The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

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ABSTRACT

BACKGROUND

Somatic mutations have the potential to encode "non-self" immunogenic antigens. We hypothesized that tumors with a large number of somatic mutations due to mismatch-repair defects may be susceptible to immune checkpoint blockade.

METHODS

We conducted a phase 2 study to evaluate the clinical activity of pembrolizumab, an anti–programmed death 1 immune checkpoint inhibitor, in 41 patients with progressive metastatic carcinoma with or without mismatch-repair deficiency. Pembrolizumab was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days in patients with mismatch repair–deficient colorectal cancers, patients with mismatch repair–proficient colorectal cancers, and patients with mismatch repair–deficient cancers that were not colorectal. The coprimary end points were the immune-related objective response rate and the 20-week immune-related progression-free survival rate.

RESULTS

The immune-related objective response rate and immune-related progression-free survival rate were 40% (4 of 10 patients) and 78% (7 of 9 patients), respectively, for mismatch repair–deficient colorectal cancers and 0% (0 of 18 patients) and 11% (2 of 18 patients) for mismatch repair–proficient colorectal cancers. The median progression-free survival and overall survival were not reached in the cohort with mismatch repair–deficient colorectal cancer but were 2.2 and 5.0 months, respectively, in the cohort with mismatch repair–proficient colorectal cancer (hazard ratio for disease progression or death, 0.10 [P<0.001], and hazard ratio for death, 0.22 [P=0.05]). Patients with mismatch repair–deficient noncolorectal cancer had responses similar to those of patients with mismatch repair–deficient colorectal cancer (immune-related objective response rate, 71% [5 of 7 patients]; immune-related progression-free survival rate, 67% [4 of 6 patients]). Whole-exome sequencing revealed a mean of 1782 somatic mutations per tumor in mismatch repair–deficient tumors, as compared with 73 in mismatch repair–proficient tumors (P=0.007), and high somatic mutation loads were associated with prolonged progression-free survival (P=0.02).

CONCLUSIONS

This study showed that mismatch-repair status predicted clinical benefit of immune checkpoint blockade with pembrolizumab. (Funded by Johns Hopkins University and others; ClinicalTrials.gov number, NCT01876511.)

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This article was published on May 30, 2015, at NEJM.org.

N Engl J Med 2015;372:2509-20.
DOI: 10.1056/NEJMoa1500596

The programmed death 1 (PD-1) pathway is a negative feedback system that represses Th1 cytotoxic immune responses and that, if unregulated, can damage the host.[1-3] It is up-regulated in many tumors and in their surrounding microenvironment. Blockade of this pathway with antibodies to PD-1 or its ligands has led to remarkable clinical responses in patients with many different types of cancer, including melanomas, non–small-cell lung cancer, renal-cell carcinoma, bladder cancer, and Hodgkin's lymphoma.[4-10] The expression of PD-1 ligands (PD-L1 or PD-L2) on the surface of tumor cells or immune cells is an important — but not a definitive — predictive biomarker of response to PD-1 blockade.[4,6-8,11]

In reports of the effects of PD-1 blockade in human tumors, only 1 of 33 patients with colorectal cancer had a response to this treatment, in contrast to substantial fractions of patients with melanomas, renal-cell cancers, and lung tumors who have a response.[10,12] What was different about this single patient? We hypothesized that this patient had mismatch-repair deficiency, because mismatch-repair deficiency occurs in a small fraction of advanced colorectal cancers,[13,14] somatic mutations found in tumors can be recognized by the patient's own immune system,[15] and mismatch repair–deficient colorectal cancers have 10 to 100 times as many somatic mutations as mismatch repair–proficient colorectal cancers.[16-18] Moreover, mismatch repair–deficient cancers contain prominent lymphocyte infiltrates, a finding consistent with an immune response.[19-22] In addition, two of the tumor types that were most responsive to PD-1 blockade in a study by Topalian et al.[10] had high numbers of somatic mutations as a result of exposure to cigarette smoke (lung cancers) or ultraviolet radiation (melanomas).[23,24] Our hypothesis was correct: the tumor of the single patient with colorectal cancer who had a response to PD-1 blockade was mismatch repair–deficient.[25] Therefore, we hypothesized that mismatch repair–deficient tumors are more responsive to PD-1 blockade than are mismatch repair–proficient tumors.

To test this hypothesis, we initiated a phase 2 clinical trial to evaluate immune checkpoint blockade in patients whose tumors had or did not have mismatch-repair deficiency. Because mismatch-repair deficiency in tumors arises through two routes,[26-28] we recruited patients with hereditary nonpolyposis colorectal cancer (also known as the Lynch syndrome), which results from an inherited germline defect in one of four mismatch-repair genes followed by a second inactivating somatic change in the remaining wild-type allele. We also recruited patients with sporadic mismatch repair–deficient tumors, in which both alleles of a mismatch-repair gene are inactivated by somatic mutations or by epigenetic silencing.[29] In either case, the neoplasms that arise harbor hundreds or thousands of mutations.[16,18]

METHODS

PATIENTS

Patients with treatment-refractory progressive metastatic cancer were recruited from three centers for this phase 2 study (Table 1). Three cohorts were evaluated: cohort A included patients with mismatch repair–deficient colorectal adenocarcinomas, cohort B included patients with mismatch repair–proficient colorectal adenocarcinomas, and cohort C included patients with mismatch repair–deficient cancers of types other than colorectal.

STUDY OVERSIGHT

The protocol, available with the full text of this article at NEJM.org, was approved by the institutional review board at each site, and the study was conducted in accordance with the provisions of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All the patients provided written informed consent before study entry. The first author (the principal investigator) and the last author (the Investigational New Drug sponsor) were responsible for oversight of the study. Merck donated the study drug and reviewed the final drafts of the protocol and of this manuscript before submission; they did not participate in the analysis of the data.

STUDY DESIGN

This phase 2 trial was conducted with the use of a Green–Dahlberg two-stage design and included the three parallel cohorts described above. The study agent, pembrolizumab, was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days (Fig. S1 in

Table 1. Demographic and Baseline Characteristics of the Patients.*

Characteristic	Mismatch Repair–Deficient Colorectal Cancer (N=11)	Mismatch Repair–Proficient Colorectal Cancer (N=21)	Mismatch Repair–Deficient Noncolorectal Cancer (N=9)	P Value†
Median age (range) — yr	46 (24–65)	61 (32–79)	57 (34–92)	0.02
Sex — no. (%)				0.72
Female	5 (45)	8 (38)	4 (44)	
Male	6 (55)	13 (62)	5 (56)	
Race — no. (%)‡				0.66
White	8 (73)	17 (81)	8 (89)	
Black	1 (9)	3 (14)	0	
Other	2 (18)	1 (5)	1 (11)	
ECOG performance status — no. (%)§				0.07
0	0	6 (29)	2 (22)	
1	11 (100)	15 (71)	7 (78)	
Cancer type — no. (%)				>0.99
Colon	9 (82)	18 (86)	0	
Rectal	2 (18)	3 (14)	0	
Ampullary or cholangiocarcinoma	0	NA	4 (44)	
Endometrial	0	NA	2 (22)	
Small bowel	0	NA	2 (22)	
Gastric	0	NA	1 (11)	
Histologic grade — no. (%)				0.20
Well or moderately differentiated	7 (64)	18 (86)	4 (44)	
Poorly differentiated	4 (36)	3 (14)	3 (33)	
Other	0	0	2 (22)	
Stage IV cancer — no. (%)	11(100)	21 (100)	9 (100)	>0.99
Liver metastases — no. (%)	6 (55)	11 (52)	6 (67)	>0.99
Median time since initial diagnosis (range) — mo	31 (6–95)	58 (27–192)	23 (2–105)	0.07
Previous therapies — no. (%)				0.89
1	0	0	1 (11)	
2	3 (27)	4 (19)	5 (56)	
3	3 (27)	5 (24)	1 (11)	
>4	5 (45)	12 (57)	2 (22)	
Detected germline mutation or known Lynch syndrome — no. (%)				<0.001
Yes	9 (82)	0	4 (44)	
No	2 (18)	21 (100)	4 (44)	
Unknown	0	0	1 (11)	
BRAF wild type — no. (%)				0.64
Yes	8 (73)	11 (52)	4 (44)	
No	0	1 (5)	0	
Unknown	3 (27)	9 (43)	5 (56)	
KRAS wild type — no. (%)				0.72
Yes	6 (55)	13 (62)	4 (44)	
No	5 (45)	8 (38)	1 (11)	
Unknown	0	0	4 (44)	

* NA denotes not applicable.
† P values are for the comparison between the cohort with mismatch repair–deficient colorectal cancer and the cohort with mismatch repair–proficient colorectal cancer.
‡ Race was self-reported.
§ Eastern Cooperative Oncology Group (ECOG) performance status is a measure of a patient's ability to perform activities of daily living; values range from 0 to 5, with higher scores indicating greater impairment.

Supplementary Appendix 1, available at NEJM.org). Pembrolizumab is a humanized monoclonal anti–PD-1 antibody of the IgG4 kappa isotype that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2 (Fig. S1 in Supplementary Appendix 1).

Safety assessments were performed before each treatment. At the start of each treatment cycle, the total tumor burden was assessed by means of measurement of serum biomarkers. Radiographic assessments were performed at 12 weeks and every 8 weeks thereafter. Further details concerning the clinical protocol are available at NEJM.org.

ANALYSIS OF MISMATCH-REPAIR STATUS

Tumors with genetic defects in mismatch-repair pathways are known to harbor hundreds to thousands of somatic mutations, especially in regions of repetitive DNA known as microsatellites. The accumulation of mutations in these regions of the genome is termed microsatellite instability.[26-28] Mismatch-repair status was assessed in tumors with the use of the MSI Analysis System (Promega), through the evaluation of selected microsatellite sequences that are particularly prone to copying errors when mismatch repair is compromised.[26-28] Additional details are provided in Supplementary Appendix 1.

GENOMIC AND BIOINFORMATIC ANALYSES

Primary tumor samples and matched normal peripheral-blood specimens were obtained from a subgroup of patients with mismatch repair–deficient carcinomas and a subgroup with mismatch repair–proficient carcinomas, for whom sufficient tumor tissue was available for exome sequencing[30] and HLA haplotyping. To assess the potential for mutant peptide binding, somatic exome data combined with each individual patient's major histocompatibility complex (MHC) class I HLA haplotype were applied to an epitope prediction algorithm.[31,32] This algorithm provided an estimate of the total number of mutation-associated neoantigens in each tumor. Additional details are provided in Supplementary Appendix 1.

STATISTICAL ANALYSIS

The primary end points for cohorts A and B were the immune-related objective response rate and the 20-week immune-related progression-free survival rate, assessed with the use of immune-related response criteria.[33] The primary end point for cohort C was the immune-related progression-free survival rate at 20 weeks (Fig. S1 in Supplementary Appendix 1). Immune-related criteria (i.e., one of the types of criteria used to evaluate immune-based therapies) are based on radiographic responses, and unlike Response Evaluation Criteria in Solid Tumors (RECIST), they capture newly developed lesions detected on radiography in the measurement of tumor burden; these criteria are defined and compared with RECIST, version 1.1, in Table S1 in Supplementary Appendix 1. The response rate and 20-week progression-free survival rate were evaluated and reported in this study with the use of both RECIST, version 1.1, and immune-related response criteria. Progression-free survival and overall survival were summarized by means of the Kaplan–Meier method. Details of the hypothesis, the decision rules for the rejection of the null hypotheses, decision rules for early discontinuation of the study in a cohort because of efficacy or futility, and statistical methods are provided in Supplementary Appendix 1.

RESULTS

PATIENTS

A total of 41 consecutive patients were enrolled in the study and treated during the period from September 2013 through January 2015 (Table 1). Recruitment included patients in pursuit of a clinical trial option who were known to have tumors with mismatch-repair defects or who had tumors of unknown status who were then tested. One patient in the cohort with mismatch repair–deficient colorectal cancer was enrolled under an institutional review board eligibility waiver allowing a grade 3 bilirubin level (i.e., higher than the cutoff specified in the inclusion criteria). A total of 32 patients with colorectal cancer were enrolled in cohorts A and B. All patients with colorectal cancer had received two or more previous chemotherapy regimens (a median of four regimens), except for 1 patient with mismatch repair–proficient cancer who had received one chemotherapeutic and one (non–PD-1–based) immunotherapeutic regimen.

Nine patients with mismatch repair–deficient solid tumors other than colorectal cancer were enrolled in cohort C. All patients in cohort C had

Table 2. Objective Responses According to RECIST Criteria.

Type of Response	Mismatch Repair–Deficient Colorectal Cancer (N=10)	Mismatch Repair–Proficient Colorectal Cancer (N=18)	Mismatch Repair–Deficient Noncolorectal Cancer (N=7)
Complete response — no. (%)	0	0	1 (14)*
Partial response — no. (%)	4 (40)	0	4 (57)†
Stable disease at week 12 — no. (%)	5 (50)	2 (11)	0
Progressive disease — no. (%)	1 (10)	11 (61)	2 (29)
Could not be evaluated — no. (%)‡	0	5 (28)	0
Objective response rate (95% CI) — %	40 (12–74)	0 (0–19)	71 (29–96)
Disease control rate (95% CI) — %§	90 (55–100)	11 (1–35)	71 (29–96)
Median duration of response — wk	Not reached	NA¶	Not reached
Median time to response (range) — wk	28 (13–35)	NA¶	12 (10–13)

* The patient had a partial response at 12 weeks, which then became a complete response at 20 weeks.
† One patient had a partial response at 12 weeks.
‡ Patients could not be evaluated if they did not undergo a scan at 12 weeks because of clinical progression.
§ The rate of disease control was defined as the percentage of patients who had a complete response, partial response, or stable disease for 12 weeks or more.
¶ The median time to response was not applicable (NA) because no responses were observed among patients with mismatch repair–proficient colorectal cancer.

received one or more previous therapeutic regimens (a median of two regimens).

PRIMARY END POINT

The immune-related objective response rate in cohort A was 40% (4 of 10 patients; 95% confidence interval [CI], 12 to 74), and the immune-related progression-free survival rate at 20 weeks was 78% (7 of 9 patients; 95% CI, 40 to 97) (Table S2 in Supplementary Appendix 1); the corresponding rates in cohort C were 71% (5 of 7 patients; 95% CI, 29 to 96) and 67% (4 of 6 patients; 95% CI, 22 to 96). In cohort B, which included patients with mismatch repair–proficient colorectal cancers, the immune-related objective response rate was 0% (95% CI, 0 to 20), and the immune-related progression-free survival rate at 20 weeks was 11% (2 of 18 patients; 95% CI, 1 to 35). Both cohorts with mismatch repair–deficient cancers (cohorts A and C) reached the prespecified point at which the protocol indicated that the study reached its primary efficacy end point when 4 patients were free from disease progression at 20 weeks and objective responses on the basis of immune-related response criteria were observed in 4 patients (Table S2 and the Methods section in Supplementary Appendix 1).

The median follow-up was 36 weeks (range, 5 to 55) for patients with mismatch repair–deficient colorectal cancer (cohort A), 20 weeks (range, 4 to 52) for patients with mismatch repair–proficient colorectal cancer (cohort B), and 21 weeks (range, 0.1 to 49) for patients with mismatch repair–deficient noncolorectal cancer (cohort C). All patients for whom the 20-week immune-related progression-free survival rate could be evaluated were followed for at least 20 weeks.

RADIOGRAPHIC EVALUATION

Of the 10 patients with mismatch repair–deficient colorectal cancer (cohort A) who could be evaluated for RECIST, 4 (40%; 95% CI, 12 to 74) had objective responses according to these criteria (Table 2 and Fig. 1, and Fig. S2 in Supplementary Appendix 1). Patients were considered not to have been evaluated unless they underwent a radiographic scan at 12 weeks. The rate of disease control, which was defined as the percentage of patients who had an objective response or whose disease was stable, was 90% in cohort A (9 of 10 patients; 95% CI, 55 to 100). Of the 7 patients in cohort C who could be evaluated, 5 (71%; 95% CI, 29 to 96) had objective responses as defined by RECIST (Table 2 and Fig. 1, and Fig. S2 in Supplementary Appendix 1), and the rate of disease control was 71% (5 of 7 patients; 95% CI, 29 to 96).

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