

# A Phase II Study of Avelumab Monotherapy in Patients with Mismatch Repair–Deficient/Microsatellite Instability–High or *POLE*-Mutated Metastatic or Unresectable Colorectal Cancer

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## Purpose

We evaluated the efficacy and safety of avelumab, an anti-PD-L1 antibody, in patients with metastatic or unresectable colorectal cancer (mCRC) with mismatch repair deficiency (dMMR)/microsatellite instability-high (MSI-H) or *POLE* mutations.

## Materials and Methods

In this prospective, open-label, multicenter phase II study, 33 patients with mCRC harboring dMMR/MSI-H or *POLE* mutations after failure of  $\geq 1$ st-line chemotherapy received avelumab 10 mg/kg every 2 weeks. dMMR/MSI-H was confirmed with immunohistochemical staining (IHC) by loss of expression of MMR proteins or polymerase chain reaction (PCR) for micro-satellite sequences. *POLE* mutation was confirmed by next-generation sequencing (NGS). The primary endpoint was the objective response rate (ORR) by Response Evaluation Criteria in Solid Tumors ver. 1.1.

## Results

The median age was 60 years, and 78.8% were male. Thirty patients were dMMR/MSI-H and three had *POLE* mutations. The ORR was 24.2%, and all of the responders were dMMR/MSI-H. For 21 patients with MSI-H by PCR or NGS, the ORR was 28.6%. At a median follow-up duration of 16.3 months, median progression-free survival and overall survival were 3.9 and 13.2 months in all patients, and 8.1 months and not reached, respectively, in patients with MSI-H by PCR or NGS. Dose interruption and discontinuation due to treatment-related adverse events occurred in four and two patients, respectively, with no treatment-related deaths.

## Conclusion

Avelumab displayed antitumor activity with manageable toxicity in patients with previously treated mCRC harboring dMMR/MSI-H. Diagnosis of dMMR/MSI-H with PCR or NGS could be complementary to IHC to select patients who would benefit from immunotherapy.

## Key words

Colorectal neoplasms, Mismatch repair deficiency, Microsatellite instability, *POLE* mutation, Avelumab

## Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide and the third most common cancer in Korea [1]. Standard palliative treatment for metastatic or unresectable CRC (mCRC) is fluorouracil-based combination chemotherapy (with oxaliplatin or irinotecan), with or without agents targeting angiogenesis (bevacizumab) or epidermal growth factor receptor (cetuximab). The available therapeutic options for later-line chemotherapy are limited;

regorafenib and TAS-102 showed only a modest clinical benefit in these patients. The objective response rate (ORR) with regorafenib and TAS-102 was approximately 1%, and median progression-free survival (PFS) was around 2 months for both treatments [2,3]. The long-term outcomes of mCRC are still poor [1], and novel therapeutic approaches are needed.

Growing evidence suggests that patients with mCRC harboring deficient mismatch repair protein (dMMR)/microsatellite instability–high (MSI-H) can obtain clinical benefit from immune checkpoint inhibitors (ICIs) [4-7]. Pembroliz-

zumab and nivolumab, which are anti-programmed death 1 (anti-PD-1) inhibitors, improved ORR and PFS in selected patients with dMMR/MSI-H mCRC [4-7]. Failure to repair DNA replication-associated errors in dMMR/MSI-H mCRC is associated with high mutation loads, tumor neoantigen loads, and dense immune cell infiltration [8]. In fact, the whole-exome sequences revealed higher somatic mutation loads (1,782 mutations per tumor) in patients with dMMR/MSI-H than in patients with proficient MMR (73 mutations per tumor) ( $p=0.007$ ), and a greater density of CD8-positive lymphocytes and a higher expression of PD-ligand 1 (PD-L1) were observed in patients with dMMR/MSI-H than in patients with proficient MMR [4]. However, the clinical benefit of ICIs is confined to a small proportion of patients, because dMMR/MSI-H is identified in only about 5% in patients with mCRC [9]. This raises the need to expand the number of potential candidates for immunotherapy.

The *POLE* gene is located in 12q24.33 and encodes the proofreading (exonuclease) subunit of polymerase epsilon (POLE) with 2,286 amino acids [10]. This *POLE* mutation has been reported in approximately 3% of proficient MMR CRC and represents high somatic mutation loads [10]. According to the Cancer Genome Atlas, up to one-quarter of hypermutated CRC carry somatic mutations in *POLE* [10]. Because high mutation loads are considered a mechanism of the response of dMMR/MSI-H to ICIs, *POLE*-mutated cancer may also be susceptible to ICIs. However, to date, clinical data regarding the response to ICIs in *POLE*-mutated cancer are lacking.

Treatment with avelumab, an anti-PD-L1 inhibitor, achieved an ORR of 33% in patients with previously treated metastatic Merkel cell carcinoma and was approved for the treatment of metastatic Merkel cell carcinoma in early 2017 [11]. Subsequently, avelumab has shown promising antitumor activity in various solid tumors, such as genitourinary tract [12,13], gynecologic [14], and lung [15], but its activity in mCRC harboring dMMR/MSI-H has not been investigated. This study aimed to evaluate the efficacy and safety of avelumab in patients with previously treated CRC with deficient MMR/MSI-H as well as those with *POLE* mutations.

## Materials and Methods

### 1. Study design and patients

This study is a prospective, open-label, multicenter phase II study conducted as a substudy of the K-MASTER project, a nationwide, government-funded precision medicine initiative [16]. Eligible patients were aged  $\geq 20$  years and had histologically or cytologically confirmed metastatic or unresectable adenocarcinoma of the colon or rectum after failure of first-line or later chemotherapy, including fluoropyrimidine, oxaliplatin, or irinotecan, with or without targeted agents (bevacizumab or cetuximab). Patients were enrolled if

dMMR/MSI-H was confirmed by either immunohistochemistry (IHC) or polymerase chain reaction (PCR) by local test at each site, or if *POLE* mutation was confirmed by next-generation sequencing (NGS) certified by the Ministry of Food and Drug Safety, Korea. MMR protein was determined to be deficient by loss of expression of one or more of the following on IHC: MLH1, MSH2, MSH6, and PMS2. MSI-H was diagnosed by PCR if two or more microsatellite markers (BAT-25, BAT-26, D2S123, D5S346, and D17S250) were detected. *POLE* mutations included hotspots such as P286R and other sites. Eligible patients had at least one measurable disease, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate organ function. Any prior treatment with anti-PD-1 or PD-L1 inhibitors was not permitted, and prior immunosuppressive treatment or the last dose of chemotherapy should not be administered within 28 days before the first dose of study drug. Prior radiotherapy was permitted if it was not administered to the target lesions selected for this study.

### 2. Treatment and evaluation

Avelumab was administered at 10 mg/kg intravenously every 2 weeks until disease progression, unacceptable toxicity, or patient refusal occurred. Dose modification was not allowed, but dose delay was permitted at the investigator's discretion in case of clinically significant events. Study treatment was discontinued if there were more than 4 weeks of delay. Response assessment was performed by computed tomography (CT) scan according to Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.1 every 6 weeks. After the end of treatment, patients were followed up for disease status and survival information every 3 months. The medical histories of all patients were obtained before treatment, including physical examination, complete blood count with differential count, serum chemistry, electrolytes, coagulation, carcinoembryonic antigen, thyroid function test (thyroid-stimulating hormone and free thyroxine), urinalysis, electrocardiogram, chest X-ray, CT scan, and other scans if clinically indicated. Adverse events were assessed every cycle according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 4.0.

### 3. Statistical analysis

The sample size was calculated using the Simon two-stage optimal design. The target response rate was set to 30%, and a rate of 10% or below was considered failure, allowing early termination of any ineffective treatment early in the study. With a one-sided type I error of 5% and a power of 0.8, the planned study was to proceed in two steps. If a tumor response occurred in at least two patients after the first 10 patients were listed, the study proceeded to the second stage with 19 additional patients. A total of 29 patients were required, and enrollment of 33 patients was planned,

**Table 1.** Baseline characteristics of the study patients

Characteristic	No. (%)
<b>Age, median (range, yr)</b>	60 (25-88)
<b>Sex</b>	
Male	26 (78.8)
Female	7 (21.2)
<b>ECOG performance status</b>	
0	10 (30.3)
1	23 (69.7)
<b>Primary tumor</b>	
Right-sided colon	22 (66.7)
Left-sided colon	5 (15.2)
Rectum	6 (18.2)
<b>Histology</b>	
Well differentiated	6 (18.2)
Moderately differentiated	19 (57.6)
Poorly differentiated	5 (15.2)
Not assessable	3 (9.1)
<b>RAS status</b>	
Wild	11 (33.3)
Mutant	20 (60.6)
Not done	2 (6.1)
<b>BRAF status</b>	
Wild	22 (66.7)
Mutant	4 (12.1)
Not done	7 (21.2)
<b>Sites of metastasis</b>	
Liver	15 (45.5)
Lung	11 (33.3)
Lymph node, abdomen	20 (60.6)
Peritoneum/Omentum	9 (27.3)
Bone	2 (6.1)
<b>Family history of cancer in any first-degree relative</b>	7 (21.2)
<b>Previous chemotherapy regimen</b>	
FOLFOX	24 (72.7)
FOLFIRI	15 (45.5)
XELOX	4 (12.1)
Capecitabine	10 (30.3)
Others	2 (6.1)
<b>Previous targeted treatment</b>	
Bevacizuamb	25 (75.8)
Cetuximab	2 (6.1)
<b>Prior radiotherapy</b>	7 (21.2)
<b>Prior surgery</b>	
Primary site resection	31 (93.9)
Metastectomy	9 (27.3)

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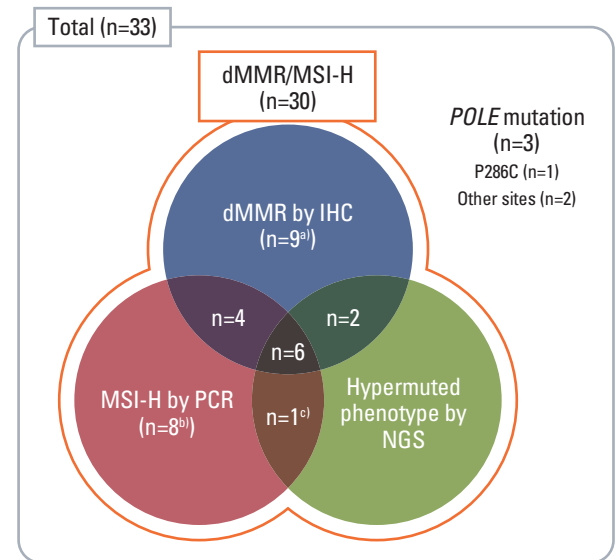
given a dropout rate of 10%.

The primary endpoint was the ORR by RECIST ver. 1.1; the secondary endpoints included the disease control rate (DCR), PFS, overall survival (OS), and adverse events. DCR

**Table 1.** Continued

Characteristic	No. (%)
<b>Prior lines of therapy</b>	
1	16 (48.5)
2	11 (33.3)
≥ 3	6 (18.2)

ECOG, Eastern Cooperative Oncology Group; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; XELOX, capecitabine and oxaliplatin.

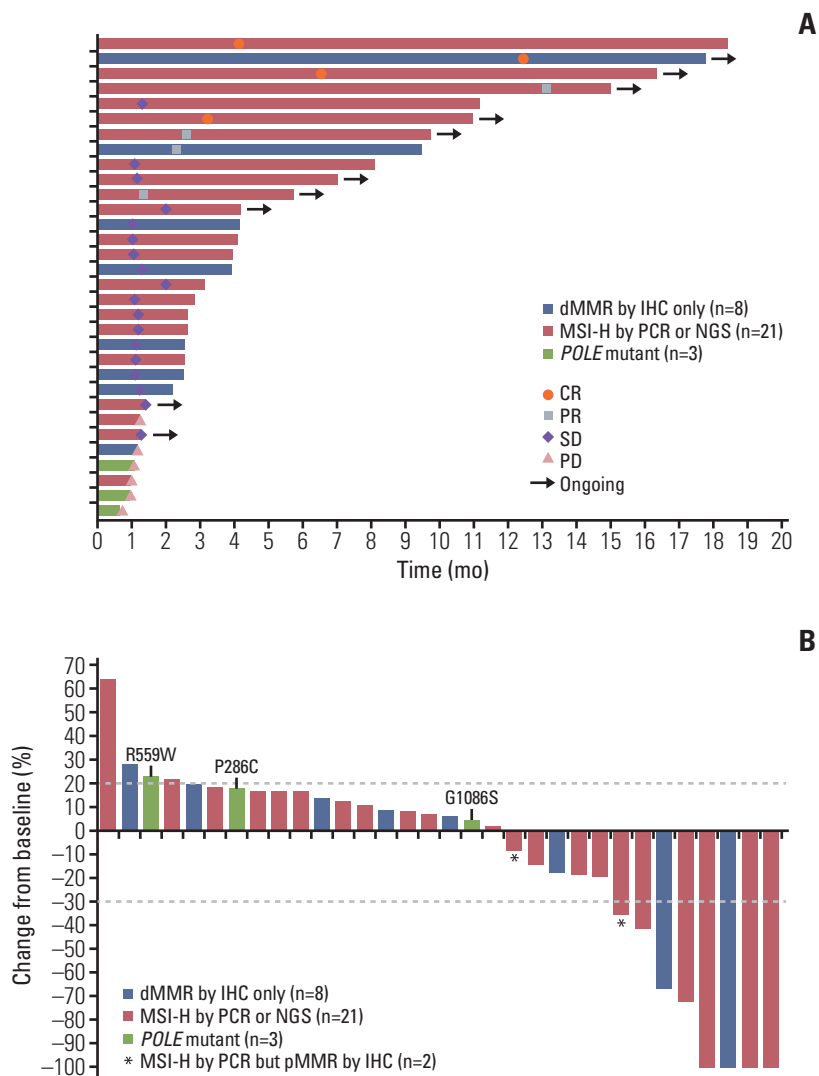


**Fig. 1.** Status of mismatch repair by immunohistochemistry (IHC) or microsatellite instability (MSI) by polymerase chain reaction (PCR) and *POLE* mutation. dMMR, mismatch repair deficiency; MSI-H, MSI-high; MSS, microsatellite stable; NGS, next-generation sequencing; p-MMR, proficient-microsatellite instability. <sup>a</sup>Six of nine were MSS by PCR or NGS, <sup>b</sup>One of eight was p-MMR by IHC, <sup>c</sup>p-MMR by IHC.

was defined as the proportion of patients with complete response (CR), partial response (PR), or stable disease (SD) sustained for ≥ 6 weeks. PFS was calculated from the first date of avelumab administration to the date of disease progression or death from any cause. OS was calculated from the first date of avelumab administration to the date of death from any cause. Survival rates were estimated using the Kaplan-Meier method. A two-sided p-value < 0.05 was considered to indicate statistical significance, and all statistical analyses were performed using IBM SPSS Statistics for Windows ver. 21.0 (IBM Corp., Armonk, NY).

**4. Ethical statement**

All procedures followed were in accordance with the ethi-



**Fig. 2.** Antitumor activity of avelumab in patients with metastatic or unresectable colorectal cancer harboring deficient mismatch repair (dMMR)/microsatellite instability-high (MSI-H) or *POLE* mutations. (A) Treatment duration of avelumab for all patients. (B) Best change from baseline in target lesion size after avelumab. CR, complete response; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD, progressive disease; p-MMR, proficient-microsatellite instability; PR, partial response; SD, stable disease.

cal standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1964 and later versions. The study protocol was approved by the institutional review board of each participating center and the Korean Cancer Study Group (KCSG; protocol No. KCSG-CO-17-07). This trial was registered on <http://www.clinicaltrials.gov> with the identifier NCT0315-0706. Informed consent or a substitute was obtained from all patients before inclusion in the study.

## Results

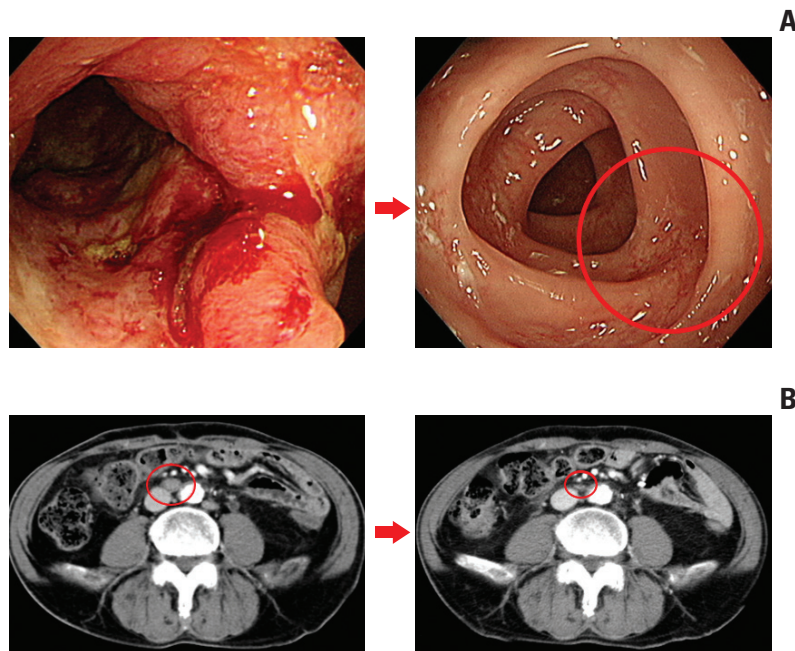
### 1. Patient characteristics

Between May 2017 and April 2019, a total of 34 patients were initially enrolled at seven clinical sites in South Korea; one patient failed the screening tests, and thus, 33 patients with histologically or cytologically confirmed, previously treated, metastatic or unresectable CRC were enrolled in the final analysis. Table 1 summarizes the baseline characteristics of the 33 study patients. The median age was 60 years (range, 25 to 88), and 78.8% were male. Two-thirds of the patients (n=22, 66.7%) had right-sided colon cancer, and 20 patients (60.6%) had RAS mutation. Seven patients had a family his-

**Table 2.** Clinical response to avelumab monotherapy

Response	All patients (n=33)		MSI high by PCR or NGS (n=21)	
	No. (%)	95% CI (%)	No. (%)	95% CI (%)
Complete response	4 (12.1)	0.97-23.2	3 (14.3)	0-29.3
Partial response	4 (12.1)	0.97-23.2	3 (14.3)	0-29.3
Stable disease	18 (54.5)	37.5-71.5	13 (61.9)	41.1-82.7
Progressive disease	6 (18.2)	5.0-31.4	2 (9.5)	0-22.0
Not assessable <sup>a)</sup>	1 (3.0)	0-8.8	0	-
Objective response rate	8 (24.2)	9.4-38.6	6 (28.6)	9.3-47.9
Disease control rate	26 (78.8)	64.9-92.7	19 (90.5)	77.9-103

MSI, microsatellite instability; PCR, polymerase chain reaction; NGS, next-generation sequencing. <sup>a)</sup>Lost to follow-up (n=1).



**Fig. 3.** Representative images of two patients who achieved partial response during avelumab monotherapy. (A) Endoscopic images of a 44-year-old man with initially metastatic T-colon cancer harboring microsatellite instability-high (MSI-H) by both immunohistochemistry (IHC) and next-generation sequencing. (B) Computed tomography scans of 75-year-old man with recurrent rectal cancer harboring MSI-H by IHC.

tory of cancer in a first-degree relative.

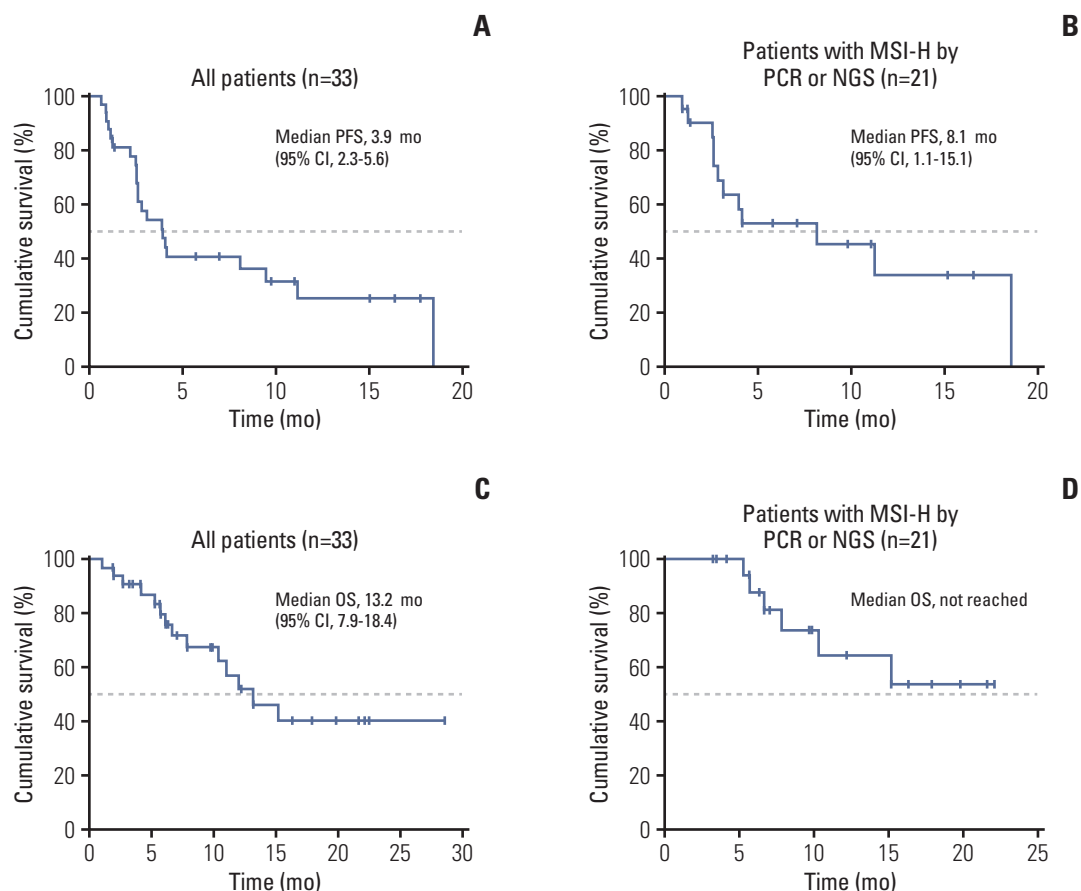
## 2. Deficient MMR or MSI high and *POLE* mutation

Among the 33 patients, 30 patients had dMMR/MSI-H CRC, and three patients had *POLE*-mutated CRC. *POLE* mutation was identified at the P286C, R559W, and G1086S sites. NGS revealed hypermutated phenotype CRC in only one patient with a P286C mutation, but the other sites (R559W and G1086S) were not associated with high tumor mutation burden (TMB) in each different NGS panel (S1 Table).

Among 30 patients with dMMR/MSI-H, IHC, and PCR were performed both in 23 patients, respectively. Both tests at the same time were performed in 16 patients, among whom

10 (62.5%) showed concordance between IHC and PCR. NGS, which was not mandatory for detecting dMMR/MSI-H, was performed in 12 patients with dMMR/MSI-H.

The distribution of patients according to different detection methods is shown in Fig. 1. Twenty-one patients were diagnosed with dMMR by IHC, and 19 were diagnosed with MSI-H by PCR. Discordance between the IHC and PCR results was found as follows: six of nine patients with dMMR by IHC only showed microsatellite stable (MSS) CRC by PCR or NGS, and one of eight patients with MSI-H by PCR only showed proficient MMR by IHC. One patient who was diagnosed as MSI-H by PCR and NGS was diagnosed as proficient MMR by IHC.



**Fig. 4.** Median progression-free survival (PFS) (A, B) and overall survival (OS) (C, D) in all patients (A, C) and patients with microsatellite instability high (MSI-H) (B, D) by polymerase chain reaction (PCR) or next-generation sequencing (NGS). CI, confidence interval.

### 3. Clinical response to avelumab

Avelumab was discontinued mainly due to disease progression (n=22, 66.7%), followed by loss to follow-up (n=1, 0.03%); the remaining 10 patients were treated with ongoing avelumab (Fig. 2A). The median time to response was 4.2 months, and the median duration of response was 13.9 months (Fig. 2A). Among the 33 patients, four (12.1%) had CR, four (12.1%) had PR, 18 (54.5%) had SD, 6 (18.2%) had progressive disease (PD), and one (3.0%) was not assessable (Table 2). The ORR and DCR were 24.2% and 78.8%, respectively. Of 21 patients with MSI-H by PCR or NGS, three (14.3%) had CR, three (14.3%) had PR, 13 (61.9%) had SD, and two (9.5%) had PD (Table 2). The ORR and DCR were 28.6% and 90.5%, respectively. All responders had dMMR/MSI-H CRC, and no patients with a *POLE* mutation achieved response.

The best percentage changes from baseline in target lesion size are shown in Fig. 2B. Six of eight patients with CR or PR showed MSI-H by PCR or NGS. Among six patients with PD, two patients with *POLE* mutations (G1086S and P286C) had PD based on unequivocal progression of nontarget lesions, and all three patients with *POLE* mutations had PD without

any tumor shrinkage. One patient with proficient MMR by IHC only but MSI-H by PCR achieved PR. Representative images of two responders are shown in Fig. 3.

Given the discrepancies between IHC and PCR results and the need to investigate their association with the response to avelumab, IHC results were separately reviewed in available eight of nine patients with dMMR by IHC only at each clinical site. dMMR was revised to proficient MMR by IHC in five patients, who also showed MSS by PCR or NGS. Four of them did not achieved response; however, the other patient achieved CR despite proficient MMR revised after review of the IHC result and MSS by PCR.

### 4. PFS and OS

With a median follow-up duration of 16.3 months (95% confidence interval [CI], 5.6 to 27.1 months), median PFS was 3.9 months (95% CI, 2.3 to 5.6 months) in all patients and 8.1 months (95% CI, 1.1 to 15.1 months) in patients with MSI-H by PCR or NGS (Fig. 4A and B). Median OS was 13.2 months (95% CI, 7.9 to 18.4 months) in all patients and not reached in patients with MSI-H by PCR or NGS (Fig. 4C and D). Overall, the 12-month PFS and OS rates were 36.4% and 66.7%,

**Table 3.** Treatment-related adverse events

Event	All patients (n=33, 100%)	
	Any grade	Grade ≥ 3
Any TRAE	24 (72.7)	6 (18.2)
Myalgia	6 (18.2)	0
Chills	5 (15.2)	0
Infusion-related reaction	5 (15.2)	0
Pruritus	5 (15.2)	0
Thyroid dysfunction	4 (12.1)	0
Skin rash	4 (12.1)	0
Diarrhea	3 (9.1)	2 (6.1)
Fever	3 (9.1)	0
Increased AST or ALT	3 (9.1)	0
Hypomagnesemia	3 (9.1)	0
Fatigue	2 (6.1)	0
Anorexia	2 (6.1)	0
Hyperglycemia	2 (6.1)	1 (3.0)
Increased amylase or lipase	1 (3.0)	2 (6.1)
Nausea	1 (3.0)	0
Sweating	1 (3.0)	0
Dry skin	1 (3.0)	0
Anemia	1 (3.0)	0
Hyperbilirubinemia	0 (0.0)	1 (3.0)

Values are presented as number (%). TRAE, treatment-related adverse event; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

respectively, and 47.6% and 76.2%, respectively, in patients with MSI-H by PCR or NGS.

### 5. Treatment-related adverse events

Treatment-related adverse events (TRAEs) with avelumab are shown in Table 3. TRAEs of any grade were observed in 24 patients (72.7%). Common TRAEs of any grade included myalgia (n=6, 18.2%), chills (n=5, 15.2%), infusion-related reaction (n=5, 15.2%), pruritus (n=5, 15.2%), thyroid dysfunction (n=4, 12.1%), and skin rash (n=4, 12.1%). Grade 3 or 4 TRAEs occurred in six patients (18.2%). Dose interruption due to TRAEs occurred in four patients (12.1%): grade 3 hyperglycemia (n=1), grade 3 lipase increase (n=1), grade 2 aspartate aminotransferase increase (n=1), and grade 2 fever (n=1). There were two discontinuations of treatment due to treatment-related grade 3 hyperbilirubinemia (n=1) and probable treatment-related grade 4 tumor bleeding (n=1) after response to avelumab as a serious adverse event. There were no deaths due to TRAE.

## Discussion

In this open-label, multicenter, phase II study, avelumab showed promising antitumor activity and manageable tox-

icity in patients with mCRC harboring dMMR/MSI-H or *POLE* mutation. The ORR and median PFS were 24.2% and 3.9 months, respectively, and six of eight responders were continuing avelumab treatment with durable response at the end of the analysis. Of note, the ORR and median PFS were 28.6% and 8.1 months, respectively, in patients with MSI-H by PCR or NGS, which were thought to be more reliable methods of determining the MSI status than IHC. Although no patients with *POLE* mutations had response to avelumab, the limitations of small sample size and variation in mutation sites need to be taken into account. TRAEs of any grade and TRAEs of grade 3 or 4 were observed in 72.7% and 18.2% of patients, respectively, which was consistent with previous studies [17]. There were two discontinuations of treatment, one because of a TRAE and the other because of a serious adverse event, probably related to treatment. There were no treatment-related deaths.

The efficacy of avelumab (an anti-PD-L1 inhibitor) for mCRC with dMMR/MSI-H, specifically in patients with MSI-H by PCR or NGS, is comparable to that of pembrolizumab and nivolumab (anti-PD-1 inhibitors) in this setting. In the KEYNOTE-016 trial, only patients with MSI-H CRC had the objective response to pembrolizumab (ORR 40%), whereas none of those with MSS CRC had the objective response [4]. A subsequent multicenter trial of pembrolizumab for dMMR/MSI-H CRC, the KEYNOTE-164 phase II study, resulted in an ORR of 33% and a median PFS of 4.1 months; the 12-month PFS and OS rates were 34% to 41% and 72% to 76%, respectively, according to prior line of treatment [6,7]. Nivolumab also showed promising antitumor activity in terms of ORR (31.1%) in dMMR/MSI-H CRC, and the 12-month PFS and OS rates were 50% and 73%, respectively [5]. All these favorable results in dMMR/MSI-H CRC contrast sharply with those of later-line conventional chemotherapy for overall mCRC with treatments such as regorafenib or TAS-102, which resulted in ORR of only 1% and median PFS of around 2 months [2,3].

Several phase I results from the JAVELIN Solid Tumor Trials have shown promising ORRs and disease stabilization with avelumab in various types of advanced tumors. Specifically, among 53 patients with metastatic or locally advanced previously treated solid tumors, four (8%) achieved responses and 30 (57%) had SD [18]. The ORR with avelumab ranged from 6.7% to 18.2%, depending on tumor type such as metastatic or unresectable previously treated renal cell carcinoma [12], urothelial carcinoma [13], non-small cell lung cancer [15], and ovarian cancer [14]. In a phase II study of 88 patients with chemotherapy-refractory metastatic Merkel cell carcinoma [11], the ORR with avelumab was 33%, including a CR rate of 11.4%. To date, avelumab has been approved for treatment of previously treated metastatic Merkel cell carcinoma, urothelial carcinoma, and renal cell carcinoma in combination with axitinib. Several explorations to expand

its use in various clinical settings are ongoing. The present study adds to the evidence of clinical activity of avelumab by showing durable objective responses in mCRC with dMMR/MSI-H.

dMMR/MSI-H is an established biomarker for the efficacy of ICIs in mCRC, and its predictive value has also been confirmed in other various tumors. MSI-H metastatic gastric cancer (mGC) had a higher ORR with pembrolizumab than did MSS mGC (57% vs. 9%) in the KEYNOTE-059 study [19]. Recently, the clinical benefit of pembrolizumab was demonstrated among patients with dMMR/MSI-H non-CRC, including endometrial cancer, gastric cancer, cholangiocarcinoma, and pancreatic cancer in terms of ORR (34.3%) and median PFS (4.1 months) [20]. Although the exact mechanism is unknown, there are several proposed explanations for T-cell checkpoint blockade [21]. Since dMMR/MSI-H results in diverse neoantigens, T-cell epitopes that are newly formed as a consequence of tumor-specific mutations, which can increase neoantigen-driven T-cell response. Another possible explanation is that dMMR/MSI-H is associated with the activation of signaling pathways through altered cytokines or chemokines, resulting in the tumor microenvironment becoming more inflamed. Cellular stress induced by dMMR/MSI-H promotes innate immune cells, such as T cells and natural killer cells, or tumor recognition.

However, not all patients with mCRC harboring dMMR/MSI-H respond to ICIs, and the TMB varies even within dMMR/MSI-H mCRC. The updated analysis from the initial single-center study showed an ORR of 50% [4], but subsequent multicenter phase II trials revealed ORR of around 30% [5-7]. In the present study, the ORR was 28.6% in patients with MSI-H by PCR or NGS. Approximately 37 to 41 mutations per megabase may be a cutoff value, and low TMB was significantly associated with poor response to ICIs and worse PFS within the dMMR/MSI-H population, which may be an explanation for the heterogeneity in response in recent clinical trials of dMMR/MSI-H CRC [22]. Moreover, substantial genomic variation is observed within dMMR/MSI-H tumors. In particular, the genome-wide intensity of MSI and the accumulation of insertion-deletion mutational loads are responsible for a wide diversity of clinical benefits with ICIs [23], and the activated WNT/ $\beta$ -catenin signaling pathway is associated with immune escape, despite a high TMB and high numbers of tumor-infiltrating lymphocytes [24].

In fact, a considerable portion of the primary resistance of mCRC treated with ICIs may be due to misdiagnosis of dMMR/MSI-H. One study showed that three of five patients (60%) who had PD at the first evaluation were reassessed as MSS by central review with PCR, contrary to the diagnosis of dMMR/MSI-H by local assessment [25]. In the Check-Mate-142 study, there were discrepancies between local and central assessments in 14 of 74 patients (19%) [5]. Most of the patients with assessments (10/14) were initially determined

as dMMR by IHC at the local laboratory, but central review with PCR reclassified them as MSS [5]. In this study, there were some discrepancies between IHC and PCR results, and six of nine patients with dMMR by IHC only showed MSS by PCR or NGS, leading to different ORRs depending on how dMMR/MSI-H CRC was defined. After review of the available IHC results in eight of these patients, five patients were revised to proficient MMR, while their PCR or NGS showed all MSS. The reliability and reproducibility of IHC results have always been a concern, because IHC results are largely affected by intra- and interobserver variation and tissue preservation status [26], and local assessment without central review contributed to these results. Likewise, in the recent phase II study with a similar design, differences in ORRs with avelumab in patients with previously treated endometrial cancer were observed between dMMR determined by IHC only and MSI-H determined by NGS (26.7% vs. 30%) [27]. dMMR/MSI-H could be misdiagnosed if IHC results are not supported by PCR or NGS, so the IHC test alone should be carefully interpreted by experienced pathologists. Further, IHC tests on tumor samples achieved after chemoradiotherapy, old samples, or poorly preserved samples are associated with a high risk of unreliable results.

*POLE*-mutated CRC has been characterized by young age, male predominance, right-sided CRC, earlier stage of disease, and excellent prognosis [10]. *POLE* has a crucial role in chromosomal DNA replication by its proofreading capacity and is known to be mutually exclusive with dMMR/MSI-H [10]. Because of high immunogenicity and enrichment of mutation-associated neoantigens, *POLE*-mutated cancer has been considered a candidate for treatment with ICIs. However, there are limited data, and there is only one case report [28] of a patient with mCRC harboring a *POLE* mutation (V411L) and MSS. This patient had a response after three cycles of pembrolizumab, and CD8 infiltrating lymphocytes with PD-1 expression were observed in the primary colon tumor. All responders in previous reports had mutations at the P286R or V411L sites, which are considered hotspots of *POLE* mutation [10]. Unfortunately, three patients with *POLE* mutations did not respond to ICIs in the present study. One patient with a P286C mutation was associated with a hypermutation phenotype by NGS, but the other two (with R559W and G1086S mutations) did not show high TMB, although they were tested with different panels (S1 Table), and the identified sites of *POLE* mutation might not have been hotspots, which could have led to negative results. Further clinical studies with larger sample sizes are necessary to evaluate the activity of ICIs and its association with sites in *POLE*-mutated CRC.

One of the responders initially showed dMMR by IHC and MSS by PCR at the time of enrollment. However, the IHC result was revised to proficient MMR in the post hoc review. Although NGS could not be performed due to an inadequate



amount of tumor tissue, leaving the *POLE* mutation status of this patient unknown, he achieved CR and has continued avelumab treatment for approximately 18 months (Fig. 3). In this regard, there may be unknown factors to explain the mechanism of response to ICIs other than MMR/MSI status or *POLE* mutation, such as PD-L1 or PD-L2 amplification [29,30], although we could not explore the cause of responsiveness in this patient due to the lack of adequate tumor tissue.

This study has several limitations. The consistency of eligibility and response evaluation could not be fully ensured, because central adjudication of the dMMR/MSI status of tumor tissue and central review of the radiologic response were not performed. Tumor samples were not collected prospectively for research purposes, and therefore translational studies, such as investigation of TMB, tumor-infiltrating lymphocytes, PD-L1 expression, or transcriptome, have not yet been performed. Further *post hoc* studies are planned with available tissue samples to elucidate their association with the response to ICIs.

In conclusion, avelumab is a promising anti-PD-L1 inhibitor in patients with metastatic or unresectable CRC harboring dMMR/MSI-H or *POLE* mutations. For the determination of dMMR/MSI-H, the conventional IHC method alone appears to be insufficient to select patients who would benefit from immunotherapy. Further studies to identify accurate strategies to select optimal candidates for immunotherapy are needed.

#### Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (<https://www.e-crt.org>).

#### Conflicts of Interest

Avelumab was kindly provided by Merck Korea, Seoul, Korea; an affiliate of Merck KGaA, Darmstadt, Germany, as part of an alliance between Merck KGaA and Pfizer. Merck KGaA, Darmstadt, Germany and Pfizer reviewed the manuscript for medical accuracy only before journal submission.

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