Multicenter Phase I/II Trial of Napabucasin and Pembrolizumab in Patients with Metastatic Colorectal Cancer (EPOC1503/SCOOP Trial) AC

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ABSTRACT

Purpose: This is a phase I/II trial to assess the efficacy and safety of napabucasin plus pembrolizumab for metastatic colorectal cancer (mCRC).

Patients and Methods: Phase I was conducted to determine the recommended phase 2 dose (RP2D) in a dose escalation design of napabucasin (240 to 480 mg twice daily) with 200 mg pembrolizumab every 3 weeks. Phase II included cohort A ($n = 10$, microsatellite instability high, MSI-H) and cohort B ($n = 40$, microsatellite stable, MSS). The primary endpoint was immune-related objective response rate (irORR). PD-L1 combined positive score (CPS), genomic profiles, and the consensus molecular subtypes (CMS) of colorectal cancer were assessed.

Results: A total of 55 patients were enrolled in this study. In phase I, no patients experienced dose-limiting toxicities, and napabucasin 480 mg was determined as RP2D. The irORR was 50.0% in cohort A and 10.0% in cohort B. In cohort B, the irORR was 0%, 5.3%, and 42.9% in CPS < 1, 1≤ CPS <10, and CPS ≥ 10, respectively. Patients with objective response tended to have higher tumor mutation burden than those without. Of evaluable 18 patients for CMS classification in cohort B, the irORR was 33.3%, 0%, 33.3%, and 33.3% in CMS1, CMS2, CMS3, and CMS4, respectively. The common grade 3 or higher treatment-related adverse events included fever (10.0%) in cohort A and decreased appetite (7.5%) and diarrhea (5.0%) in cohort B.

Conclusions: Napabucasin with pembrolizumab showed antitumor activity with acceptable toxicities for patients with MSS mCRC as well as MSI-H mCRC, although it did not meet the primary end point. The impact of related biomarkers on the efficacy warrants further investigations in the additional cohort.

See related commentary by Nusrat, p. 5775

Introduction

Immune checkpoint inhibitors targeting the programmed cell death-ligand 1 (PD-L1) and programmed cell death-1 (PD-1) pathway have shown durable responses in patients with microsatellite instability high (MSI-H) metastatic colorectal cancer (mCRC), a population that constitutes 3% to 5% of patients with mCRC (1–3). In contrast, anti–PD-1/PD-L1 antibodies have little clinical benefit in patients with microsatellite-stable (MSS) mCRC (1, 4, 5). Although most patients with MSS mCRC do not have T-cell infiltration and are considered the so-called "cold tumors" (6), the mechanism underlying immune resistance in such tumors remains poorly understood. Currently, several immune checkpoint inhibitor therapies in combination with chemotherapies or targeted agents are being explored in patients with MSS mCRC to turn cold into hot tumors. However, a recent phase III trial of atezolizumab (a PD-L1 inhibitor) plus cobimetinib (an MEK inhibitor) has failed to improve overall survival (OS) compared with regorafenib in the third- or later-line setting (5). Therefore, the development of novel combination therapies to overcome resistance of PD-1 blockade for MSS mCRC is urgently required.

STAT3 has been previously reported as a potential key driver of immune evasion (7, 8). Wang and colleagues showed that STAT3 activity in the tumor microenvironment can mediate immune evasion by blocking the production of proinflammatory cytokines and chemokines that activate immunity and dendritic cells, leading to tumor-specific T-cell responses (7). Previous research also reported that STAT3 signal was associated with increased expressions of the immune checkpoint modulators such as PD-L1 and indoleamine 2,3-dioxygenase 1 (IDO1; refs. 9, 10). Napabucasin is a small-molecule inhibitor of STAT3 that is activated by NAD(P)H:quinone oxidoreductase 1, an antioxidant enzyme overexpressed in many solid tumors (11). Although a phase III trial of napabucasin monotherapy in patients with mCRC did not show the improvement of survival compared with placebo (12), another phase III trial is ongoing with napabucasin in combination with the standard chemotherapy in patients with mCRC, based on promising results of a phase Ib/II clinical study (13). Napabucasin also reduced PD-L1 and IDO1 protein

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

The development of novel combination therapies to overcome resistance of PD-1 blockade for microsatellite-stable (MSS) metastatic colorectal cancer (mCRC) is urgently required. The present study is a phase I/II study to assess the efficacy and safety of napabucasin plus pembrolizumab in patients with mCRC. Our trial demonstrated antitumor activity of napabucasin plus pembrolizumab for patients with MSS mCRC as well as patients with microsatellite instability high mCRC together with related biomarkers such as PD-L1, tumor mutation burden, and the consensus molecular subtypes of colorectal cancer. The impact of related biomarkers on the efficacy warrants further investigations in the additional cohort. Most treatment-related adverse events were manageable without unexpected safety signals.

levels in colon cancer cells stimulated by IFNγ (14), which might reinvigorate the antitumor T-cell response in combination with PD-1 blockade.

The present study is a phase I/II study to assess the efficacy and safety of napabucasin plus pembrolizumab in patients with mCRC.

Patients and Methods

Study design and patients

This is a multicenter phase I/II trial of napabucasin plus pembrolizumab in patients with mCRC. Phase I was conducted to determine the recommended phase 2 dose (RP2D) in a 3 plus 3 cohort-based dose escalation design of napabucasin with pembrolizumab. Phase II was composed of cohort A (MSI-H) and cohort B (MSS). Patients had to fulfill the following criteria: (i) 20 years or older of age; (ii) phase I: histologically confirmed gastrointestinal cancer refractory or intolerant to standard chemotherapy, phase II: histologically confirmed mCRC refractory or intolerant to one or more regimens of the following standard chemotherapies; fluoropyrimidine, irinotecan, and oxaliplatin irrespective of combination with angiogenesis inhibitors; (iii) Eastern Cooperative Oncology Group performance status of 0 or 1; (iv) adequate organ function; and (v) phase II cohort B only: measurable disease by RECIST version 1.1. This study was approved by the Institutional Review Board of each institution and conducted under the Declaration of Helsinki. All patients provided written-informed consent for participation in the study. The study protocol was registered at the ClinicalTrials.gov (NCT02851004).

Procedure

In phase I, patients received oral napabucasin 240 mg b.i.d. on level 1 and 480 mg b.i.d. on level 2 in combination with i.v. pembrolizumab 200 mg every 3 weeks with one cycle as 3 weeks. The first cycle of 28 days consisted of a 7-day napabucasin run-in followed by a 21-day concomitant dosing period. Dose-limiting toxicities (DLT) were evaluated during the first cycle as DLT evaluation period. DLTs were defined as any of the following toxicities judged to be caused due to protocol treatment: grade 4 hematologic toxicity; grade 3 thrombocytopenia requiring transfusions; grade ≥3 febrile neutropenia; liver disorder [aspartate aminotransferase/alanine aminotransferase > 5 x upper limited normal (ULN) and total bilirubin > 3 x ULN]; uncontrollable nonhematologic toxicity of grade ≥3 despite maximal supportive care; and grade 3 or higher immune-related adverse events lasting 8 days or more despite administration of corticosteroid. In phase II, all patients received the RP2D. Dose modifications of study drugs are described in detail in the protocol. Treatment continued until tumor progression, unacceptable toxic effects, or withdrawal of consent.

Computed tomography or magnetic resonance imaging was carried out at baseline and every 6 weeks until 24 weeks (thereafter every 9 weeks until week 42, and every 12 weeks after week 48). Tumor responses were evaluated using immune-related response criteria (irRECIST) or RECIST version 1.1. Adverse events were classified according to the Common Terminology Criteria for Adverse Events version 4.0. Baseline molecular characteristics, such as MSI or mismatch repair (MMR) status and *RAS* or *BRAF* mutational status in mCRC, were analyzed in each institution using formalin-fixed paraffin-embedded tissue specimens from archival tissue samples (Supplementary Methods).

A *post-hoc* exploratory biomarker analyses were performed using either archival or pretreatment biopsy samples if available. PD-L1 expression was assessed by the pathologist (T. Kuwata) using the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies) and measured using the combined positive score (CPS), defined as the number of PD-L1–positive cells (tumor cells, lymphocytes, and macrophages) as a proportion of the total number of tumor cells multiplied by 100. Mutational status including tumor mutation burden (TMB) and the consensus molecular subtypes (CMS) of colorectal cancer was assessed by whole-exome sequencing (WES) and RNA sequencing as previously described (15). The detailed methods of WES and RNA sequencing are available in Supplementary Methods.

Outcomes

The primary endpoint was immune-related objective response rate (irORR) based on irRECIST (16). According to the irRECIST, disease progression detected by the imaging evaluation was confirmed by the following imaging evaluation at 4 weeks or later, if patients were clinically stable. Secondary endpoints included ORR based on RECIST version 1.1, the disease control rate (DCR), progression-free survival (PFS), immune-related progression-free survival (irPFS) at 12 weeks (16), OS, duration of response (DOR), and adverse events. ORR was defined as the proportion of patients with the best overall response of the complete response (CR) or partial response (PR). DCR was defined as the proportion of patients with the best overall response of CR, PR, or stable disease (SD). PFS was defined as the time from the date of registration until the date of disease progression or the date of death from any cause, whichever occurs first. OS was defined as the time from the date of registration until the date of death from any cause. DOR was defined as the time from the date of first documented objective response until disease progression or the date of death from any cause. A *post-hoc* exploratory endpoint included biomarker analysis such as PD-L1 expression, mutational status, and CMS in archival or pretreatment biopsy samples.

Statistical analysis

In phase II, required sample size for cohort A (10 patients) was determined in an exploratory manner. The enrollment in cohort A was anticipated to be quite slow, as patients with MSI-H form a small minority of mCRC. Therefore, sample size for cohort A was not set up based on the statistical grounds. In cohort B, an optimal two-stage design proposed was applied (17). A stop for inefficacy was planned to be declared if the 12-week irPFS rate was 0% at the first stage (13 patients). Pembrolizumab monotherapy or napabucasin monotherapy provided almost no objective response for patients with MSS mCRC (4, 12). Considering the side effects of napabucasin plus

pembrolizumab, we thought that at least 15% extra in irORR was necessary as clinically meaningful antitumor activity to proceed to a confirmatory trial. Therefore, the primary endpoint (irORR) in cohort B was assessed setting the threshold and expected values as 5% and 20%. A total of 40 patients (13 at the first stage and 27 at the second stage) were calculated as required with one-sided alpha of 5% and power of 90%. The primary endpoint would be met if 5 or more patients achieved immune-related objective response (irCR or irPR) among 40 patients. Confidence intervals (CI) of ORR, DCR, and irPFS were constructed using Clopper and Pearson method. PFS, OS, and DOR were estimated using the Kaplan–Meier method. All the efficacy endpoints were planned to be analyzed for all the patients who received RP2D determined in phase I. All statistical analyses were performed using SAS Release version 9.4 (SAS Institute).

Results

Phase I and patient's deposition

A total of 55 patients were enrolled between November 2016 and June 2018 in this study. In phase I, 5 patients were enrolled in level 1 and 3 patients in level 2 (Supplementary Fig. S1 and Supplementary Table S1). All patients were MSS mCRC. Two patients in level 1 were excluded from DLT evaluation because of disease progression during DLT evaluation period. No DLTs were observed at either level, and then napabucasin 480 mg b.i.d. (level 2) with pembrolizumab was determined as RP2D. Safety profiles are shown in Supplementary Table S2. Two patients (1 in level 1 and 1 in level 2) had SD, and the remaining 6 had PD. One patient with SD > 4 months in level 2 showed a decrease in tumor size from baseline.

Phase II

Patients

In phase II cohort B, 3 of 13 patients enrolled in the first stage achieved irPFS at 12 weeks. Therefore, we enrolled additional 27 patients for the second stage (Supplementary Fig. S1). **Table 1** summarizes baseline characteristics of 10 patients in cohort A (MSI-H) and 40 patients in phase II cohort B (MSS). Among the first enrolled 11 patients with left-sided colon cancer in cohort B, no patients achieved irPFS at 12 weeks (3 SD and 8 PD), whereas 3 of 4 patients with right-sided colon cancer achieved irPFS at 12 weeks (1 PR and 2 SD) at the time. Thus, we hypothesized that the study treatment might be more effective for patients with right-sided colon cancer than those with left-sided colon cancer, leading to the restriction of the enrollment in cohort B to patients with right-sided colon cancer. We did this restriction simply based on clinical findings in the middle of this study. Also, we did not make an amendment to the protocol regarding this restriction, as it was just a practical change. As a result, 27 (67.5%) patients had right-sided colon cancer, and 13 (32.5%) patients had left-sided colon cancer in cohort B.

Data cutoff for the analysis of safety and efficacy analysis was August 31, 2018, with a median follow-up of 10.4 months (range, 3.3–14.1 months) in cohort A and 6.3 months (range, 1.1–15.4 months) in cohort B. The median number of treatment cycle was 14.5 (range, 1–20) in cohort A and 2.5 (range, 1–14) in cohort B. At the data cutoff, 7 (70%) patients in cohort A and 2 (5%) patients in cohort B were ongoing treatment. All other 3 patients in cohort A discontinued the protocol treatment due to disease progression. Other 38 patients in cohort B discontinued the protocol treatment due to disease progression ($n = 37$) and treatment-related adverse events ($n = 1$).

Table 1. Baseline characteristics.

Characteristics	Cohort A (MSI-H) ($n = 10$, %)	Cohort B (MSS) ($n = 40$, %)
Median age, years (range)	53 (30–77)	63 (25–79)
Sex		
Male	5 (50.0)	17 (42.5)
Female	5 (50.0)	23 (57.5)
ECOG PS		
0	10 (100)	34 (85.0)
1	0 (0)	6 (15.0)
Primary site		
Right-sided colon	10 (100)	27 (67.5)
Left-sided colon	0 (0)	13 (32.5)
Metastatic organ		
Liver	2 (20.0)	28 (70.0)
Lung	2 (20.0)	29 (72.5)
Lymph node	7 (70.0)	17 (42.5)
Peritoneum	7 (70.0)	12 (30.0)
Number of prior chemotherapy		
≤2	6 (60.0)	12 (30.0)
≥3	4 (40.0)	28 (70.0)
Prior regimens		
Fluoropyrimidine	10 (100)	40 (100)
Oxaliplatin	10 (100)	40 (100)
Irinotecan	10 (100)	40 (100)
Angiogenesis inhibitor	9 (90.0)	40 (100)
Anti-EGFR inhibitor	2 (20.0)	17 (42.5)
Regorafenib	1 (10.0)	17 (42.5)
TAS-102	0 (0)	23 (57.5)
RAS status		
Wild-type	5 (50.0)	14 (35.0)
Mutant	5 (50.0)	25 (62.5)
Unknown	0 (0)	1 (2.5)
BRAF V600E status		
Wild-type	5 (50.0)	28 (70.0)
Mutant	2 (20.0)	2 (5.0)
Unknown	3 (30.0)	10 (25.0)
MSI or MMR status		
MSI-high or MMR-deficient	10 (100)	0 (0)
MSS or MMR-proficient	0 (0)	40 (100)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Antitumor activity and tumor biomarkers

The irORR was 50.0% (5 of 10 patients; 95% CI, 18.7–81.3) in cohort A and 10.0% (4 of 40 patients; 95% CI, 2.8–23.7) in cohort B (**Table 2** and **Fig. 1**). Thus, the primary endpoint was not met. The ORR was completely consistent with the irORR. The median DOR

Table 2. Tumor responses according to irRECIST.

	Cohort A (MSI-H) ($n = 10$)	Cohort B (MSS) ($n = 40$)
Best response, *n* (%)		
CR	1 (10.0)	0 (0)
PR	4 (40.0)	4 (10.0)
SD	4 (40.0)	14 (35.0)
Disease progression	1 (10.0)	21 (52.5)
Not evaluated	0 (0)	1 (2.5)
ORR, % (95% CI)	50.0 (18.7–81.3)	10.0 (2.8–23.7)
DCR, % (95% CI)	90.0 (55.5–99.7)	45.0 (29.3–61.5)
DOR, months, median (95% CI)	NR (7.9–NR)	9.0 (1.7–9.0)

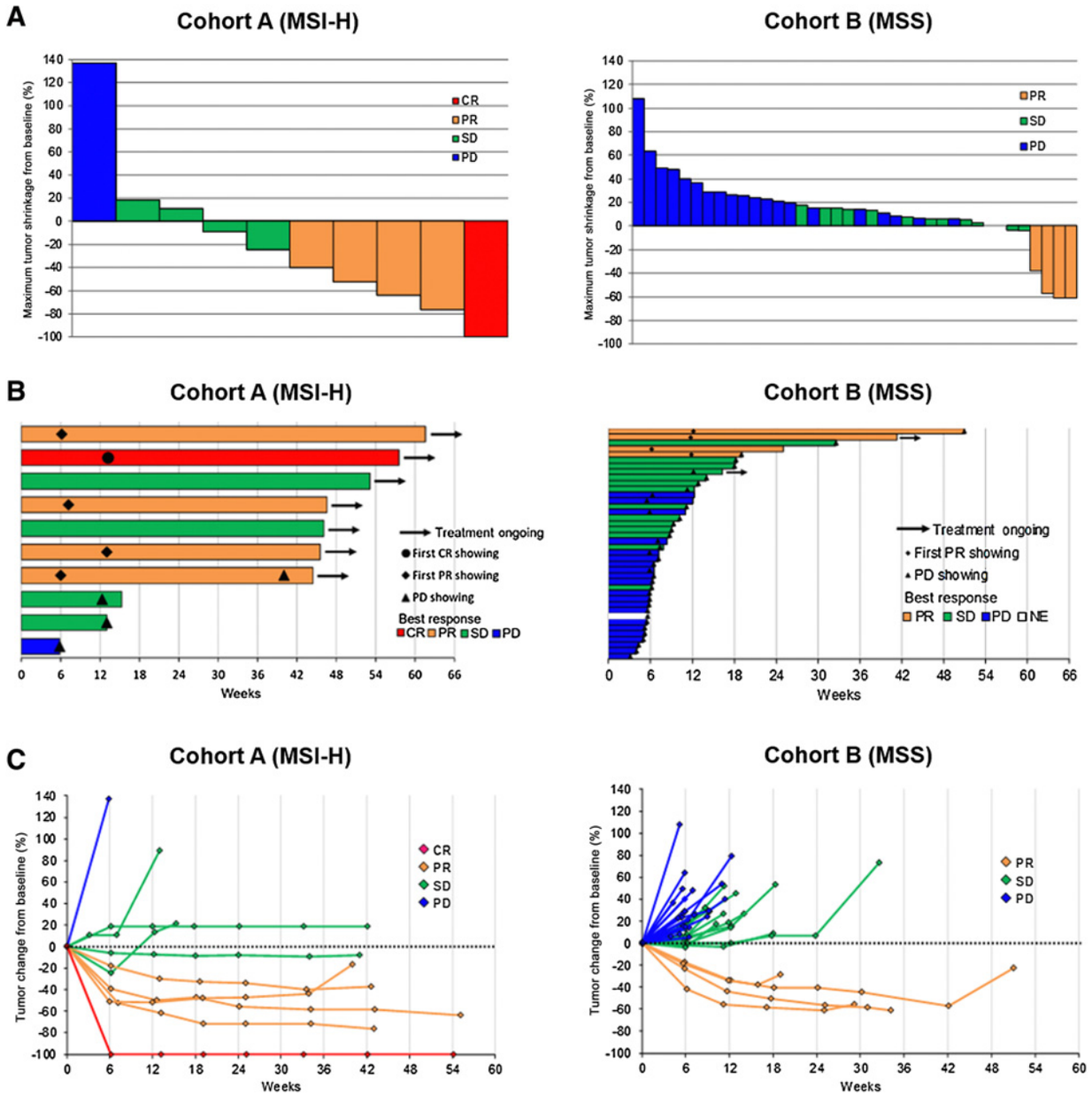

Figure 1.

A, Waterfall plot of maximum percent change in tumor size from baseline as measured by irRECIST. **B,** Time on treatment. **C,** Longitudinal change in irRECIST percentage from baseline. PD, progressive disease.

was not reached (NR; 95% CI, 7.9–NR) in cohort A and 9.0 months (95% CI, 1.7–9.0) in cohort B. All 4 patients with objective response in cohort B had right-sided colon, resulting in the irORR of 14.8% (4 of 27 patients) in patients with right-sided colon and 0% (0 of 13 patients) in patients with left-sided colon. None of 4 patients with objective response in cohort B had undergone radiation before starting the trial. Among the 4 patients with objective response, 1 had *RAS/BRAF* wild-type tumor, 2 had *RAS* mutations, and the remaining 1 had *BRAF* V600E mutation. More detailed characteristics and computed tomography during the study treatment of the 4 responders in cohort B are shown in Supplementary Table S3 and Supplementary Fig. S2. The DCR was 90.0% (95% CI, 55.5–99.7) in cohort A and 45.0% (95% CI, 29.3–61.5) in cohort B. Two patients with SD showed a decrease in tumor size from baseline, and 3 patients showed SD > 4 months in cohort B (**Fig. 1**).

The median PFS was NR (95% CI, 1.4–NR) in cohort A and 1.6 months (95% CI, 1.4–2.1) in cohort B (Supplementary Fig. S3). The median OS was NR (95% CI, 3.3–NR) in cohort A and 7.3 months (95% CI, 5.3–11.8 months) in cohort B.

PD-L1 CPS could be evaluated in 10 patients in cohort A and 36 patients in cohort B. All patients in cohort A had CPS ≥ 1. In cohort B,

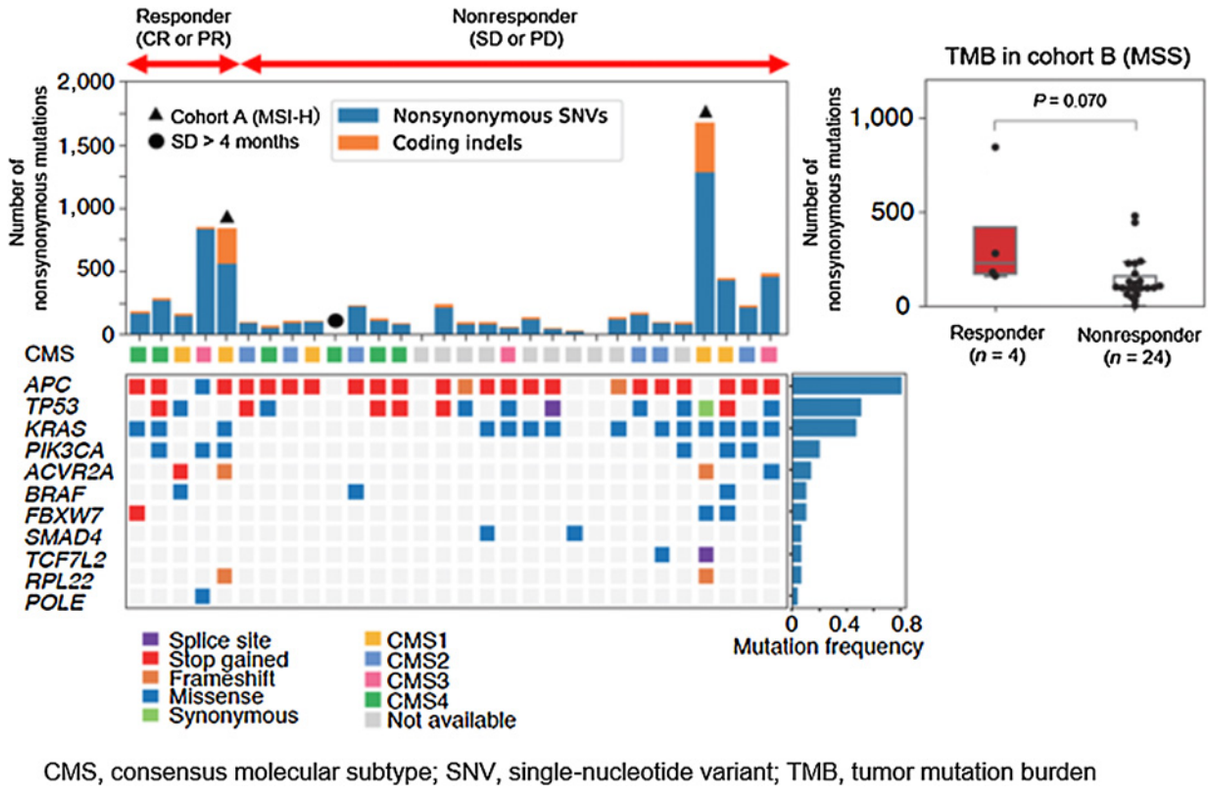

Figure 2.
Genomic profiles and the CMS of colorectal cancer.

the irORR was 0% (0 of 10 patients), 5.3% (1 of 19 patients), and 42.9% (3 of 7 patients) in CPS <1, 1≤ CPS <10, and CPS ≥10, respectively (Supplementary Table S4). TMB could be evaluated in 2 patients in cohort A and 28 patients in cohort B. Patients with objective response tended to have higher TMB than those without in cohort B, although the difference was not significant (**Fig. 2**). CMS could be evaluated in 2 patients in cohort A and 18 patients in cohort B, all of which were analyzed using primary tumor samples. Of evaluable 18 patients for CMS classification in cohort B, CMS1, CMS2, CMS3, and CMS4 were detected in 3, 6, 3, and 6 cases, respectively (**Fig. 2**). The irORR was 33.3% (1 of 3 patients), 0% (0 of 6 patients), 33.3% (1 of 3 patients), and 33.3% (2 of 6 patients) in CMS1, CMS2, CMS3, and CMS4, respectively. One CMS3 patient with PR had *POLE* mutation, whereas 1 CMS1 and 2 CMS4 patients with PR did not. One patient with SD > 4 months in cohort B showing tumor shrinkage also had CMS 4. Of evaluable 2 patients for CMS in cohort A, both the patents had CMS1. One showed PR, and the other with *STK11* mutation showed PD.

Safety

Adverse events are shown in **Table 3**. The most common grade 3 or higher treatment-related adverse events included fever (10.0%) in cohort A and diarrhea (5.0%) and decreased appetite (7.5%) in cohort B, without unexpected safety signals. Severe adverse events were observed in 1 (10.0%) patient in cohort A and 7 (17.5%) patients in cohort B, and 4 events in cohort B were related to study treatment. No treatment-related deaths occurred. One patient with PR in cohort B discontinued the study treatment due to grade 3 rash. Treatment-related adverse events led to at least one dose reduction of napabucasin in 8 (80%) in cohort A and 17 (42.5%) patients in cohort B.

Discussion

Our trial demonstrated antitumor activity of napabucasin plus pembrolizumab for patients with MSS mCRC as well as patients with MSI-H mCRC together with related biomarkers such as PD-L1, TMB, and CMS, although it did not meet the primary end point. To the best of our knowledge, this is the first report of a study evaluating the combination activity of napabucasin plus pembrolizumab for patients with mCRC. Most treatment-related adverse events were manageable without unexpected safety signals.

In the multicohort KEYNOTE-028 trial, pembrolizumab monotherapy provided the ORR of 0% for patients with MSS mCRC with PD-L1–positive status (4). Also, no patients achieved objective response in the phase III trial comparing napabucasin monotherapy with placebo for mCRC (12). In contrast, the irORR was 10% with the median DOR of 9.0 months for patients with MSS mCRC in this study (phase II cohort B). These results suggested favorable outcomes of napabucasin plus pembrolizumab for MSS mCRC than those of single agents in previous studies, though cross trial comparison should be carefully interpreted because this study is the phase I/II trial with selected population. Encouraging antitumor activity of this combination was also observed in cohort A with the irORR of 50% and the median DOR of NR, comparable with that of pembrolizumab monotherapy for MSI-H mCRC in the KEYNOTE-164 trial (18), which warrants further investigations in a larger cohort.

Table 3. Treatment-related adverse events (≥ 10% or any toxicities with grade 3 or higher).

	Cohort A (MSI-H) $n = 10$		Cohort B (MSS) $n = 40$	
Adverse event, *n* (%)	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
All events	10 (100)	1 (10)	40 (100)	10 (25)
Diarrhea	8 (80)	0 (0)	35 (87.5)	2 (5.0)
Decreased appetite	0 (0)	0 (0)	13 (32.5)	3 (7.5)
Nausea	4 (40)	0 (0)	11 (27.5)	1 (2.5)
Vomiting	1 (10)	0 (0)	7 (17.5)	0 (0)
Fever	4 (40)	1 (10)	6 (15.0)	0 (0)
Malaise	0 (0)	0 (0)	6 (15.0)	0 (0)
Fatigue	0 (0)	0 (0)	5 (12.5)	0 (0)
Hypothyroidism	2 (20)	0 (0)	5 (12.5)	0 (0)
Rash	2 (20)	0 (0)	3 (7.5)	1 (2.5)
Abdominal pain	2 (20)	0 (0)	2 (5.0)	1 (2.5)
Anemia	0 (0)	0 (0)	2 (5.0)	1 (2.5)
Hypokalemia	0 (0)	0 (0)	2 (5.0)	1 (2.5)
Alanine aminotransferase increased	0 (0)	0 (0)	2 (5.0)	0 (0)
Infusion-related reaction	1 (10)	0 (0)	1 (2.5)	0 (0)
Hearing impaired	0 (0)	0 (0)	1 (2.5)	1 (2.5)
Colitis	0 (0)	0 (0)	1 (2.5)	1 (2.5)
Serum amylase increased	0 (0)	0 (0)	1 (2.5)	1 (2.5)
Alkaline phosphatase increased	0 (0)	0 (0)	1 (2.5)	1 (2.5)
Platelet count decreased	1 (10)	0 (0)	0 (0)	0 (0)
Cheilitis	1 (10)	0 (0)	0 (0)	0 (0)

An exploratory analysis in this study showed a trend of higher irORR with PD-L1 CPS ≥1 or CPS ≥10 population than CPS <1 population, which were similar to that of previous trials with pembrolizumab for several malignancies (19, 20). Previous studies reported that inhibition of STAT3 activation shows antitumor activity by suppressing polarization of tumor-associated macrophages, which is more frequently observed in PD-L1–positive population than negative population (21, 22).These findings suggest that napabucasin plus pembrolizumab might be more effective for PD-L1–positive population. Patients with objective response tended to have higher TMB than those without in this study, which were consistent with the results of previous reports of anti–PD-1 therapies (23, 24). Optimal cutoff value as well as impact of TMB on the efficacy of this combination warrants further investigations in larger cohorts. Except for one CMS 3 patient with *POLE* mutation, which is reported to be associated with hypermutated phenotype and response to anti–PD-1 monotherapy (25), the remaining 3 patients with objective response had CMS4 ($n = 2$) or CMS1 ($n = 1$) in cohort B. One MSS patient with SD > 4 months showing tumor shrinkage also had CMS 4. It is well known that STAT3 induces angiogenic molecules such as vascular endothelial growth factor directly (26). CMS4 is immune-excluded tumors; immune system is engaged, but microenvironment such as angiogenesis or TGFβ prevents activity (15).Thus, inhibition of angiogenesis via STAT3 signal by napabucasin might enhance antitumor T-cell response of pembrolizumab in patients with CMS4. Considering that CMS1 is inflamed tumors with infiltrating activated lymphocytes as well as high expressions of immune checkpoint molecules (15), patients with CMS1 might also respond to this combination via the reduction of immune checkpoint molecules by napabucasin. Interestingly, a higher ORR was observed in patients with right-sided primary compared with those with left-sided primary in this study. Right-sided colon is reported to be associated with higher frequency of CMS1 and increased infiltration of immune cells with enhanced cytotoxic function compared with left-sided colon (15, 27), which might be one of the reasons for the different response to this combination according to tumor sidedness.

The safety profiles in this study were almost similar to napabucasin monotherapy or pembrolizumab monotherapy (1, 4, 12, 18, 19). Common toxicities such as diarrhea, decreased appetite, nausea, and vomiting predominantly due to napabucasin were manageable with dose reductions, dose interruptions, and supportive care.

This study had several limitations. The patient population was small, and the study was not randomized as the phase I/II trial. Also, we limited the enrollment in cohort B to patients with right-sided colon cancer during this study, leading to patient selection bias. Also, we did not analyze PD-L1 CPS, cancer genome alterations including TMB, and CMS in all the patients enrolled in this study. Furthermore, we did not show predictive biomarkers directly related to napabucasin. These issues warrant further evaluations in the additional cohort. However, it is notable that 4 patients with mCRC MSS responded to this combination in association with tumor biomarkers, which might provide new insight into further development of immune checkpoint inhibitors for mCRC.

In conclusion, the combination of napabucasin plus pembrolizumab had antitumor activity and manageable safety profiles in patients with mCRC. The impact of related biomarkers or tumor sidedness on the efficacy of this combination is under investigation in the additional cohort.

Disclosure of Potential Conflicts of Interest

A. Kawazoe reports personal fees from Taiho, personal fees from Ono, and grants from MSD outside the submitted work. Y. Kuboki reports grants and personal fees from Taiho Pharma, grants from Takeda Pharma, grants from Ono Pharma, grants from AstraZeneca, grants from Daiichi-Sankyo, grants from Boehringer Ingelheim, grants from Amgen, grants from Chugai Pharma, grants from GSK, grants from Incyte, personal fees from Sanofi, personal fees from Eli Lilly, and personal fees from Bayer outside the submitted work. E. Shinozaki reports other from MSD (clinical trial fee) and other from Dainippon Sumitomo Pharma (clinical trial fee) outside the submitted work. H. Hara reports grants from Astellas, grants from AstraZeneca, grants from BeiGene, grants from Boehringer Ingelheim, grants and personal fees from Chugai, grants and personal fees from Daiichi Sankyo, grants from Dainippon Sumitomo, grants from Eisai, grants from Incyte, personal fees from Kyowa Hakko Kirin, personal fees from Lilly, grants from LSK BioPharma, grants and personal fees from Merck Biopharma, grants and personal fees from MSD, grants and personal fees from Ono, grants from Pfizer, personal fees from Sanofi, grants and personal fees from Taiho, personal fees from Takeda, personal fees from Yakult, personal fees from Bayer, and personal fees from Bristol-Myers Squibb outside the submitted work. T. Nishina reports grants and personal fees from Taiho Pharmaceutical, grants and personal fees from Merck Biopharma, grants and personal fees from Bristol-Myers Squibb, grants and personal fees from Ono Pharmaceutical, grants from MSD, grants from Daiichi Sankyo, grants from Dainippon Sumitomo Pharma, grants from Astrazeneca, grants from Astellas pharma, personal fees from Chugai Pharmaceutical, and personal fees from Nippon kayaku outside the submitted work. Y. Komatsu reports grants from Dainihon-Sumitomo (institution) and grants from MSD (institution) during the conduct of the study. M. Wakabayashi reports personal fees from Chugai Pharmaceutical Co., Ltd. (lecture fees) and personal fees from Johnson and Johnson K.K. MEDICAL COMPANY (lecture fees) outside the submitted work. S. Nomura reports personal fees from AstraZeneca, personal fees from Chugai, personal fees from Taiho Pharmaceutical, and personal fees from Pfizer outside the submitted work. A. Sato reports other from MSD (Investigational Drug Supply) and grants and other from Dainippon Sumitomo Pharma (Investigational Drug Supply and study grant) during the conduct of the study; grants and other from Eizai Pharma (for Investigator Initiated Trial), grants and other from Ono Pharma (for Investigator Initiated Trial), grants and other from Taiho Pharma (for Investigator Initiated Trial), grants and other from Takeda Pharma (for Investigator Initiated Trial), grants and

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Authors' Contributions

A. Kawazoe: Writing-original draft. **Y. Kuboki:** Conceptualization. **E. Shinozaki:** Writing-review and editing. **H. Hara:** Writing-review and editing. **T. Nishina:** Writing-review and editing. **Y. Komatsu:** Writing-review and editing. **S. Yuki:** Writing-review and editing. **M. Wakabayashi:** Data curation, software, formal analysis. **S. Nomura:** Writing-review and editing. **A. Sato:** Writing-review and editing. **T. Kuwata:** Supervision. **M. Kawazu:** Formal analysis, methodology. **H. Mano:** Supervision. **Y. Togashi:** Supervision. **H. Nishikawa:** Supervision. **T. Yoshino:** Conceptualization.

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