB.[30] Downregulation of *MLH1* expression was observed in TMB-high lung adenocarcinoma.[31] Moreover, increases in TMB and the number of neo-antigens were observed in MLH1-deficient cancer cells, including breast cancer.[32] Inactivation of MMR triggered immune surveillance in murine models suggested that inactivation of DNA repair may enhance the immunogenicity of MSS tumors.[32,33] These data appear to provide novel insights regarding the significance of MMR deficiency as a surrogate marker for identifying patients who would benefit from ICI, rather than elucidating roles that have been demonstrated in LS and MSI-H tumors at various oncogenic stages. Therefore, we believe that assessing MMR in breast cancer, regardless of MSI status, merits further investigation to establish its clinical value.

MMR proteins were found to be expressed heterogeneously within a tumor, which was consistent with the aforementioned report stating that MMR evaluation should be conducted employing surgical specimens.[13] We observed a complete loss of PMS2 in Case 2 with MedCa (Figure S2B). We suspect that PMS2 expression might have been present in other sections due to heterogeneity within a single tumor, although we used the surgical section with the largest amount of tumor tissue, because complete loss of PMS2 alone is difficult to explain biologically.

For MedCa, PD-L1 expression in IC was significantly higher than that in TIL-high TNBC ($P<.001$; Table 4). We previously revealed that CD8-positive TILs predominated in MedCa compared with TIL-high TNBC, regardless of CD4-positive TIL subsets, suggesting that the immune response against TC is boosted.[17] Therefore, we suspect that our data showed more active local immune responses in MedCa and this factor might be one of the reasons why patients with this histological type had better outcomes, although details of mechanisms by which MedCa elicits cell-mediated immunity should be further examined. Moreover, we can reasonably speculate that ICI would be effective for tumors of this type.

The major strength of the current study was sample selection. By evaluating surgical specimens, we were able to select more accurately TIL-high tumors and assess MMR protein expression, avoiding possible bias driven by intratumor heterogeneity, which might have influenced the results, compared with analyzing tissue microarray samples, as in previous studies.[11-13] Nevertheless, we identified no MSI-H cases among the TIL-high TN breast cancers. Major limitations

of the current study include its small sample size and the exclusion of patients who received neo-adjuvant chemotherapy for more advanced tumors. Further investigations, including a study with a larger number of patients employing samples from such cases, are necessary to obtain conclusive evidence. However, again, it must be kept in mind that intratumor heterogeneity of MMR proteins might be an obstacle when biopsy specimens are used. Moreover, even when a surgical specimen is examined by highly experienced specialists, interobserver and intraobserver differences in evaluation results among pathologists are still possible and warrant further consideration.

Our results revealed that MSI-H breast cancers do not correspond to TIL-high tumors. Our negative results might aid other researchers and clinicians when planning further studies related to MSI in breast cancer. Furthermore, in the current study, MLH1 protein expression in TNBC was inversely correlated with PD-L1 expression in IC. Therefore, we believe that examining MMR proteins, while not a purpose of MSI screening, may merit additional study as these proteins might provide information for predicting which patients are likely to benefit from ICI.

ACKNOWLEDGMENTS

The authors sincerely appreciate Bierta Barfod for her help with language editing. This study was supported by the Japan Society for the Promotion of Science (YH, grant 18K16287 and KN, grant 19K16780).

DISCLOSURE

The authors have no conflict of interest to declare.

ORCID

Yoshiya Horimoto https://orcid.org/0000-0001-8935-0768

REFERENCES

1. Hampel H, Frankel W, Panescu J, et al. Screening for lynch syndrome (Hereditary Nonpolyposis Colorectal Cancer) among endometrial cancer patients. *Cancer Res*. 2006;66:7810-7817.
2. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for lynch syndrome among patients with colorectal cancer. *J Clin Oncol*. 2008;26:5783-5788.
3. Hause RJ, Pritchard CC, Shendure J, et al. Classification and characterization of microsatellite instability across 18 cancer types. *Nat Med*. 2016;22:1342.
4. Bonneville R, Krook MA, Kautto EA, et al. Landscape of microsatellite instability across 39 cancer types. *JCO Precision Oncol*. 2017;1:1-15.
5. Latham A, Srinivasan P, Kemel Y, et al. Microsatellite instability is associated with the presence of lynch syndrome pan-cancer. *J Clin Oncol*. 2017;37:286-295.
6. Saeki H, Hlaing MT, Horimoto Y, et al. Usefulness of immunohistochemistry for mismatch repair protein and microsatellite instability examination in adenocarcinoma and background endometrium of sporadic endometrial cancer cases. *J Obstet Gynaecol Res*. 2019;45:2037-2042.
7. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252-264.
8. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500:415-421.
9. Salem ME, Puccini A, Grothey A, et al. Landscape of tumor mutation load, mismatch repair deficiency, and PD-L1 expression in a large patient cohort of gastrointestinal cancers. *Mol Cancer Res*. 2018;16:805-812.
10. Korehisa S, Oki E, Iimori M, et al. Clinical significance of programmed cell death-ligand 1 expression and the immune microenvironment at the invasive front of colorectal cancers with high microsatellite instability. *Int J Cancer*. 2018;142:822-832.
11. Wen YH, Brogi E, Zeng Z, et al. DNA mismatch repair deficiency in breast carcinoma: a pilot study of triple-negative and non–triple-negative tumors. *Am J Surgical Pathology*. 2012;36:1700-1708.
12. Mills AM, Dill EA, Moskaluk CA, et al. The relationship between mismatch repair deficiency and PD-L1 expression in breast carcinoma. *Am J Surg Pathol*. 2018;42:183-191.
13. Fusco N, Lopez G, Corti C, et al. Mismatch repair protein loss as a prognostic and predictive biomarker in breast cancers regardless of microsatellite instability. *JNCI Cancer Spectrum*. 2018;2:pky056.
14. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med*. 2018;379:2108-2121.
15. Michael-Robinson JM, Biemer-Hüttmann A-E, Purdie DM, et al. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. *Gut*. 2001;48:360-366.
16. Pakish JB, Zhang Q, Chen Z, et al. Immune microenvironment in microsatellite-instable endometrial cancers: hereditary or sporadic origin matters. *Clin Cancer Res*. 2017;23:4473-4481.
17. Igari F, Sato E, Horimoto Y, et al. Diagnostic significance of intratumoral CD8+ tumor-infiltrating lymphocytes in medullary carcinoma. *Hum Pathol*. 2017;70:129-138.
18. Friedman K, Brodsky AS, Lu S, et al. Medullary carcinoma of the colon: a distinct morphology reveals a distinctive immunoregulatory microenvironment. *Mod Pathol*. 2016;29:528-541.
19. Tsuda H, Tsugawa K, Akiyama F, et al. Histological classification of breast tumors in the General Rules for Clinical and Pathological Recording of Breast Cancer. *Breast Cancer*. 2020;27:309-321.
20. Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol*. 2015;26:259-271.
21. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*. 2004;96:261-268.
22. Schmitt FC, Soares R, Gobbi H, et al. Microsatellite instability in medullary breast carcinomas. *Int J Cancer*. 1999;82:644-647.
23. Lee S-C, Berg KD, Sherman ME, et al. Microsatellite instability is infrequent in medullary breast cancer. *Am J Clin Pathol*. 2001;115:823-827.
24. Kurata K, Kubo M, Kai M, et al. Microsatellite instability in Japanese female patients with triple-negative breast cancer. *Breast Cancer*. 2020;27(3):490-498.
25. Polak P, Kim J, Braunstein LZ, et al. A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. *Nat Genet*. 2017;49:1476-1486.
26. Luchini C, Bibeau F, Ligtenberg M, et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. *Ann Oncol*. 2019;30:1232-1243.
27. Vanderwalde A, Spetzler D, Xiao N, et al. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med*. 2018;7:746-756.

28. An JY, Kim H, Cheong J-H, et al. Microsatellite instability in sporadic gastric cancer: its prognostic role and guidance for 5-FU based chemotherapy after R0 resection. *Int J Cancer*. 2012;131:505-511.
29. Hortobagyi GN, Chen D, Piccart M, et al. Correlative analysis of genetic alterations and everolimus benefit in hormone receptor-positive, human epidermal growth factor receptor 2–negative advanced breast cancer: results from BOLERO-2. *J Clin Oncol*. 2016;34:419-426.
30. Salem M, Grothey A, Goldberg R, et al. Association between tumor mutation burden (TMB) and MLH1, PMS2, MSH2, and MSH6 alterations in 395 microsatellite instability-high (MSI-High) gastrointestinal (GI) tumors. *Ann Oncol*. 2018;29:v109.
31. Jia M, Yao L, Yang Q, et al. Association of MSH2 expression with tumor mutational burden and the immune microenvironment in lung adenocarcinoma. *Front Oncol*. 2020;10:168.
32. Germano G, Lamba S, Rospo G, et al. Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. *Nature*. 2017;552:116-120.
33. American Association for Cancer Research. Loss of DNA repair drives neoantigen renewal and inhibits tumor growth. *Cancer Discov*. 2018;8:11.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Horimoto Y, Thinzar Hlaing M, Saeki H, et al. Microsatellite instability and mismatch repair protein expressions in lymphocyte-predominant breast cancer. *Cancer Sci*. 2020;111:2647–2654. https://doi.org/10.1111/cas.14500