The genomes of mismatch repair–deficient tumors all harbor hundreds to thousands of somatic mutations, regardless of their cell of origin. We therefore sought to investigate the effects of PD-1 blockade (by the anti–PD-1 antibody pembrolizumab) in mismatch repair–deficient tumors independent of the tissue of origin. In the current study, we prospectively evaluated the efficacy of PD-1 blockade in a range of different subtypes of mismatch repair–deficient cancers (ClinicalTrials.gov number NCT01876391).

Eighty-six consecutive patients were enrolled between September 2013 and September 2016 (table S1). The data cutoff was 19 December 2016. All patients received at least one prior therapy and had evidence of progressive disease prior to enrollment. Twelve different cancer types were enrolled in the study (Fig. 1). All enrolled patients had evidence of mismatch repair deficiency as assessed by either polymerase chain reaction or immunohistochemistry. For most cases, germline sequencing of MSH2, MSH6, PMS2, and MLH1 was performed to determine whether the mismatch repair deficiencies were associated with a germline change in one of these genes (i.e., whether the patients had Lynch syndrome) (table S2). Germline sequence changes diagnostic of Lynch syndrome were noted in 32 cases (48%), with MSH2 being the most commonly mutated gene. In seven additional cases where germline testing was not performed, the patient reported a family history consistent with a diagnosis of Lynch syndrome.

Adverse events during treatment were manageable and resembled those found in other clinical studies using pembrolizumab (table S3). Although 74% of patients experienced an adverse effect, most were low-grade. Endocrine disorders, mostly hypothyroidism, occurred in 21% of patients and were easily managed with thyroid hormone replacement.

Seventy-eight patients had disease that could be evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) (Table 1). Objective radiographic responses were noted in 46 of the 86 patients (53%; 95% confidence interval, 42 to 64%), with 21% (n = 18) achieving a complete radiographic response. Disease control (measured as partial response + complete response + stable disease) was achieved in 66 of the 86 patients (77%; 95% CI, 66 to 85%). Radiographic responses could be separated into two classes. First, in 12 cases, scans at 20 weeks showed stable disease, which eventually converted to an objective response (measured as tumor size reduction in response to therapy, according to RECIST criteria). Second, in 11 additional cases, we observed...
an initial partial response or stable disease at the 20-week scan that later converted to a complete response while treatment was continued. The average time to any response was 21 weeks; the average time to complete response was 42 weeks (Fig. 1).

Of note, the objective response rate was similar between colorectal cancer and other cancer subtypes. Specifically, we observed objective responses in 52% (95% CI, 36 to 68%) of patients with colorectal cancers and in 54% (95% CI, 39 to 69%) of the patients with cancers originating in other organs (tables S4 and S5). There was also no significant difference in the objective response rate between Lynch syndrome–associated and non-Lynch syndrome–associated tumors [46% (95% CI, 30 to 63%) versus 59% (95% CI, 41 to 76%), respectively; \( P = 0.27 \)].

Neither median progression-free survival (PFS) nor median overall survival (OS) has yet been reached (median follow-up time of 12.5 months; Fig. 1), and the study is ongoing. However, the estimates of PFS at 1 and 2 years were 64% and 53%, respectively. The estimates of OS at 1 and 2 years were 76% and 64%, respectively, which is markedly higher than expected considering the advanced state of disease in this cohort ([21]).

The PFS and OS were not significantly different in patients with colorectal cancers relative to those with other cancer types (fig. S1). Neither PFS (hazard ratio (HR) = 1.2 (95% CI, 0.582 to 2.512); \( P = 0.61 \)) or OS (HR = 1.71 (95% CI, 0.697 to 4.196); \( P = 0.24 \)) were influenced by tumors associated with Lynch syndrome.

Eleven patients achieved a complete response and were taken off therapy after 2 years of treatment. No evidence of cancer recurrence has been observed in those patients with an average time off therapy of 8.3 months. Seven other patients had residual disease by imaging, but pembrolizumab was discontinued after reaching the 2-year milestone or because of intolerance to therapy. To date, the average time off
therapy for this group was 7.6 months. As of the data cutoff, none of these patients has shown evidence of progression since discontinuation of pembrolizumab.

Twenty patients with measurable radiographic disease underwent percutaneous biopsies between 1 month and 5 months after the initiation of therapy. Twelve of these biopsies demonstrated no evidence of tumor cells and were shown to have varying degrees of inflammation, fibrosis, and mucin, consistent with an ongoing immune response (fig. S2). The other eight cases showed residual tumor cells. The absence of cancer cells in posttreatment biopsies was a strong predictor of PFS (HR for PFS = 0.189 [95% CI, 0.046 to 0.767]; P = 0.012), with median PFS of 25.9 months versus 2.9 months for biopsies with evidence of residual tumor. Although there was no significant difference in OS between patients whose biopsies were positive or negative for tumor cells, median OS has not yet been reached in patients with negative biopsies (table S6).

Primary clinical resistance to initial therapy with pembrolizumab, as measured by progressive radiographic disease on the first study scan, was noted in 12 patients (14%) (Table 1). After determining the exonic sequences of tumor and matched normal DNA from three of these patients, we compared them to the exomes of 15 primary tumors from patients who had achieved objective responses to the therapy (table S7). The three therapy-insensitive tumors harbored an average of 1413 nonsynonymous mutations, not significantly different from the number in patients with objective responses (1644 nonsynonymous mutations; P = 0.67, Student’s t test). The gene (B2M) encoding β2-microglobulin, a protein required for antigen presentation (22), was not mutated in any of the primary tumors from the resistant group (table S8).

Only five cases of acquired resistance were noted, where patients developed progressive disease after an initial objective response to pembrolizumab. Three of these cases were atypical in that the tumors emerged in occult sites such as the brain (two cases) or bone (one case). All three cases were treated with local therapy (radiotherapy or surgery), and the patients survived and continued treatment with pembrolizumab. However, in accordance with study design, these three patients are listed in Fig. 1 as having progressive disease.

We performed exome sequencing of biopsies of brain metastases from two patients and compared the results with those of their primary tumors (fig. S3 and table S7). In the first case, the primary duodenal tumor and brain metastasis shared 397 nonsynonymous somatic mutations, providing unequivocal evidence that the metastasis was derived from the primary duodenal tumor rather than from an independent tumor. Moreover, the metastasis harbored 1010 nonsynonymous new mutations not present in the primary tumor, while the primary tumor harbored 904 mutations not present in the metastasis (table S9). In the second case, the primary colorectal tumor and brain metastasis shared 548 nonsynonymous somatic mutations, similarly providing unequivocal evidence of a genetic relationship between the two lesions. The brain metastasis harbored 221 nonsynonymous mutations not present in the primary colorectal tumor, while the primary tumor harbored 100 mutations not present in the metastasis (table S10). Of note, the brain metastases from both of these patients contained mutations in the B2M gene. In the patient with the colorectal tumor, a truncating mutation (L15Fb*41) in the B2M gene was identified in the metastasis but not in the primary tumor. The primary duodenal tumor harbored a truncating mutation in β2-microglobulin (V69Wf*34), whereas the metastasis retained this mutation and acquired a second B2M mutation (12L>P; table S7).

We also evaluated the exomes of three primary tumors from patients who originally had stable disease by RECIST criteria at 20 weeks, but whose disease progressed within 8 months of initiating therapy. The average mutational burden was 1647 for this group, similar to those of the other patients described above. Interestingly, two of these three tumors harbored mutations of B2M (table S7).

We next sought to directly test the hypothesis that checkpoint blockade induces peripheral expansion of tumor-specific T cells and that mismatch repair–deficient tumors harbor functional MANA-specific T cells. Deep sequencing of T cell receptor CDR3 regions (TCR-seq) has emerged as a valuable technique to evaluate T cell clonal representation in both tumors and peripheral blood. We performed TCR-seq on tumors from three responding patients (obtained from archival surgical resections) and identified intratumoral clones that were selectively expanded in the periphery (Fig. 2A). These clones were present at very low frequency (often undetectable) in the peripheral blood before pembrolizumab treatment, but many rapidly increased after treatment initiation, followed by a contraction that generally occurred before radiologic responses were observed. To characterize functional T cell clones specific for mutant peptides, we obtained peripheral blood from one of the patients (subject 19). We tested the patient’s posttreatment peripheral blood for reactivity against the 15 top candidate MANAs as identified via a neoantigen prediction algorithm (specified by the patient’s human leukocyte antigen (HLA) class I alleles; see supplementary materials) with an interferon-γ (IFN-γ) ELISpot assay. Counts of spot-forming cells or cytokine activity analyses revealed T cell responses against 7 of 15 peptides (Fig. 2, B and C). We next interrogated the expanded lymphocyte populations against these seven peptides with TCR-seq. Clonal T cell expansion was noted in response to three of the seven peptides (Fig. 2D), with specificity demonstrated by a lack of expansion in response to any other peptide tested (fig. S4). In the peripheral blood, T cell expansion to these three mutant peptides resulted in 142 unique TCR sequences, seven of which were found in the tumor sample (two from MANA1, three from MANA2, and two from MANA4) (Fig. 2D). Of note, the mutant peptides that scored positive all resulted from frameshift mutations—the type of mutation that is most characteristic of mismatch repair–deficient cancers.

All seven of the MANA-reactive TCRs were detectable in peripheral blood at very low frequency (less than 0.02%) before treatment. However, four of the clones rapidly increased in frequency in the peripheral blood after anti–PD-1 treatment (Fig. 2E). Similar to results from the three patients...
Fig. 2. TCR clonal dynamics and mutation-associated neoantigen recognition in patients responding to PD-1 blockade. (A) TCR sequencing was performed on serial peripheral T cell samples obtained before and after PD-1 blockade. Tumor tissue with mismatch repair deficiency was obtained from three responding patients. Shown for each patient are 15 TCR clones with the highest relative change in frequency after treatment (left) that were also found in the original tumor (right panels). (B) Whole-exome sequencing was performed on tumor and matched normal tissue from patient 19. Somatic alterations were analyzed using a neoantigen prediction pipeline to identify putative MANAs. Reactivity to 15 candidate MANAs was tested in a 10-day cultured IFN-γ ELISpot assay. Data are shown as the mean number of spot-forming cells (SFC) per 10⁶ T cells (left) or mean cytokine activity (right) of triplicate wells ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. (C) Seven candidate MANAs were selected for TCR analysis on the basis of ELISpot reactivity. (D) MANA-specific T cell responses were identified against three of seven candidate MANAs (MANA1, MANA2, and MANA4) after 10 days of in vitro stimulation (left panels). MANA-specific clones were identified by significant expansion in response to the relevant peptide and no significant expansion in response to any other peptide tested (fig. S3). Data are shown as the relative change in TCR clone frequency compared to the frequency of that clone after identical culture without peptide. These T cell clones were also found in the original tumor biopsy (right panels). (E) Frequency of MANA-specific clones, carcinoembryonic antigen (CEA), and radiographic response in the tumor [from (D)] were tracked in the peripheral blood before treatment and at various times after pembrolizumab treatment. (F) In vitro binding and stability assays demonstrate the affinity kinetics of each relevant MANA and the corresponding wild-type peptide (when applicable) for their restricting HLA class I allele. The A*02:01-restricted influenza M GILGFVTL epitope was used as a negative control for each assay; known HLA-matched epitopes were used as positive controls when available. Data are shown as counts per second with increasing peptide concentration for binding assays (top) or counts per minute over time for stability assays (bottom). Data points indicate the mean of two independent experiments ± SD. Amino acid abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
analyzed above, the frequencies of these functionally validated MANA-specific T cell clones peaked soon after treatment and corresponded with normalization of the systemic tumor marker, predating objective radiographic response by several weeks. This peak in T cell clonal expansion was followed by decreases in frequency, reminiscent of T cell responses to acute viral infections (Fig. 2E). Because all the MANAs were from frameshift mutations, only MANA2 had a similar wild-type counterpart (differing in the two C-terminal amino acids). The corresponding wild-type peptide bound to HLA with less than 1% of the affinity of the mutant peptide counterpart (Fig. 2F), consistent with the mutation conferring enhanced HLA binding.

To estimate the proportion of cancer patients for whom the results of this study might be applicable, we evaluated 12,019 cancers representing 92 distinct tumor types for mismatch repair deficiency using a next-generation sequencing–based approach (Fig. 3). In accordance with a recent independent estimate using a different approach (29), we found that >2% of adenocarcinomas of the endometrium, stomach, small intestine, colon and rectum, cervix, prostate, bile duct, and liver, as well as neuroendocrine tumors, uterine sarcomas, and thyroid carcinomas, were mismatch repair–deficient. Among these 11 tumor types, 10% of stage I to stage III cancers and 5% of stage IV cancers were mismatch repair–deficient. This represents roughly 40,000 annually stage I to III diagnoses and 20,000 stage IV diagnoses in the United States alone. Because genetic and immunohistochemical tests for mismatch repair deficiency are already widely available, these results tie immunity, cancer genetics, and therapeutics together in a manner that will likely establish a new standard of care. In the future, testing for mismatch repair deficiency in patients who are refractory to other treatments might be considered in order to identify those who may benefit from PD-1 pathway blockade, regardless of tumor type.

REFERENCES AND NOTES

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SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/357/6349/409/suppl/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S10
References (24–36)
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Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade


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Predicting responses to immunotherapy

Colon cancers with loss-of-function mutations in the mismatch repair (MMR) pathway have favorable responses to PD-1 blockade immunotherapy. In a phase 2 clinical trial, Le et al. showed that treatment success is not just limited to colon cancer (see the Perspective by Goswami and Sharma). They found that a wide range of different cancer types with MMR deficiency also responded to PD-1 blockade. The trial included some patients with pancreatic cancer, which is one of the deadliest forms of cancer. The clinical trial is still ongoing, and around 20% of patients have so far achieved a complete response. MMR deficiency appears to be a biomarker for predicting successful treatment outcomes for several solid tumors and indicates a new therapeutic option for patients harboring MMR-deficient cancers.

Science, this issue p. 409; see also p. 358

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