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CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Luis Diaz	Non provisional Application Number (if known):	
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims.

3. The applicable box is checked below:

I. ☒ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original non provisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original non provisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original non provisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Cameron Luitjens/	Date September 27, 2018
Name (Print/Typed) Cameron M. Luitjens, Ph.D., J.D.	Practitioner Registration Number 58,674

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☒ *Total of 1 forms are submitted.

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CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

ABSTRACT

Blockade of immune checkpoints such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) shows promise in patients with cancer. Inhibitory antibodies directed at these receptors have been shown to break immune tolerance and promote anti-tumor immunity. These agents work particularly well in patients with a certain category of tumor. Such tumors may be particularly susceptible to treatment because of the multitude of neoantigens which they produce.

We claim:

1. A method for treating cancer in a patient in need thereof, wherein the patient has been determined to have a tumor that exhibits a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status, the method comprising:

administering an effective amount of an anti-PD-1 antibody to the patient;

wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered the anti-PD-1 antibody, wherein the reference patient has a tumor that does not exhibit a MSI-high or a MMR deficiency status;

and wherein the patient has received a prior cancer therapy drug.

2. The method of claim 1, wherein the patient has been determined to have a tumor that exhibits a MSI-high status when instability of a microsatellite marker in a DNA sequence has been detected in a tumor sample obtained from the patient.

3. The method of claim 2, wherein the microsatellite marker is BAT-25, BAT-26, MONO-27, NR-21 or NR-24.

4. The method of claim 1, wherein the patient has been determined to have a tumor that exhibits a MMR deficiency status when deficiency of a mismatch repair marker in a DNA sequence has been detected in a tumor sample obtained from the patient.

5. The method of claim 4, wherein the mismatch repair marker is POLE, POLD1, or MYH.

6. The method of claim 1, wherein the MSI-high status of the tumor is detected by immunohistochemistry.

7. The method of claim 1, wherein the cancer in the patient has progressed after the patient received the prior cancer therapy drug.

8. The method of claim 1, wherein the outcome that is improved is an improved objective response rate (ORR), an improved progression-free survival (PFS), or an improved overall survival.

9. The method of claim 8, wherein the ORR is an immune-related ORR (irORR), the PFS is an immune-related progression-free survival (irPFS), or both.

10. The method of claim 8, wherein the outcome is assessed in the patient at approximately 20 weeks after administering the anti-PD-1 antibody.

11. The method of claim 1, wherein the anti-PD-1 antibody is administered by intravenous infusion.

12. The method of claim 1, wherein the anti-PD-1 antibody is pembrolizumab.

13. The method of claim 1, wherein the cancer is a metastatic cancer.

14. The method of claim 1, wherein the cancer is a metastatic colorectal cancer.

15. The method of claim 12, wherein the metastatic colorectal cancer is unresectable.

16. The method of claim 1, wherein the prior cancer therapy drug was fluoropyrimidine, oxaliplatin, or irinotecan.

17. A method for treating cancer in a patient in need thereof, the method comprising:

detecting a high microsatellite instability (MSI-high) or a mismatch repair (MMR) deficiency status in a tumor sample from the patient;

wherein the tumor sample exhibits an instability of one or more microsatellite markers or a deficiency of one or more mismatch repair markers;

administering an effective amount of an anti-PD-1 antibody to the patient;

wherein the patient exhibits an outcome that is improved as compared to a corresponding outcome that would be observed in a reference patient that has been administered the anti-PD-1 antibody, wherein reference patient has a tumor that does not exhibit an instability of the one or more microsatellite markers or a deficiency of the one or more mismatch repair markers; and

wherein the patient has received a prior cancer therapy drug.

18. The method of claim 17, wherein the microsatellite marker is BAT-25, BAT-26, MONO-27, NR-21, or NR-24.

19. The method of claim 17, wherein the mismatch repair marker is POLE, POLD1, or MYH.

20. The method of claim 17, wherein the MSI-high status of the tumor is detected by immunohistochemistry.

21. The method of claim 17, wherein the cancer is a metastatic cancer.

22. The method of claim 17, wherein the cancer is a metastatic colorectal cancer.

23. The method of claim 22, wherein the metastatic colorectal cancer is unresectable.

24. The method of claim 22, wherein the metastatic colorectal cancer in the patient has progressed after the patient received the prior cancer therapy drug.

25. The method of claim 17, wherein the outcome that is improved is an improved objective response rate (ORR), an improved progression-free survival (PFS), or an improved overall survival.

26. The method of claim 25, wherein the ORR is an immune-related ORR (irORR), the PFS is an immune-related progression-free survival (irPFS), or both.

27. The method of claim 25, wherein the outcome is assessed in the patient at 20 weeks after administering the anti-PD-1 antibody.

28. The method of claim 17, wherein the anti-PD-1 antibody is administered by intravenous infusion.

29. The method of claim 17, wherein the anti-PD-1 antibody is pembrolizumab.

30. The method of claim 17, wherein the prior cancer therapy is fluoropyrimidine, oxaliplatin, or irinotecan.

CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

CROSS REFERENCE TO RELATED APPLICATIONS

[01] This application is a continuation of U.S. Application No. 15/523,451, filed May 1, 2017, which is a National Stage application under 35 U.S.C. § 371 of International Application No. PCT/US2015/060331, having an International Filing Date of November 12, 2015, which claims the benefit of priority of U.S. Provisional Application No. 62/190,977, filed July 10, 2015 and U.S. Provisional Application No. 62/079,357, filed November 13, 2014, each of which are incorporated herein by reference in their entirety.

[02] This invention was made with government support under CA43460 and CA62924 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

[03] This invention is related to the area of cancer. In particular, it relates to cancer therapy.

BACKGROUND OF THE INVENTION

[04] Microsatellite instability (MSI) is the accumulation of sequencing errors in microsatellites. This occurs in tumors with deficiency in DNA mismatch repair. MSI is present in Lynch Syndrome which is an inherited cancer syndrome that predisposes patients to colon, endometrial, gastric cancer, ovarian, small intestine, liver, hepatobiliary, upper urinary tract, brain, and prostate cancer. MSI is also present in 10-20% of sporadic colorectal, gastric, prostate, lung, ampullary, and endometrial cancers. Between 0.3% and 13% of pancreatic cancers are reported to be MSI as well.

[05] The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis

in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in solid malignancies such as ovarian, colorectal and pancreatic cancer, hepatocellular carcinoma, malignant MEL and RCC. TILs can be expanded *ex vivo* and re-infused, inducing durable objective tumor responses in cancers such as melanoma.

[06] The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. The ligands for PD- 1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in various tumors. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. High expression of PD-L1 on tumor cells (and to a lesser extent of PD-L2) has been found to correlate with poor prognosis and survival in various cancer types, including renal cell carcinoma (RCC), pancreatic carcinoma, hepatocellular carcinoma, ovarian carcinoma and non-small cell lung cancer (NSCLC). Furthermore, PD-1 has been suggested to regulate tumor-specific T cell expansion in patients with malignant MEL. The observed correlation of clinical prognosis with PD-L1 expression in multiple cancers suggests that the PD- 1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

[07] Blockade of immune checkpoints such as cytotoxic T-lymphocyte antigen-4 (CTLA- 4) and programmed death-1 (PD-1) is showing promise in patients with cancer. CTLA-4

and PD-1 are upregulated on activated T cells and provide inhibitory signals to T cells undergoing activation. Inhibitory antibodies directed at these receptors have been shown to break immune tolerance and promote anti-tumor immunity. MK-3475 is a humanized monoclonal IgG4 antibody against PD-1 and is showing activity in multiple tumor types including melanoma and non-small cell lung cancer (NSCLC). Previously, activity of a different PD-1 blocking antibody, BMS-936558, a fully humanized monoclonal IgG4 antibody, also showed activity in melanoma, NSCLC, and a complete response in a single patient with colorectal cancer.

[08] MK-3475 (previously known as SCH 900475) is a potent and highly-selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. MK-3475 contains the S228P stabilizing mutation and has no antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) activity. MK-3475 strongly enhances T lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. In T- cell activation assays using human donor blood cells, the EC50 was in the range of 0.1 to 0.3 nM. MK-3475 also modulates the level of interleukin-2 (IL-2), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and other cytokines. The antibody potentiates existing immune responses only in the presence of antigen and does not nonspecifically activate T- cells.

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

[10] We were intrigued that, in reports of the effects of PD-1 blockade in human tumors, only one of 33 colorectal cancer (CRC) patients responded to this treatment, in contrast to substantial fractions of patients with melanomas, renal cell cancers, and lung tumors.[10,12]. What was different about this single patient? We hypothesized that this patient had MMR-deficiency, because MMR-deficiency occurs in a small fraction of advanced CRCs,[13,14] somatic mutations found in tumors can be recognized by the patient's own immune system,[15] and MMR-deficient cancers have 10- to 100-fold more somatic mutations than MMR-proficient CRC.[16-18] Moreover, MMR-deficient cancers contain prominent lymphocyte infiltrates, consistent with an immune response[19-22]. And 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 UV radiation (melanomas)[23,24]. Our hypothesis was correct: the tumor of the single CRC patient who responded to PD-1 blockade was MMR-deficient[25]. We therefore hypothesized that MMR-deficient tumors are more responsive to PD-1 blockade than are MMR-proficient tumors.

[11] 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 MMR-deficiency. Since MMR deficiency in tumors arises through two routes[26-28], we recruited patients with Hereditary Non-Polyposis Colorectal Cancer (HNPCC, also known as Lynch Syndrome), which results from an inherited germline defect in one of four MMR genes followed by a second inactivating somatic change in the remaining wild-type allele. We also recruited patients with sporadic MMR-deficient tumors, where both alleles of a MMR 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].

[12] There is a continuing need in the art to improve cancer treatments so that the lives of patients are not curtailed and so that the quality of life is not diminished.

SUMMARY OF THE INVENTION

[13] According to one embodiment of the invention a method of treating a cancer patient is provided. The cancer patient has a high mutational burden, such as found in microsatellite instable cancer (MSI). An immune checkpoint inhibitory antibody is administered to the cancer patient.

[14] According to another embodiment of the invention a method of treating a cancer patient is provided. A sample from a cancer patient is tested for one or more microsatellite markers selected from the group consisting of BAT-25, BAT-26, MON0-27, NR-21, NR-24, Penta C, and Penta D, and determined to have microsatellite instability. The cancer is selected from the group consisting of: colon, gastric, endometrial, cholangiocarcinoma, pancreatic, and prostate cancers. An anti-PD-1 antibody is administered to the cancer patient.

[15] According to another embodiment of the invention a method is provided for categorizing a tumor of a human. A sample from the human is tested to evaluate stability of one or more microsatellite markers. Microsatellite instability is determined in the sample. The tumor is identified as a good candidate for treatment with an immune checkpoint inhibitory antibody.

[16] According to yet another embodiment of the invention a method is provided for categorizing a tumor of a human. A sample from the human is tested to evaluate stability of one or more microsatellite markers. Microsatellite stability in the sample is determined. The tumor is identified as a bad candidate for treatment with an immune checkpoint inhibitory antibody.

[17] These and other embodiments which will be apparent to those of skill in the art upon reading the specification provide the art with methods for treating microsatellite instable cancers.

BRIEF DESCRIPTION OF THE DRAWINGS

[18] Figs. 1A-1B. Clinical Responses to pembrolizumab. (Fig. 1A) Biochemical Responses. Serum protein biomarker levels were measured with each cycle and the values represent percent change from baseline. Patients were included if baseline tumor marker values were greater than the upper limit of normal. CA-125 was used for a patient with endometrial cancer; CA19-9 was used for one cholangiocarcinoma and one ampullary cancer; and CEA was used for all other patients. Green, red, and black lines represent patients with MMR-deficient CRCs, MMR-proficient CRCs, and MMR-deficient non-CRC, respectively. (Fig. 1B) Radiographic responses. Tumor responses were measured at regular intervals and values show the best fractional change of the sum of longest diameters (SLD) from the baseline measurements of each measurable tumor.

[19] Figs. 2A-2D. Clinical benefit to pembrolizumab according to MMR status. Kaplan-Meier curves are shown for (Fig. 2A) progression-free survival in the colorectal cancer cohorts, (Fig. 2B) overall survival in the colorectal cancer cohorts, (Fig. 2C) progression-free survival of patients with MMR-deficient cancers other than colorectal (median PFS=5.4 months; 95% CI, 3% to not estimable), and (Fig. 2D) overall survival of patients with MMR-deficient cancers other than colorectal. In both cohorts with MMR-deficient tumors (CRC and non-CRC), median overall survival was not reached. Patients in the cohort with MMR-proficient cancers had a median PFS of 2.2 months (95% CI 1.4 to 2.8%) and a median OS of 5.0 months (95% CI 3.0 to not estimable).

[20] Fig. 3 (Figure S2.) Spider plot of radiographic response**.** Tumor responses were measured at regular intervals and values show percent change of the sum of longest diameters (SLD) from the baseline measurements of each measurable tumor. Patients were only included if baseline and on study treatment scans were available. Green and red represent patients with MMR-deficient and proficient CRCs, respectively. Blue represents patients with MMR-deficient cancers other than CRC.

[21] Figs. 4A-4B **(**Figure S3). MMR-proficient and deficient CRCs have comparable time on treatment and duration of metastatic disease prior to study enrollment**.** Kaplan-Meier estimates of (Fig. 4A) time on therapy immediately prior to study enrollment (HR 0.81, 95% CI 0.38 to 1.752, p=.60) and (Fig. 4B) duration of metastatic disease prior to enrollment (HR 1.13, 95% CI 0.49 to 2.62, p=.78) on this pembrolizumab study were comparable between the MMR-deficient and proficient CRC cohorts. The short duration on prior therapy is expected in a treatment refractory CRC population.

[22] Fig. 5 **(**Figure S4.) Waterfall plot of biochemical response**.** Serum protein biomarker levels were measured with each cycle and the values represent best percent change from baseline. Patients were included if baseline tumor marker values were greater than the upper limit of normal. CA-125 was used for a patient with endometrial cancer; CA19-9 was used for 1 cholangiocarcinoma and 1 ampullary cancer; and CEA was used for all other patients. Green and red represent patients with MMR-deficient and proficient CRCs, respectively. Blue represents patients with MMR-deficient cancers other than CRC.

[23] Figs. 6A-6B (Figure S5.) Somatic mutations in MMR-deficient and proficient tumors. Total somatic mutations per tumor identified by exome sequencing of tumor and matched normal DNA (Fig. 6A) and correlation with objective responses (Fig. 6B) (non-parametric Wilcoxon test , p= 0.007 and Jonckheere-Terpstra test for trend, p=0.02).

[24] Fig. 7 (Figure S6). Immunohistochemistry of CD8 and PD-L1 Expression**.** The invasive front (yellow dashed line) from a MMR-deficient CRC (subject #16, top) and MMR-proficient CRC (subject #3, bottom). The yellow dashed line separates tumor (T) and normal (N) tissue. There is marked expression of PD-L1 (blue arrows) and CD8 (brown dots) in the MMR-deficient tumor (top panels) patient while there is very little expression of either marker in the MMR-proficient tumor (bottom panels). Representative images of

tumor infiltrating lymphocytes (TIL) in another MMR-deficient CRC (subject #19, top) and MMR-proficient CRC (subject #3, bottom) immunolabeled with an antibody to CD8 (brown dots). Note the infiltration of CD8 cells in the MMR-deficient tumor. Invasive front original magnification 10x and TIL 20x.

[25] Fig. 8 (Figure S7.) CD8 and PD-L1 Expression in the MMR-deficient and MMR-proficient tumor microenvironment**.** T cell density units are cells/mm2 of tumor. Invasive front refers to the immune cells (TILs and macrophages) at the junction of the tumor and normal tissue. P-values obtained using an unpaired t-test.

[26] Fig. 9 (Figure S8.) CD8 expression and clinical benefit to pembrolizumab**.** Correlation between the intratumoral $CD8^+$ T cell density (cells/mm2) and objective response (Jonckheere-Terpstra test for trend, p=0.02).

[27] Fig. 10 (Table S1.) Comparison of immune-related and RECIST response criteria (adapted from Wolchok et al. Clin Can Res 2009;15:7412-20.)

[28] Fig. 11 (Table S2.) Immune-Related response to treatment

[29] Fig. 12 (Table S4.) Correlation of total somatic mutations and mutation associated neoantigens (MANA) with clinical outcomes

[30] Fig. 13 (Table S5.) Correlation of immune markers with clinical outcome

DETAILED DESCRIPTION OF THE INVENTION

[31] The inventors have found that immune checkpoint inhibitors work best in tumors with high mutation burdens. Furthermore, tumors deficient in mismatch repair are particularly

susceptible to a particular form of immunotherapy because this phenotype results in ongoing accumulation of mutations at a high frequency. The inventors have developed a treatment for cancer patients that display the microsatellite instability phenotype or other high mutational burden. The treatment involves an inhibitory antibody for an immune checkpoint. Such checkpoints include PD-1, IDO, CTLA-4, PD-L1, and LAG-3. Other immune checkpoints can be used as well. Antibodies can be administered by any means that is convenient, including but not limited to intravenous infusion, oral administration, subcutaneous administration, sublingual administration, ocular administration, nasal administration, etc.

[32] Microsatellite instability (MSI) tumors are deficient in DNA mismatch repair which leads to a high rate of spontaneous mutations and the potential for the expression of neo-antigens. Furthermore, similar to melanoma, in MSI positive colon cancers, there is often prominent lymphocyte infiltration. Any tumors that are MSI or otherwise high mutational burden may be treated according to the invention. They may be tested for the attribute of MSI according to any method known in the art, including but not limited that described in example 1 below. Any of one or more MSI markers can be tested to determine an MSI phenotype. Samples may be tested for high mutational burden by identifying tumors with at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, or at least 1600 mutations per tumor genome. High mutational burden means a large number of somatic mutations in the tumor relative to normal tissues of the individual. An average number of somatic mutations in a non-MSI tumor is about 70 somatic mutations.

[33] Any type of tumor that displays the MSI phenotype or a high mutational burden may be tested and/or treated according to the invention. These include without limitation cancers of the colon, gastric, endometrial, cholangiocarcinoma, pancreatic, and prostate cancer. Tumors of the ampulla, biliary, brain, including glioma, breast, lung, skin, esophagus, liver, kidney, ovaries, sarcoma, uterus, cervix, bladder, testes, oral cavity, tongue, and small and large bowel may also be tested and/or treated.

[34] Testing of MSI can be accomplished by any means known in the art. One or more of the following markers may be tested: five nearly monomorphic mononucleotide repeat markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24) and two highly polymorphic pentanucleotide repeat markers (Penta C and Penta D). In one commercial system which can be used, fluorescently labeled primers (marker panel) are used for co-amplification of all seven of the above named markers. Fragments are detected after amplification for assignment of genotype/phenotype.

[35] Samples that can be tested for MSI include tumor tissue as well as body fluids that contain nucleic acids shed from tumors. Testing for tumor DNA in such tissues and body fluids is well known.

[36] Types of antibodies which can be used include any that are developed for the immune checkpoint inhibitors. These can be monoclonal or polyclonal. They may be single chain fragments or other fragments of full antibodies, including those made by enzymatic cleavage or recombinant DNA techniques. They may be of any isotype, including but not limited to IgG, IgM, IgE. The antibodies may be of any species source, including human, goat, rabbit, mouse, cow, chimpanzee. The antibodies may be humanized or chimeric. The antibodies may be conjugated or engineered to be attached to another moiety, whether a therapeutic molecule or a tracer molecule. The therapeutic molecule may be a toxin, for example.

[37] The data from the small phase 2 trial of pembrolizumab to treat tumors with and without deficiency of MMR supports the hypothesis that MMR-deficient tumors are more responsive to PD-1 blockade than are MMR-proficient tumors. MMR-deficiency occurs in many cancers, including those of the colorectum, uterus, stomach, biliary tract, pancreas, ovary, prostate and small intestine[18,34-42]. Patients with MMR-deficient tumors of these types also benefit from anti-PD-1 therapy, as may patients whose tumors contain other DNA repair deficiencies, such as those with mutations in *POLD, POLE, or MYH.*[18,43,44]

[38] The hypothesis that MMR-deficient tumors stimulate the immune system is not a new idea[45], and has been supported by the dense immune infiltration and Th1-associated cytokine-rich environment observed in MMR-deficient tumors.[19-22,46] A recent study refined these classic observations by showing that the MMR-deficient tumor microenvironment strongly expressed several immune checkpoint ligands including PD-1, PD-L1, CTLA-4, LAG-3 and IDO, indicating that their active immune microenvironment is counterbalanced by immune inhibitory signals that resists tumor elimination[47]. That the immune infiltrate associated with MMR-deficient carcinomas was directed at neoantigens was the most likely explanation for both the old and new findings. The correlation of higher mutational load and higher response rate to anti-CTLA-4 in melanoma[41] and anti-PD-1 in lung cancer[48] provide further support for the idea that MANA recognition is an important component of the endogenous anti-tumor immune response.

[39] Based on the results of the current and previous studies, we suggest that the greatly (>20-fold) increased number of mutation-associated neoantigens resulting from MMR deficiency (Fig.. 12 (Table S4); also available on line at New England Journal of Medicine; incorporated by reference herein) is the basis for the enhanced anti-PD-1 responsiveness of this genetically defined subset of cancers. Though our estimates for the number of mutation-associated neoantigens in tumors is based only on *in silico* predictions of binding-affinity, this suggestion is consistent with the observation that MMR-proficient tumors have far less infiltration of lymphocytes than MMR-deficient tumors (Fig. 7 (S6), Fig. 8 (S7) and Fig. 13 (Table S5); available on line at New England Journal of Medicine; incorporated by reference herein). Recent studies[49,50] show that only a tiny proportion of predicted neo-epitopes are actually presented on the cell surface with MHC and are targets of endogenous T cell responses. It seems likely, though that the number of predicted mutation-associated neoantigens is proportionate to the number of actual mutation-associated neoantigens, and tumors with a high number of actual mutation-associated neoantigens are more likely to stimulate the immune system to react against the tumor. Alternative mechanisms underlying the difference in anti-PD-1 responsiveness between MMR-deficient and MMR-proficient tumors should also be

considered. For example, different signaling pathways activated in MMR-deficient and MMR-proficient tumors may result in differences in secretion of soluble factors that could result in differential activation of the PD-1 pathway within the tumor microenvironment[26-28]. Genetic differences could effect epigenetic differences that alter the expression of tumor-associated self-antigens that in turn could alter the antigenicity of the tumor. Experimental analyses of antigen-specific immune responses as well as changes in immune microenvironments should help to define the relative contribution of these factors to the striking responsiveness of MMR-deficient tumors to PD-1 antibodies.

[40] Several notable observations were made during the course of this study. First, changes in serum protein biomarkers, like CEA, corresponded with clinical benefit after a single dose of therapy. Declines in CEA levels preceded objective radiographic evidence by several months; perhaps other biomarkers such as circulating tumor DNA (ctDNA) may also be beneficial as surrogate markers of early response.[51,52] Second, our results suggest that the evaluation of tumor genomes can help guide immunotherapy. They support the view that the number and type of alterations may prove useful for judging the potential utility of immune checkpoint inhibitors, even in MMR-proficient cancers[41,48,53] Most importantly, our results demonstrate a new approach for the treatment of a specific class of tumors based solely on genetic status: *i.e.,* without regard to underlying tumor type.

[41] The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1

MSI Testing

[42] MSI testing is already standardized and performed in CLIA-certified laboratories without need for assay development. Archived tumor samples or newly obtained biopsies will be used for determining MSI. MSI status will be performed locally by CLIA certified

immunohistochemistry (IHC) or PCR based tests for eligibility. Evaluable patients will be confirmed using the MSI Analysis System from Promega at Johns Hopkins. This test will determine MSI status through the insertion or deletion of repeating units in the five nearly monomorphic mononucleotide repeat markers (BAT-25, BAT-26, MON0-27, NR-21 and NR-24). At least 2 MSI loci are required to be evaluable in Cohorts A and C. Patients may be assigned to a new cohort and/or replaced based on the Promega test results.

EXAMPLE 2

METHODS

[43] Patients

[44] Treatment-refractory progressive metastatic cancer patients for this phase 2 study were recruited from three participating centers (Table 1). Three cohorts were evaluated: Cohort A was composed of patients with MMR-deficient colorectal adenocarcinomas; Cohort B was composed of patients with MMR-proficient colorectal adenocarcinomas; and Cohort C was composed of patients with MMR-deficient cancers of types other than colorectal.

[45] Study Oversight

[46] The protocol, which can be found at NEJM.org, was approved by each site's institutional review boards, and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All the patients provided written informed consent before study entry. The principal investigator (D.L.) and study sponsor (L.A.D.) were responsible for oversight of the study. Merck donated the study drug, reviewed the final drafts of the protocol and of this manuscript. The clinical study was primarily funded through philanthropic support.

[47] Study Design

[48] This phase 2 trial was conducted using a Green-Dahlberg two-stage design and consisted of the three parallel cohorts described above. The study agent, pembrolizumab (Merck), was administered at 10 mg/kg intravenously every 14 days. 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.

[49] Safety assessments were performed before each treatment. Assessments of total tumor burden via measurements of serum biomarkers were performed at the start of each cycle. Radiologic assessments were made at 12 weeks and every 8 weeks thereafter. Further details concerning the clinical protocol are provided in the Example 3.

[50] Analysis of mismatch repair status

[51] Tumors with genetic defects in MMR pathways are known to harbor 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 (MSI) [26-28]. MMR-status was assessed using the MSI Analysis System from Promega in tumors, through the evaluation of selected microsatellite sequences particularly prone to copying errors when MMR is compromised[26-28]. See Supplementary Appendix for additional details.

[52] Genomic & Bioinformatic Analyses

[53] Primary tumor samples and matched normal peripheral-blood specimens were obtained from a subset of subjects with MMR-deficient and others with MMR-proficient carcinomas where 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 the individual patient's MHC class I HLA haplotype was applied to the 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 the

Supplementary Appendix (available on line at New England Journal of Medicine; incorporated by reference herein).

[54] Statistical Analysis

[55] The primary endpoints for Cohorts A and B were immune-related objective response rate (irORR) and immune-related progression-free survival (irPFS) rate at 20 weeks assessed using immune-related response criteria (irRC)[33].The primary endpoint for Cohort C was irPFS rate at 20 weeks. Immune-related criteria (i.e, criteria used to evaluate immune-based therapies) are based on radiographic responses, and unlike RECIST criteria, capture extent of disease after disease progression; these criteria are defined and compared to RECIST v1.1 in Fig. 10 (Table S1). Response rate and PFS rate at 20 weeks were evaluated and reported in this study using RECIST v1.1 and irRC (Fig. 10 (Table S1)). PFS and overall survival was summarized by Kaplan-Meier method. Details of the hypothesis, the decision rules to reject the null hypotheses and early-stopping rules for efficacy and futility, and statistical methods are provided in the Supplementary Appendix.

EXAMPLE 3

SUPPLEMENTARY METHODS

[56] PATIENTS

[57] To be eligible for participation in this study, patients had to be at least 18 years of age, have histologically confirmed evidence of previously-treated, progressive carcinoma. All patients underwent MMR status testing prior to enrollment. All patients had at least one measurable lesion as defined by the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, an Eastern Cooperative Oncology Group (ECOG) performance-status score of 0 or 1, and adequate hematologic, hepatic, and renal function. Eligible patients with CRC must have received at least 2 prior cancer therapies and patients with other cancer types must have received at least 1 prior cancer therapy. Patients with

untreated brain metastases, history of HIV, hepatitis B, hepatitis C, clinically significant ascites/effusions, or autoimmune disease were excluded.

[58] STUDY OVERSIGHT

[59] Initial drafts of the manuscript were prepared by a subset of the authors and all authors contributed to the final manuscript. All the authors made the decision to submit the manuscript for publication. The principal investigator and study sponsor vouch for the accuracy and completeness of the data reported as well as adherence to the protocol.

[60] HLA TYPING

[61] HLA-A, HLA-B and HLA-C Sequence Based Typing can be divided into three distinct steps, as described below. A generic, A*02 specific, B generic, B group specific, C generic and C*07 specific PCR and sequencing mixes were made in the JHU core facility. Celera's AlleleSEQR HLA-B Sequence Based Typing kit was used for B generic SBT. The HLA-A typing scheme is composed of two PCR reactions, A generic and A*02 specific. A generic amplicon encompasses partial exon 1- partial exon 5. A*02 amplicon encompasses partial intron 1 - partial exon 5. HLA-B typing scheme is composed of two PCR reactions, B generic and B group specific. The B generic PCR is a multiplexed reaction containing two PCR amplicons encompassing exon 2 – exon 3 and exon 4 – exon 7. B group specific amplicon encompasses partial intron 1 - partial exon 5. HLA-C typing scheme is composed of two PCR reactions, C generic and C*07 specific. C generic and C*07 specific amplicons encompasses exons 1 - 7.

[62] The specificity of the HLA-A and B PCR employed AmpliTaq Gold DNA polymerase. The GeneAmp High Fidelity enzyme is used for the HLA-C and C*07 PCR mixes. This enzyme is a mix of two polymerases: AmpliTaq DNA polymerase (non-proofreading polymerase) and a proofreading polymerase. This enzyme mix is necessary to produce efficient and robust amplification of the larger full length HLA-C amplicon.

[63] PCR product purification was performed using Exonuclease I and Shrimp Alkaline Phosphatase The A generic and B generic amplicons were bi-directionally sequenced for exons 2,3,4. The C generic amplicon was bi-directionally sequenced for exons 2,3 and sequenced in a single direction for exons 1,4,5,6,7. A*02 specific, B group specific and C*07 specific amplicons were sequenced in a single direction for exons 2,3. All sequencing reactions were performed with Big Dye Terminator V1.1 from Applied Biosystems and sequenced with an ABI Prism 3500XL Genetic Analyzer. Conexio Genomic's "Assign SBT" allele assignment software was used to process the data files.

[64] MISMATCH REPAIR STATUS TESTING[1,2]

[65] Six slides of tumor and normal (uninvolved lymph node or margin of resection) were cut (5 microns each), deparaffinized (xylene), and one stained with hematoxylin and eosin (H+E). A tumor area containing at least 20% neoplastic cells, designated by a board-certified Anatomic Pathologist was macrodissected using the Pinpoint DNA isolation system (Zymo Research, Irvine, CA), digested in proteinase K for 8 hours and DNA was isolated using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA). MSI was assessed using the MSI Analysis System (Promega, Madison, WI), composed of 5 pseudomonomorphic mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) to detect MSI and 2-pentanucleotide repeat loci (PentaC and PentaD) to confirm identity between normal and tumor samples, per manufacturer's instructions. Following amplification of 50-100 ng DNA, the fluorescent PCR products were sized on an Applied Biosystems 3130xl capillary electrophoresis instrument (Invitrogen, Calsbad, CA). Pentanucleotide loci confirmed identity in all cases. Controls included water as a negative control and a mixture of 80% germline DNA with 20% MSI cancer DNA as a positive control. The size in bases was determined for each microsatellite locus and tumors were designated as MSI if two or more mononucleotide loci varied in length compared to the germline DNA.

[66] SEQUENCING ANALYSIS

[67] Samples

[68] Samples provided as FFPE blocks or frozen tissue underwent pathological review to determine tumor cellularity. Tumors were macrodissected to remove contaminating normal tissue, resulting in samples containing >20% neoplastic cells. Matched normal samples were provided as blood, saliva or normal tissue obtained from surgery.

[69] Sample Preparation and Next-Generation Sequencing[3]

[70] Sample preparation, library construction, exome capture, next generation sequencing, and bioinformatics analyses of tumor and normal samples were performed at Personal Genome Diagnostics, Inc. (Baltimore, Maryland). In brief, DNA was extracted from frozen or formalin-fixed paraffin embedded (FFPE) tissue, along with matched blood or saliva samples using the Qiagen DNA FFPE tissue kit or Qiagen DNA blood mini kit (Qiagen, CA). Genomic DNA from tumor and normal samples were fragmented and used for Illumina TruSeq library construction (Illumina, San Diego, CA) according to the manufacturer's instructions or as previously described4. Briefly, 50 nanograms (ng) - 3 micrograms (μg) of genomic DNA in 100 microliters (μl) of TE was fragmented in a Covaris sonicator (Covaris, Woburn, MA) to a size of 150-450bp. To remove fragments smaller than 150bp, DNA was purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 0.9 of PCR product to beads twice and washed using 70% ethanol per the manufacturer's instructions. Purified, fragmented DNA was mixed with 36 μl of H2O, 10 μl of End Repair Reaction Buffer, 5 μl of End Repair Enzyme Mix (cat# E6050, NEB, Ipswich, MA). The 100 μl end-repair mixture was incubated at 20°C for 30 min, and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.25 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. To A-tail, 42 μl of end-repaired DNA was mixed with 5 μl of 10X dA Tailing Reaction Buffer and 3 μl of Klenow (exo-)(cat# E6053, NEB, Ipswich, MA). The 50 μl mixture was incubated at 37°C for 30 min and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.0 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. For

adaptor ligation, 25 µl of A-tailed DNA was mixed with 6.7 µl of H2O, 3.3 µl of PE-adaptor (Illumina), 10 µl of 5X Ligation buffer and 5 µl of Quick T4 DNA ligase (cat# E6056, NEB, Ipswich, MA). The ligation mixture was incubated at 20°C for 15 min and purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 0.95 and 1.0 of PCR product to beads twice and washed using 70% ethanol per the manufacturer's instructions. To obtain an amplified library, twelve PCRs of 25 µl each were set up, each including 15.5 µl of H2O, 5 µl of 5 x Phusion HF buffer, 0.5 µl of a dNTP mix containing 10 mM of each dNTP, 1.25 µl of DMSO, 0.25 µl of Illumina PE primer #1, 0.25 µl of Illumina PE primer #2, 0.25 µl of Hotstart Phusion polymerase, and 2 µl of the DNA. The PCR program used was: 98°C for 2 minutes; 12 cycles of 98°C for 15 seconds, 65°C for 30 seconds, 72°C for 30 seconds; and 72°C for 5 min. DNA was purified using Agencourt AMPure XP beads (Beckman Coulter, IN) in a ratio of 1.0 to 1.0 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. Exonic or targeted regions were captured in solution using the Agilent SureSelect v.4 kit according to the manufacturer's instructions (Agilent, Santa Clara, CA). The captured library was then purified with a Qiagen MinElute column purification kit and eluted in 17 µl of 70°C EB to obtain 15 µl of captured DNA library. (5) The captured DNA library was amplified in the following way: Eight 30uL PCR reactions each containing 19 µl of H2O, 6 µl of 5 x Phusion HF buffer, 0.6 µl of 10 mM dNTP, 1.5 µl of DMSO, 0.30 µl of Illumina PE primer #1, 0.30µl of Illumina PE primer #2, 0.30 µl of Hotstart Phusion polymerase, and 2 µl of captured exome library were set up. The PCR program used was: 98°C for 30 seconds; 14 cycles (exome) or 16 cycles (targeted) of 98°C for 10 seconds, 65°C for 30 seconds, 72°C for 30 seconds; and 72°C for 5 min. To purify PCR products, a NucleoSpin Extract II purification kit (Macherey-Nagel, PA) was used following the manufacturer's instructions. Paired-end sequencing, resulting in 100 bases from each end of the fragments for exome libraries and 150 bases from each end of the fragment for targeted libraries, was performed using Illumina HiSeq 2000/2500 and Illumina MiSeq instrumentation (Illumina, San Diego, CA).

[71] Primary Processing of Next-Generation Sequencing Data and Identification of Putative Somatic Mutations3

[72] Somatic mutations were identified using VariantDx custom software (Personal Genome Diagnostics, Baltimore, Maryland) for identifying mutations in matched tumor and normal samples. Prior to mutation calling, primary processing of sequence data for both tumor and normal samples were performed using Illumina CASAVA software (v1.8), including masking of adapter sequences. Sequence reads were aligned against the human reference genome (version hg18) using ELAND with additional realignment of select regions using the Needleman-Wunsch method 5. Candidate somatic mutations, consisting of point mutations, insertions, and deletions were then identified using VariantDx across the either the whole exome or regions of interest. VariantDx examines sequence alignments of tumor samples against a matched normal while applying filters to exclude alignment and sequencing artifacts. In brief, an alignment filter was applied to exclude quality failed reads, unpaired reads, and poorly mapped reads in the tumor. A base quality filter was applied to limit inclusion of bases with reported phred quality score > 30 for the tumor and > 20 for the normal. A mutation in the tumor was identified as a candidate somatic mutation only when (i) distinct paired reads contained the mutation in the tumor; (ii) the number of distinct paired reads containing a particular mutation in the tumor was at least 10% of read pairs; (iii) the mismatched base was not present in >1% of the reads in the matched normal sample as well as not present in a custom database of common germline variants derived from dbSNP; and (iv) the position was covered in both the tumor and normal at > 150X. Mutations arising from misplaced genome alignments, including paralogous sequences, were identified and excluded by searching the reference genome.

[73] Candidate somatic mutations were further filtered based on gene annotation to identify those occurring in protein-coding regions. Functional consequences were predicted using snpEff and a custom database of CCDS, RefSeq and Ensembl annotations using the latest transcript versions available on hg18 from UCSC (https://genome.ucsc.edu/). Predictions were ordered to prefer transcripts with canonical start and stop codons and CCDS or Refseq transcripts over Ensembl when available. Finally mutations were filtered to exclude intronic and silent changes, while retaining mutations resulting in missense

mutations, nonsense mutations, frameshifts, or splice site alterations. A manual visual inspection step was used to further remove artifactual changes.

[74] MUTANT PEPTIDE MHC BINDING PREDICTION

[75] Somatic frameshift, insertions, deletions, and missense mutations predicted to result in an amino acid change were analyzed for potential MHC class I binding based on the individual patient's HLA haplotype. Our initial analysis focused on HLA-A and HLA-B. Amino acid mutations were linked to their corresponding CCDS accession number and in instances where this was unavailable, either a Refseq or ensemble transcript was used to extract the protein sequence. To identify 8mer, 9mer, and 10mer epitopes, amino acid fragments surrounding each mutation were identified. These 15, 17, and 19 mutant amino acid fragments were analyzed by the epitope prediction program NetMHC 3.4.6 Epitopes with a predicted affinity of <50nm were considered to be strong potential binders and epitopes with a predicted affinity of <500nm were considered to be weak potential binders as suggested by the NetMHC group6.

[76] To further refine the total neoantigen burden, we repeated that same process for the complementary wild-type peptide for each mutant peptide. We then filtered for mutant peptides that were strong potential binders when the complementary wild-type peptide was predicted a weak potential binder. These mutant peptides are referred to as mutation-associated neoantigens (MANA). In the event that a patient had a (e.g., cases 1, 17 and 21) single MHC haplotype not supported by NetMHC 3.4, the individual haplotype was not included in our analysis.

[77] STATISTICAL METHODS

[78] Design of the trial[7]

[79] This trial was conducted using a parallel two-stage design to simultaneously evaluate the efficacy of MK-3475 and MSI as a treatment selection marker for anti-PD-1 therapy. It

consisted of two-stage phase 2 studies in parallel in the three cohorts of patients described in the text. The study agent, MK-3475, was administered at 10 mg/kg intravenously every 14 days.

[80] For each of Cohort A and B, the co-primary endpoints were progression-free-survival (irPFS) at 20 weeks and objective response (irOR) assessed using immune related criteria. A step-down gatekeeping procedure was used to preserve the overall type I error. A two-stage Green-Dahlberg design was used to evaluate irPFS, with interim and final analysis after 15 and 25 patients, respectively. At stage 1, $\geq$ 1 of 15 free-of-progression at 20 weeks were required to proceed to the second stage, and $\geq$ 4 of 25 free-of-progression at 20 weeks were then required to proceed to test for irOR, with $\geq$ 4 of 25 responders (irCR or irPR) indicating promising efficacy in that cohort. Each cohort could be terminated for efficacy as soon as $\geq$ 4 free-of-progression at 20 weeks and $\geq$ 4 responses were confirmed, or be terminated for futility as soon as 0 of 15 in stage 1 were free-of-progression at 20 weeks or $\geq$ 22 subjects had disease progression by 20 weeks. This design achieves 90% power to detect a 20-week irPFS rate of 25% and 80% power to detect an irOR rate (irORR) of 21%, with an overall type I error of 0.05 at the null hypothesis of 20-week irPFS rate of 5% and irORR of 5%.

[81] For Cohort C, the primary endpoint was irPFS at 20 weeks. A two-stage Green-Dahlberg two-stage design was used, with an interim and final analysis after 14 and 21 patients; at stage 1, $\geq$ 1 of 14 free-of-progression at 20 weeks were required to proceed to the second stage, with $\geq$ 4 of 21 free-of-progression at 20 weeks at the end indicating adequate efficacy in Cohort C. The cohort could be terminated as soon as $\geq$ 4 free-of-progression at 20 weeks were confirmed. The design has 81% power to detect a 20-week irPFS rate of 25% with a 5% type I error at the null hypothesis of 20-week irPFS rate of 5%.

[82] Statistical analysis

[83] Response and progression were evaluated using RECIST v1.1 and the immune-related response criteria (irRC) adopted from Wolchok et al.8, which uses the sum of the

products of bidimensional tumor measurements and incorporates new lesions into the sum. Progression-free survival (PFS) rates and irPFS rate at 20-weeks was estimated as the proportion of patients who were free-of-disease progression and alive at 20 weeks after the initiation of pembrolizumab. Patients who had disease progression prior to 20 weeks or were enrolled for >20 weeks at the time the study data were collated were included in the analysis for estimating 20-week PFS (irPFS) rate. Patients who dropped out early due to toxicities or worsening disease and therefore did not have 20-week tumor assessment were considered as having progressive disease. ORR (irORR) was the proportion of patients who achieved best overall response of CR or PR (irCR or irPR). Patients who were in the study long enough to have tumor response evaluations were included in the analysis for estimating response rates. Among those who responded (CR or PR), duration of response was the time of first RECIST response to the time of disease progression, and was censored at the last evaluable tumor assessment for responders who had not progressed.

[84] PFS and irPFS were defined as the time from the date of initial dose to the date of disease progression or the date of death due to any cause, whichever occurred first. PFS and irPFS were censored on the date of the last evaluable tumor assessment documenting absence of progressive disease for patients who were alive and progression-free. Overall survival (OS) was defined as the time from the date of initial dose to death due to any cause. For patients who were still alive at the time of analysis, the OS time was censored on the last date the patients were known to be alive. Survival times were summarized by the Kaplan-Meier method. As a post hoc analysis, log-rank tests were used to compare Cohort A and B and hazard ratios were estimated based on Cox models.

[85] The association of percent CEA decline after 1 cycle with PFS or OS was assessed using landmark analysis based on Cox regression models. For correlative studies, non-parametric Wilcoxon test was used to compare mutational load between MMR-deficient and MMR-proficient patients. The effects of baseline mutational burden and immune markers on response and survival times were examined using logistic regression and Cox regression, respectively.

[86] IMMUNOHISTOCHEMISTRY & IMAGE ANALYSIS

[87] The fraction of malignant cells exhibiting a membranous pattern of B7-H1 expression and the percentage at the invasive front were quantified by three pathologists (R.A.A., F.B., and J.M.T.) as previously reported9,10. Image analysis was used to determine the number of CD8 diaminobenzidine (DAB)-stained cells. Using the H&E-stained slide for each case, we identified the following regions: i) tumor, ii) invasive front (the boundary between malignant and non-malignant tissue), and iii) normal tissue. The CD8-stained slides were scanned at 20x equivalent magnification (0.49 micrometers per pixel) on an Aperio ScanScope AT. Regions corresponding to tumor, invasive front and normal tissue (above, from the H&E) were annotated on separate layers using Aperio ImageScope v12.1.0.5029.

[88] CD8-positive lymphocyte density was calculated in each of the above regions using a custom algorithm implemented in PIP11. Results were converted to Deepzoom images using the VIPS library12 and visualized using the OpenSeadragon viewer (http://openseadragon.github.io).

[89] References for Example 3 only.

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EXAMPLE 4

[90] Patients

[91] 41 consecutive patients were enrolled and treated between September 2013 and January 2015. (Table 1). Recruitment included patients in pursuit of a clinical trial option who were known to have tumors with mismatch repair, or who had tumors of unknown status who were then tested. One patient in the MMR-deficient CRC cohort was enrolled under an IRB eligibility waiver allowing a grade 3 bilirubin level. A total of 32 CRC patients were enrolled into Cohorts A and B. All CRC patients received $\geq$2 prior chemotherapy regimens (median=4) except for one MMR-proficient patient who had received one chemotherapeutic and one (non-PD1-based) immunotherapeutic regimen.

[92] Nine subjects diagnosed with MMR-deficient solid tumors other than CRC were enrolled onto Cohort C. All Cohort C patients received $\geq$1 prior cancer treatments (median=2).

EXAMPLE 5

[93] Primary endpoint evaluation

[94] The irORR and irPFS at 20 weeks (Fig. 11 (Table S2)) for Cohort A were 40% (4 of 10 patients; 95% CI, 12 to 74%) and 78% (7 of 9 patients; 95% CI, 40 to 97%) and for 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, comprised of patients with MMR-proficient CRCs, irORR and 20-week irPFS were 0% (95%CI, 0 to 20%) and 11% (2 of 18 patients; 95% CI, 1 to 35%). Both the MMR-deficient cohorts A and C reached their predefined early stopping rule for efficacy when four subjects were free-of-disease progression at 20 weeks and four objective responses were observed based on immune-related response criteria (Fig. 11 (Table S2); available on line at New England Journal of Medicine; incorporated by reference herein; and supplementary methods, above).

[95] The median time of follow-up for patients was 32 weeks (range, 5-51 weeks) for patients with MMR-deficient CRC (Cohort A), 12 weeks (range, 2-56 weeks) for patients with MMR-proficient CRC (Cohort B) and 12 weeks (range, 4-42 weeks) for patients with MMR-deficient non-CRC tumors (Cohort C). All patients evaluable for 20-week irPFS were followed for at least 20 weeks.

EXAMPLE 6

[96] Radiographic evaluation

[97] Of the ten evaluable MMR-deficient CRC patients in Cohort A, four (40%; 95% CI, 12-74%) achieved objective responses by RECIST criteria (Table 2, Fig. 1 and Fig. 3 (S2)). Patients were considered not evaluable unless they underwent a 12-week scan. The disease control rate was defined as the fraction of patients who achieved an objective response or whose disease was stable, and was 90% in Cohort A (9 of 10 patients; 95% CI, 55-100%).

[98] Of the seven evaluable patients with MMR-deficient cancer types other than CRC enrolled in Cohort C, five (71%; 95% CI, 29-96%) achieved objective responses (Table 2, Fig. 3 (S2) and Fig. 1) using RECIST criteria and the disease control rate was 71% (5 of 7 patients; 95% CI, 29-96%).

[99] Patients in Cohort C responded faster than patients in Cohort A (median time to response by RECIST of 12 vs. 28 weeks, p=0.03). Furthermore, all six MMR-deficient tumors that were not associated with Lynch syndrome (100%) achieved an objective response, whereas only three of eleven tumors (27%) associated with Lynch Syndrome responded (Table S3; p=0.009; available on-line at New England Journal of Medicine; and incorporated by reference herein). No other baseline characteristics showed statistically significant association with objective responses.

[100] Of the 18 patients with MMR-proficient CRCs in Cohort B, no objective responses were observed (Table 2, Fig. 3 (S2) and Fig. 1) using RECIST criteria and the disease control rate was 11% (2 of 18 patients; 95%CI, 1 to 35%).

[101] All patients who achieved a response by RECIST criteria (Fig. 11 (Table 2)) also achieved a response by immune-related response criteria (Fig. 11 (Table S2)).

EXAMPLE 7

[102] Survival

[103] In Cohort A, the patients with MMR-deficient CRC, median progression-free survival (PFS) and median overall survival (OS) were not reached (Fig. 2). In contrast, the patients with MMR-proficient cancers in Cohort B achieved a PFS of only 2.2 months (95% CI, 1.4-2.8) and a median OS of 5.0 months (95% CI, 3.0 to not estimable). In Cohort C (MMR-deficient non-CRC), the median PFS was 5.4 months (95% CI, 3 to not estimable) and the median OS was not reached.

[104] A *post hoc* (Fig. 2) comparison of the MMR-deficient and proficient CRC cohorts showed hazard ratios (HR) for disease progression (HR=0.10; 95% CI, 0.03-0.37; $p<0.001$) and overall survival (HR = 0.22; 95% CI, 0.05-1.00; p=0.05), favoring patients with MMR-deficient CRC.

[105] To evaluate whether the difference in survival might be due to prognostic differences, we measured the duration of time patients had been diagnosed with metastatic disease and the clinical performance of patients on their previous regimen prior to enrollment. We found that there was no significant difference between MMR-deficient vs. MMR-proficient CRC patients with respect to their duration of metastatic disease (p=0.77; Log-rank test) or median PFS (p=0.60, Log-rank test) on their prior regimens (Fig. 4 (S3)). We also performed an additional multivariate analysis of PFS and OS to examine the difference in outcomes between MMR-deficient CRC and MMR-proficient tumors adjusting for elapsed time since initial diagnosis. The magnitude of the hazard ratios for

PFS (HR 0.04, 95% CI 0.01-0.21, P<0.001) and OS (HR 0.18, 95% CI 0.03-1.01, P=0.05), representing the different effect of pembrolizumab between MMR-deficient and MMR-proficient tumors, was maintained after adjusting for this potential difference.

EXAMPLE 8

[106] Safety Assessment

[107] Adverse events occurring in > 5% of patients are listed in Table 3. Select adverse events included rash/pruritus (24%), thyroiditis/hypothyroidism/hypophysitis (10%), and asymptomatic pancreatitis (15%). While the numbers were small, thyroid function abnormalities were limited to the MMR-deficient cohorts (Table 3).

EXAMPLE 9

[108] Tumor markers

[109] In the two CRC cohorts, baseline CEA levels were evaluable and above the upper limit of normal (3 mg/dl), in 29 of 32 patients prior to enrollment. Major CEA declines occurred in seven of the ten patients with MMR-deficient CRC and in none of the 19 patients with MMR-proficient CRCin which CEA was evaluable (Fig. 1 and Fig. 5 (S4)). In non-CRC MMR-deficient patients, tumor marker levels (CEA, CA19-9 or CA-125) were elevated above the upper limit of normal in four patients. CA19-9 or CA-125 declines of > 70% occurred in three of these four patients. Tumor marker kinetics of all 3 cohorts are shown in Figure 1. The level of CEA decline after 1 dose (between days 14 and 28) of pembrolizumab was predictive of both progression-free (p=0.01) and overall survival outcomes (p=0.02). The CEA response occurred well in advance of radiographic confirmation of disease control (range, 10 to 35 weeks). In contrast, patients who progressed showed rapid biomarker elevation within 30 days of initiating therapy. Thus, changes in CEA levels significantly preceded and correlated with ultimate radiographic changes.

EXAMPLE 10

[110] Genomic Analysis

[111] Analysis of whole-exome sequences showed an average of 1,782 somatic mutations per tumor in MMR-deficient patients (n = 9) compared with 73 mutations per tumor in MMR-proficient patients (n=6) (non-parametric Wilcoxon test, p=0.007) (Figs. 6A-6B (S5); see also Table S3 which is available on-line at New England Journal of Medicine; incorporated by reference herein). Most (63%) of these mutations are predicted to alter amino acids.

[112] These mutations were then assessed for their immunogenic potential in the context of each patient's individual MHC haplotype. We thereby identified an average of 578 and 21 potential mutation-associated neoantigens from the tumors of MMR-deficient and MMR-proficient patients, respectively (Table S3; which is available on-line at New England Journal of Medicine; incorporated by reference herein). The fraction of potential mutation-associated neoantigens among all somatic mutations was similar in both cohorts (averaging 32% and 29% in MMR-deficient and -proficient patients, respectively). High numbers of somatic mutations and potential mutation-associated neoantigens were associated with improved progression-free survival and with a trend in favor of objective response (Fig. 13 (S5) and Fig. 12 (Table S4); also available on line at New England Journal of Medicine; incorporated by reference herein).

EXAMPLE 11

[113] Immunohistochemistry

[114] Expression of CD8 and PD-L1 were evaluated by immunohistochemistry within the tumor and at the invasive fronts of the tumor in the 30 cases in which tumor tissue was available (Fig. 7 (S6); also available on line at New England Journal of Medicine; incorporated by reference herein). Tumors from patients in Cohorts A and C contained a greater density of CD8-positive lymphoid cells than did tumors from Cohort B patients

(Fig. 8 (S7); p=0.10) and CD8-labeling was associated with a trend favoring objective response and stable disease (Fig. 9 (S8) and Fig. 13 (Table S5); also available on line at New England Journal of Medicine; incorporated by reference herein). This CD8-positive lymphoid infiltrate was especially prominent at the invasive fronts of the tumors (Fig 8 (S7); p=0.04). Significant membranous PD-L1 expression only occurred in MMR-deficient patients and was prominent on tumor infiltrating lymphocytes (TILs) and tumor-associated macrophages located at the tumors' invasive fronts (Fig. 8 (S7); p=0.04). Expression of CD8 and PD-L1 were not statistically associated with PFS or OS (Fig. 13 (Table S5)).

Table 1. Demographic and Baseline Characteristics of Patients

Characteristic	MMR-deficient CRC n=11	MRC-proficient CRC n=21	P values[1]	MMR-deficient non-CRC n=9
Age – years				
median	46	61	0.02	57
range	(24-65)	(32-79)		(34-92)
Sex – no. (%)				
Female	5(45)	8(38)	0.72	4(44)
Male	6(55)	13(62)		5(56)
Race – no. (%)				
white	8(73)	17(81)	0.66	8(89)
black	1(9)	3(14)		0(0)
other	2(18)	1(5)		1(11)
ECOG Performance Status – no. (%)[2]				
0	0(0)	6(29)	0.07	2(22)
1	11(100)	15(71)		7(78)
Diagnosis – no. (%)				
Colon	9(82)	18(86)	>0.99	0(0)
Rectal	2(18)	3(14)		0(0)
Ampullary/Cholangiocarcinoma	0(0)	N/A		4(44)
Endometrial	0(0)	N/A		2(22)
Small bowel	0(0)	N/A		2(22)
Gastric	0(0)	N/A		1(11)
Histology – no. (%)				
Well/moderately differentiated	7(64)	18(86)	0.20	4(44)
Poorly differentiated	4(36)	3(14)		3(33)
Other	0(0)	0(0)		2(22)
Stage IV – no. (%)	(11)100	21(100)	>0.99	9(100)
Liver metastases – no. (%)	6(55)	11(52)	>0.99	6(67)
Time since first diagnosis - months				
median	31	58	0.07	23
range[▽]	6-95	27-192		2-105
Prior systemic therapies – no. (%)				
0	0(0)	0(0)	0.89	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 – no. (%)				
Yes	9(82)	0(0)	<0.001	4(44)
No	2(18)	21(100)		4(44)
Unknown	0(0)	0(0)		1(11)
BRAF wild type – no. (%)				
Yes	8(73)	11(52)	0.64	4(44)
No	0(0)	1(5)		0(0)
Unknown	3(27)	9(43)		5(56)
KRAS wild type – no. (%)				
Yes	6(55)	13(62)	0.72	4(44)
No	5(45)	8(38)		1(11)
Unknown	0(0)	0(0)		4(44)

MMR, mismatch repair; CRC, colorectal cancer
[1] MMR-deficient CRC versus MMR-proficient CRC
[2] ECOG, Eastern Cooperative Oncology Group

Table 2. Objective RECIST responses

Type of Response – no. (%)	MMR-deficient CRC n=10	MRC-proficient CRC n=18	MMR-deficient non-CRC n=7
Complete Response	0(0)	0(0)	1(14)[1]
Partial Response	4(40)	0(0)	4(57)[2]
Stable Disease (Week 12)	5(50)	2(11)	0(0)
Progressive Disease	1(10)	11(61)	2(29)
Not Evaluable[3]	0(0)	5(28)	0(0)
Objective Response Rate (%)	40	0	71
95% CI	12-74	0-19	29-96
Disease Control Rate (%)[4]	90	11	71
95% CI	55-100	1-35	29-96
Duration of Response – median weeks	Not reached	N/A[5]	Not reached
Time to Response, median weeks (range)	28 (13-35)	N/A[5]	11 (10-13)

[1] Originally PR at 12 weeks that was converted to CR at 20 weeks
[2] One PR at 12 weeks
[3] Patients were considered not evaluable if they did not undergo a 12 week scan due to clinical progression.
[4] 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.
[5] No responses recorded for MMR-proficient CRC patients

Table 3. Drug-Related Adverse Events

Event – no (%)[1]	All Grades	Grade 3 or 4
	N=41	N=41
Any	40(98)	17(41)
Blood and Lymphatic		
Anemia	8(20)	7(17)
Lymphopenia	8(20)	8(20)
Cardiac		
Sinus tachycardia	4(10)	0
Dermatologic		
Dry skin	5(12)	0
Rash/pruritis	10(24)	0
Endocrine Disorders		
Thyroiditis/Hypothyroidism/Hypophysitis	4(10)	0
Gastrointestinal		
Abdominal Pain	10(24)	0
Anorexia	4(10)	0
Constipation	8(20)	0
Diarrhea	10(24)	2(5)
Dry mouth	5(12)	0
Nausea	5(12)	0
Bowel Obstruction	3(7)	3(7)
Hepatobiliary		
ALT, elevated	3(7)	2(5)
Pancreatitis[2]	6(15)	0
Metabolism and Nutrition		
Hypoalbuminemia	4(10)	4(10)
Hyponatremia	3(7)	3(7)
Musculoskeletal		
Arthralgia	7(17)	0
Myalgia	6(15)	0
Nervous System		
Dizziness	4(10)	0
Headache	7(17)	0
Psychiatric		
Insomnia	3(7)	0
Respiratory[3]		
Allergic Rhinitis	12(29)	0
Cough	4(10)	0
Dyspnea	6(15)	0
Upper Respiratory Infection	3(7)	0
Other		
Cold intolerance	6(15)	0
Edema	4(10)	0
Fatigue	13(32)	0
Fever	5(12)	0
Pain	14(34)	0

[1] Adverse Events occurring in greater than 5% of patients
[2] All cases of pancreatitis were asymptomatic
[3] One incidence of pneumonitis (2%)

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The disclosure of each reference cited is expressly incorporated herein.

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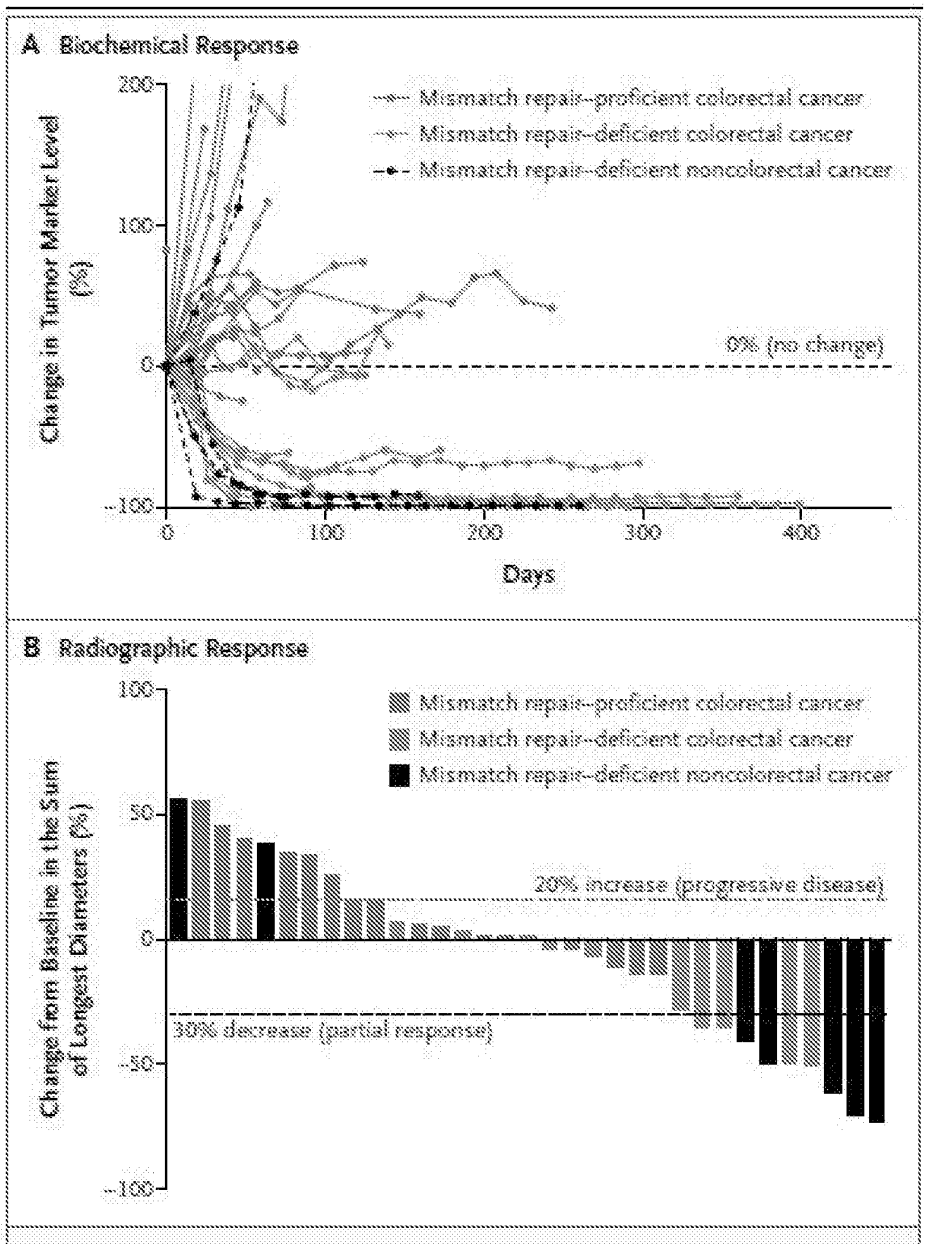
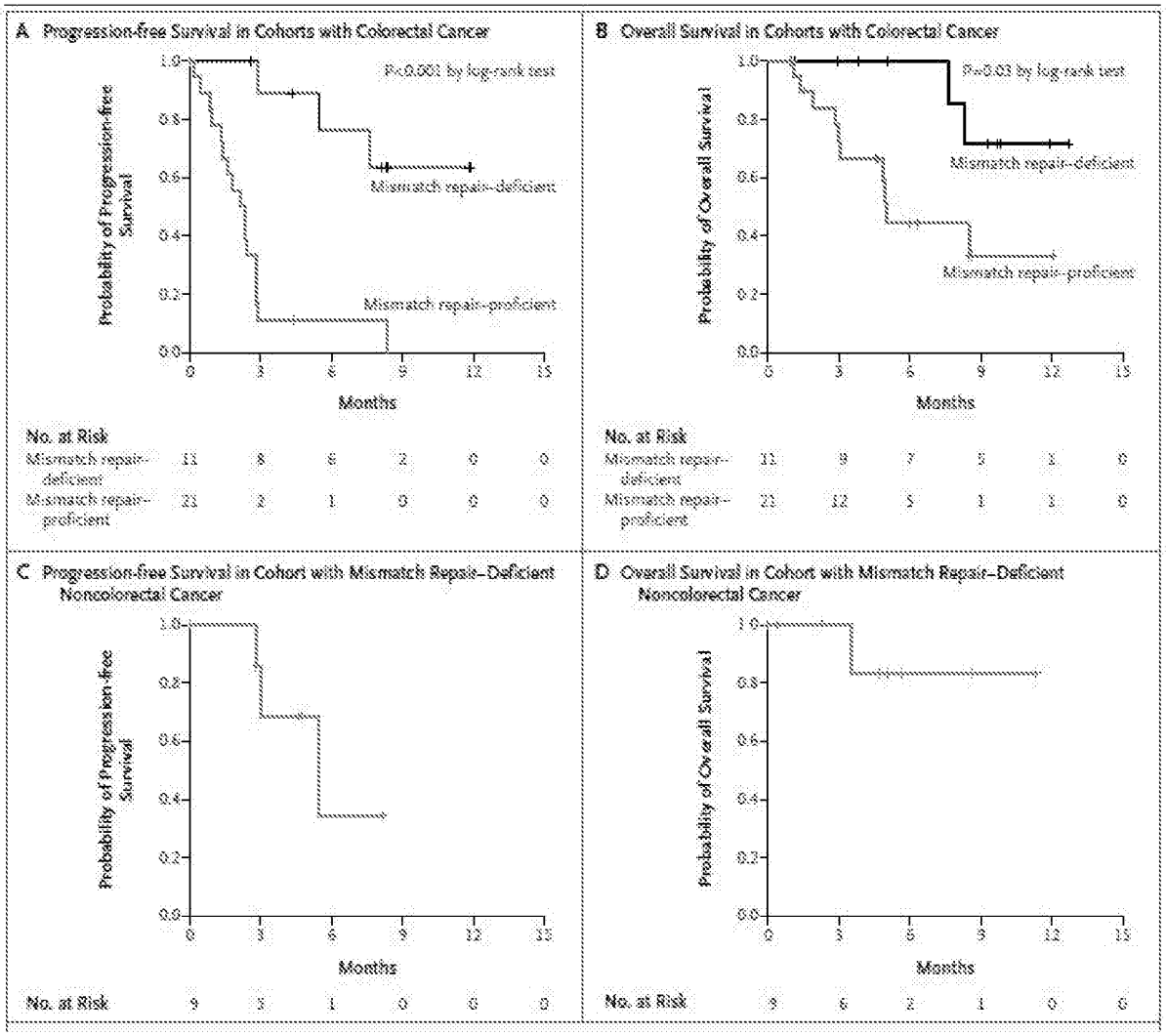


Figs. 1A and 1B


A Progression-free Survival in Cohorts with Colorectal Cancer
P<0.001 by log-rank test
Mismatch repair–deficient
Mismatch repair–proficient
Probability of Progression-free Survival
Months
No. at Risk
Mismatch repair–deficient 11 8 6 2 0 0
Mismatch repair–proficient 21 2 1 0 0 0
B Overall Survival in Cohorts with Colorectal Cancer
P=0.03 by log-rank test
Mismatch repair–deficient
Mismatch repair–proficient
Probability of Overall Survival
Months
No. at Risk
Mismatch repair–deficient 11 9 7 3 1 0
Mismatch repair–proficient 21 12 5 1 1 0
C Progression-free Survival in Cohort with Mismatch Repair–Deficient Noncolorectal Cancer
Probability of Progression-free Survival
Months
No. at Risk 9 3 1 0 0 0
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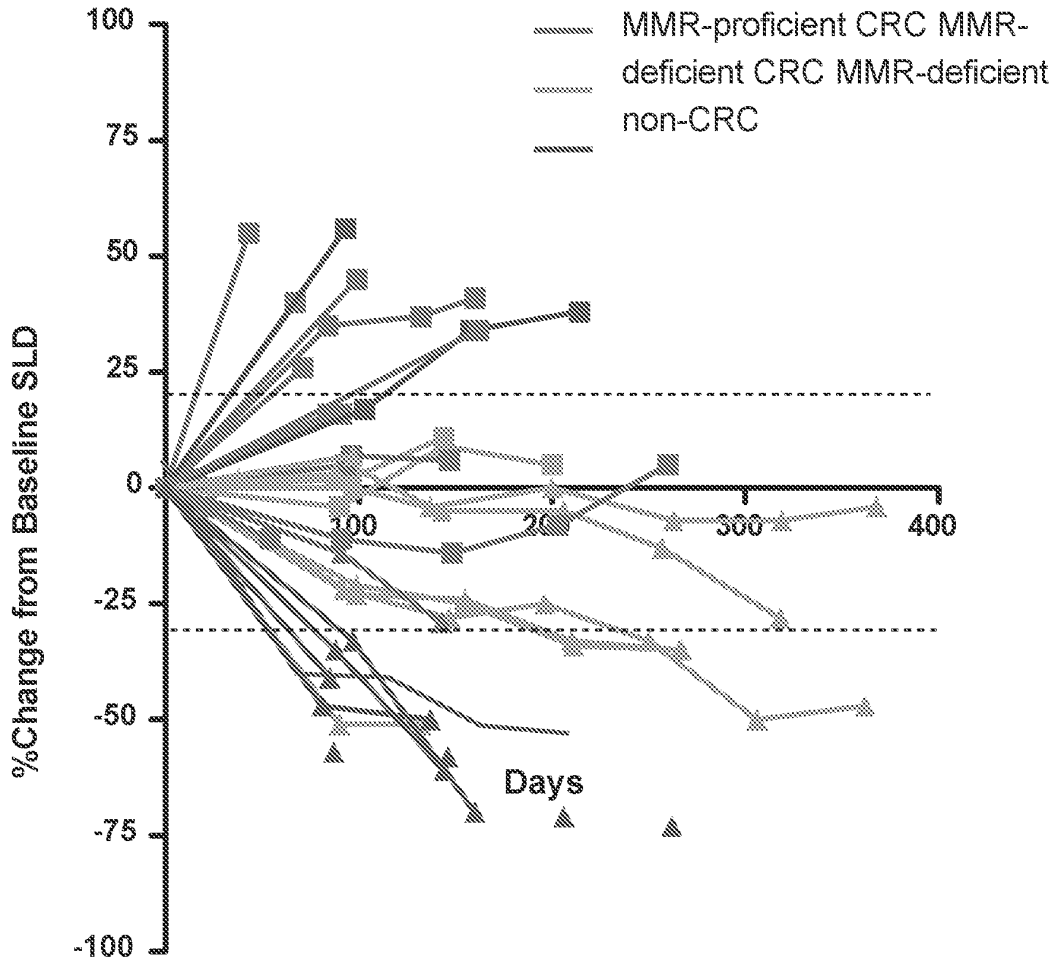
Probability of Overall Survival
Months
No. at Risk 9 6 2 1 0 0

Figs. 2A-2D


MMR-proficient CRC
MMR-deficient CRC
MMR-deficient non-CRC
%Change from Baseline SLD
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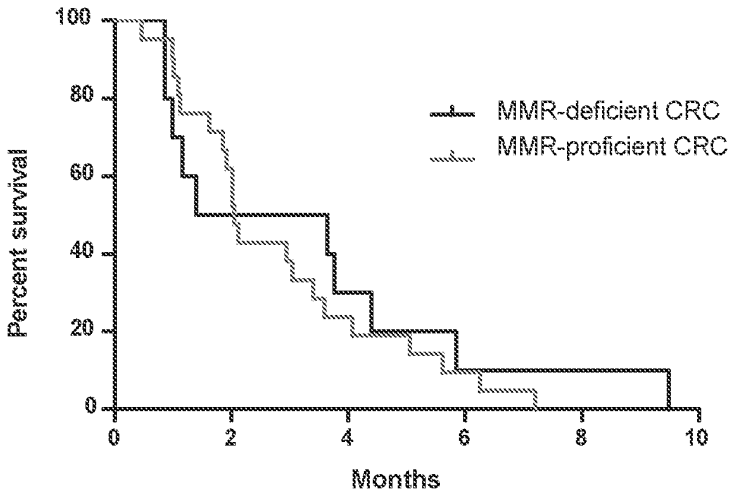
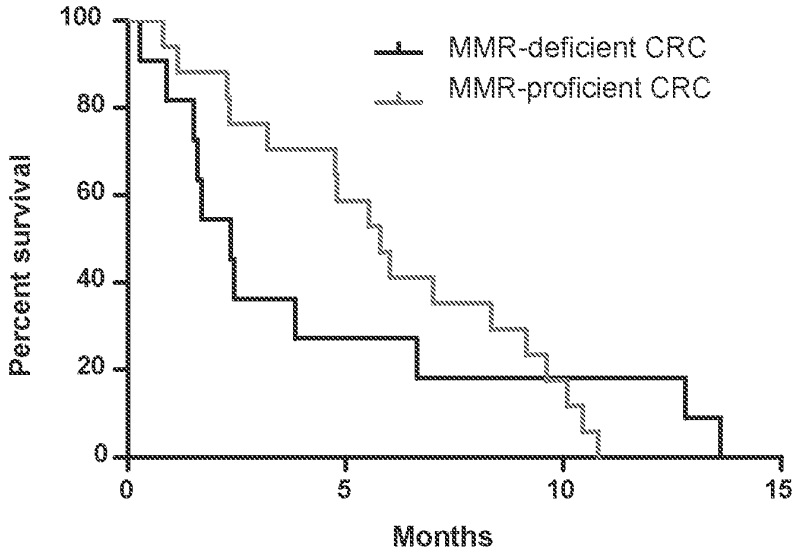
50
25
0
-25
-50
-75
-100
100
200
300
400
Days

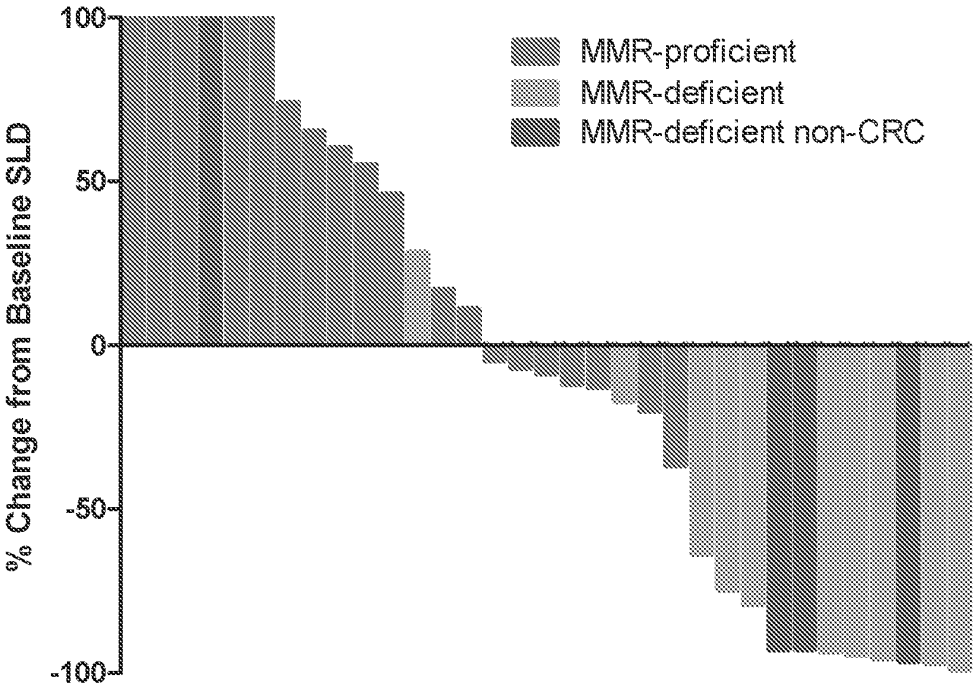
Fig. 3



Figs. 4A



Figs. 4B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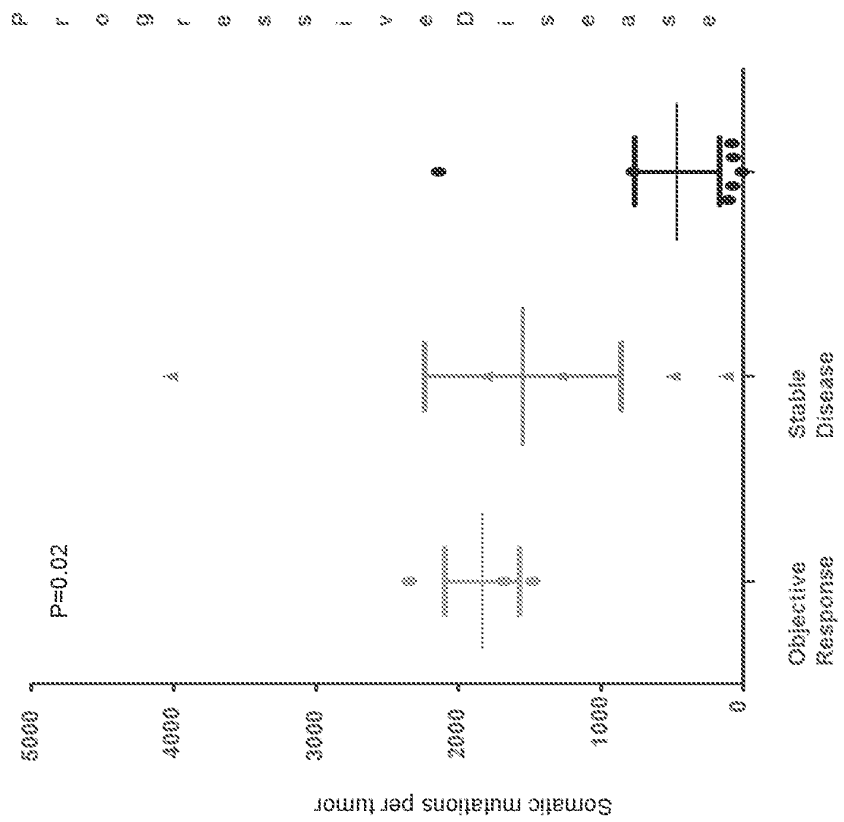
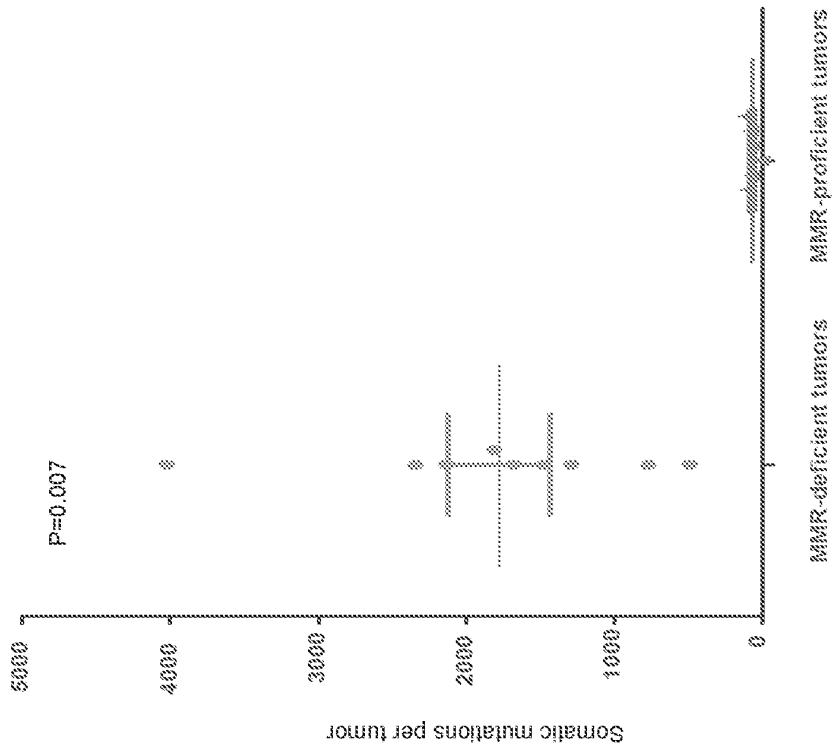
-100
% Change from Baseline SLD
MMR-proficient
MMR-deficient
MMR-deficient non-CRC

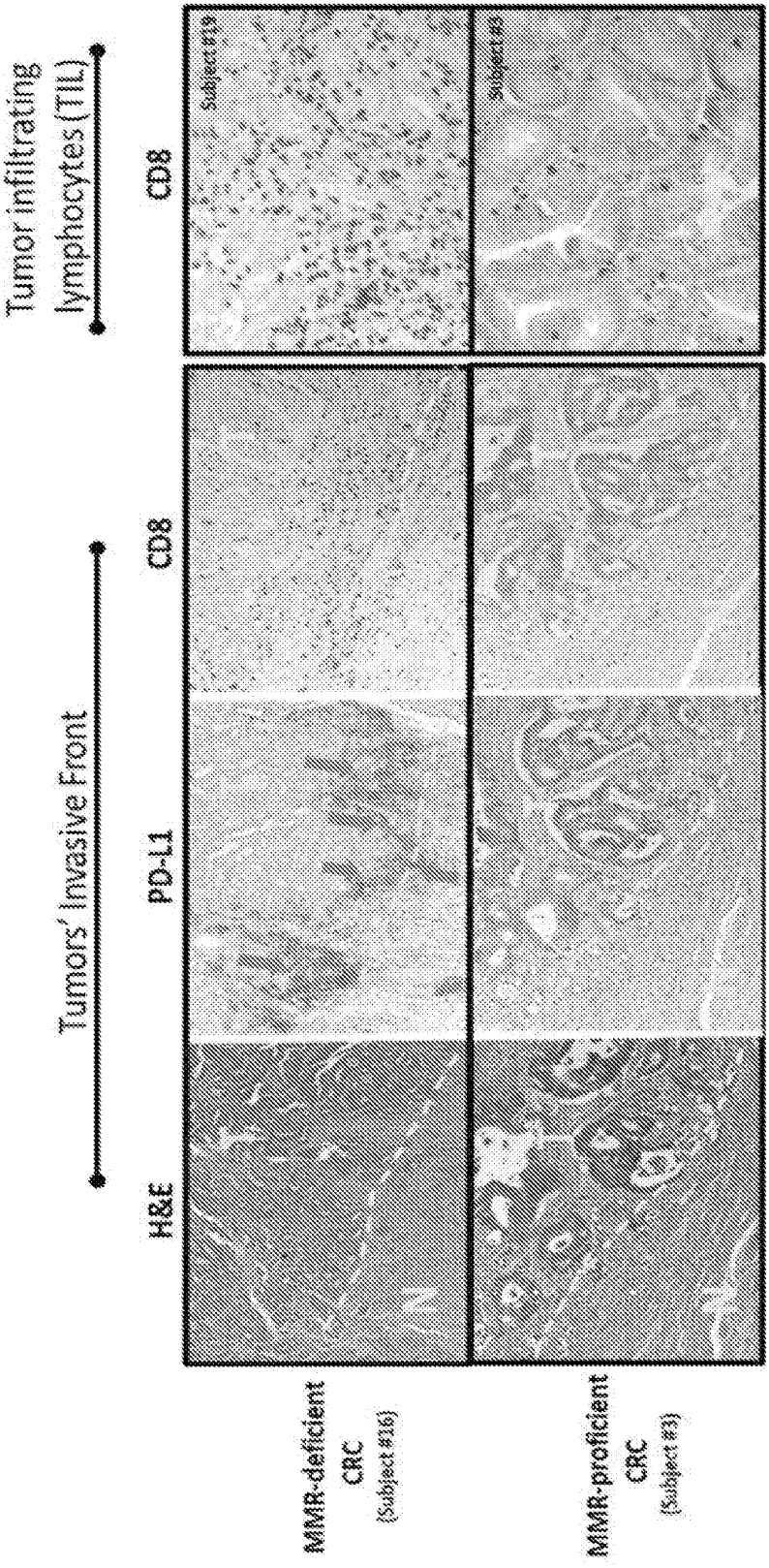
Fig. 5

Fig. 6B



Figs. 6A

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Tumors' Invasive Front
H&E
PD-L1
CD8
CD8
MMR-deficient CRC (Subject #16)
MMR-proficient CRC (Subject #3)
Subject #19
Subject #3

Fig. 7

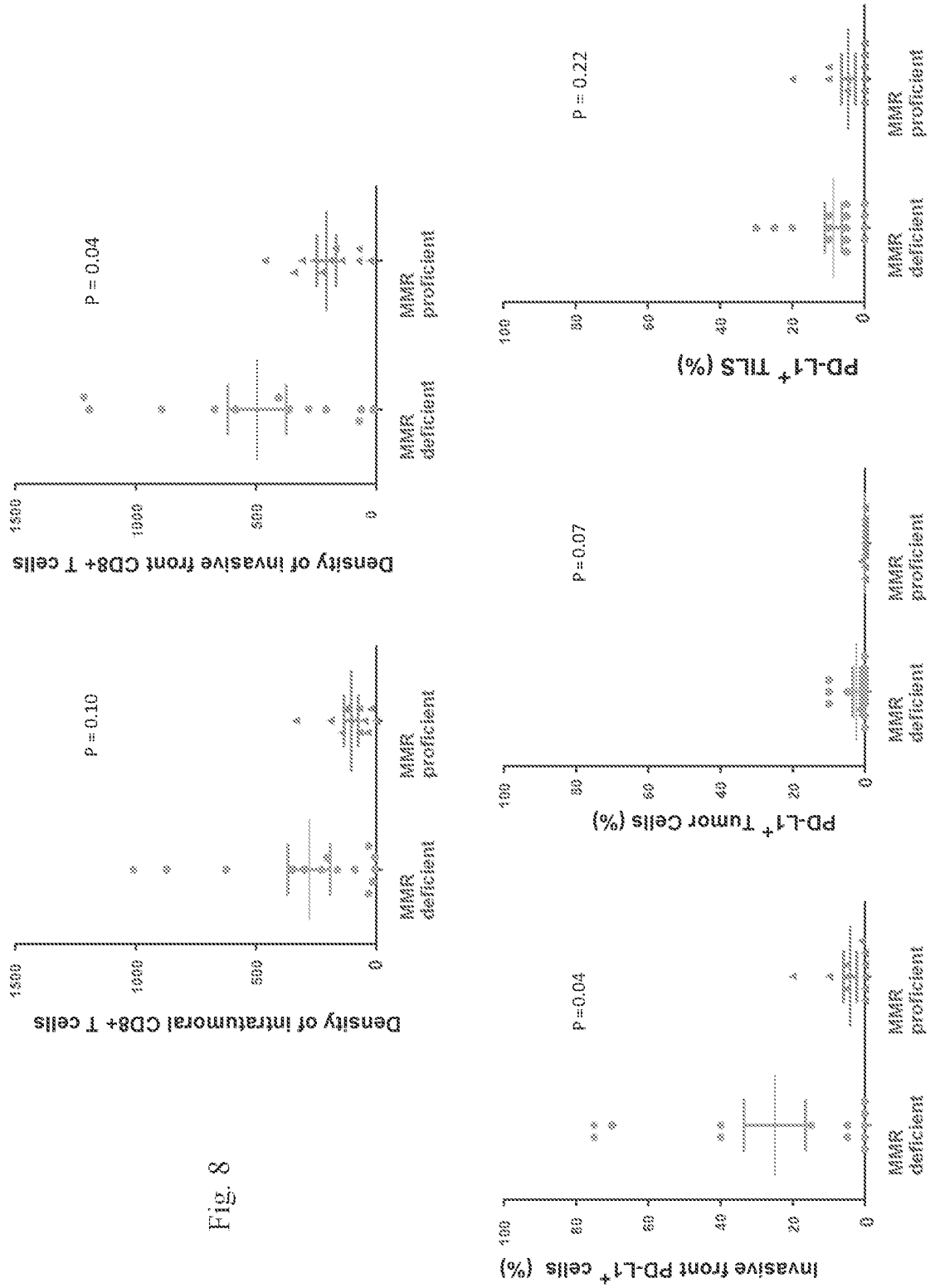
Density of intratumoral CD8+ T cells
1500
1000
500
0
P = 0.10
MMR deficient
MMR proficient
Density of invasive front CD8+ T cells
1500
1000
500
0
P = 0.04
MMR deficient
MMR proficient
Invasive front PD-L1+ cells (%)
100
80
60
40
20
0
P = 0.04
MMR deficient
MMR proficient
PD-L1+ Tumor cells (%)
100
80
60
40
20
0
P = 0.07
MMR deficient
MMR proficient
PD-L1+ TILs (%)
100
80
60
40
20
0
P = 0.22
MMR deficient
MMR proficient

Fig. 8

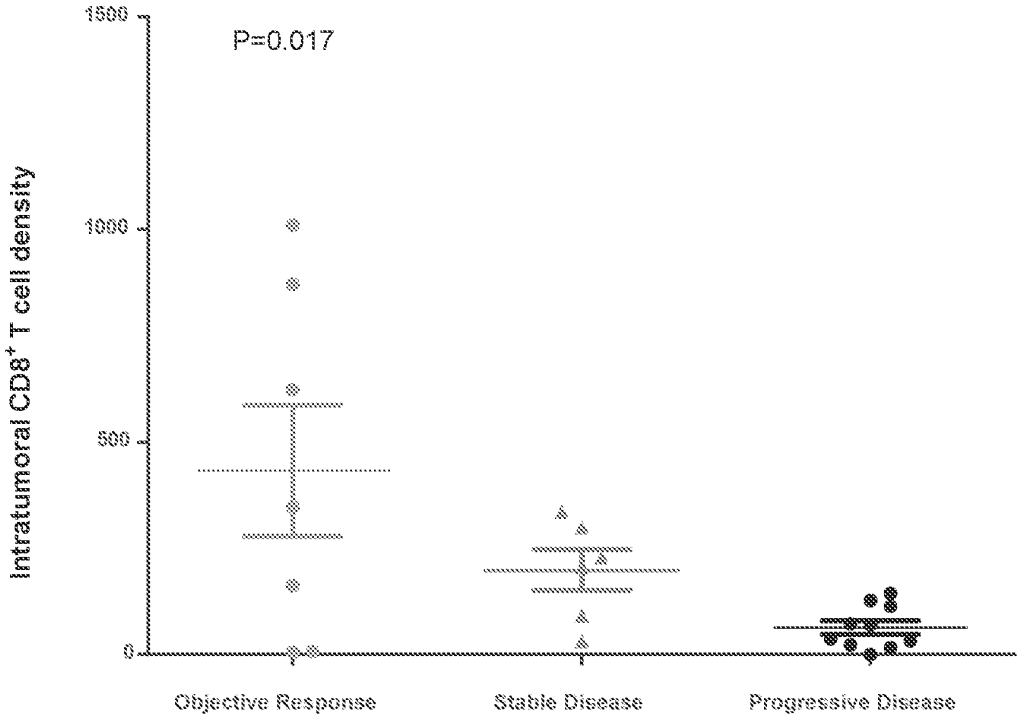
1500
1000
500
0
P=0.017
Intratumoral CD8+ T cell density
Objective Response
Stable Disease
Progressive Disease

Fig. 9

Fig. 10. Comparison of immune-related and RECIST response criteria

	CR	PR	SD	PD
RECIST 1.1	All lesions gone	> 30% decrease in sum of target lesion diameters from baseline	Neither R, or PD from nadir	> 20% increase in sum of target lesion diameters from nadir Appearance of new lesion
	irCR	**irPR**	**irSD**	**irPD**
irRC	All lesions gone	SPD of index + any new lesions decreases >50% from baseline	SPD of index + any new lesions neither irCR, irPR, or irPD	SPD of index + any new lesions increases > 25% from nadir
		New lesions allowed	New lesions allowed	irPD is based on SPD only

RECIST 1.1= Response Evaluation Criteria in Solid Tumors 1.1; irRC= Immune Related Response Criteria

CR= complete response; PR= paritial response; SD= stable disease; PD= progressive disease; SPD= sum of the product of the perpindicular diameters

Fig. 11. Immune-related response to treatment

Type of immune-related responses-no (%)	MMR-deficient CRC n=10	MMR-proficient CRC n=18	MMR-deficient non-CRC n=7
Complete Response Partial Response Stable Disease (Week 12) Progressive Disease Not Evaluable[3]	0 (0) 4 (40) 5 (50) 1 (10) 0 (0)	0 (0) 0 (0) 2 (11) 11 (61) 5 (28)	1 (14)[1] 4 (57)[2] 0 (0) 2 (29) 0 (0)
Immune-related objective response rate (%)	40	0	71
95% CI	12-74	0-19	29-96
Immune-related disease control rate (%)[4]	90	11	71
95% CI	55-100	1-35	29-96
Immune-related PFS at 20 weeks (%)	78	11	67
95% CI	40-97	1-35	22-96

[1] Originally PR at 12 that was converted to CR at 20 weeks

[2] One PR at 12 weeks

[3]Patients were considered not evaluable if they did not undergo a 12 week scan due to clinical progression. [4]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.

Fig. 12. Correlation of total somatic mutations and mutation associated neoantigens (MANA) with clinical outcomes

Trend test to compare CR/PR vs. SD vs. PD

Variable	P value
Total Somatic Mutations	0.024
Mutation-associated neoantigens (MANAs)	0.028

Association of mutations and MANA with response rates

Variable (log scale*)	Odds Ratio	95% Confidence interval Lower	Upper		P value
Total Somatic Mutations		3.312	0.500	21.924	0.214
Mutation-associated neoantigens (MANAs)		3.087	0.617	15.439	0.170

Association of mutations and MANA with PFS

Variable (log scale*)	Hazard Ratio	95% Confidence interval Lower	Upper	P value
Total Somatic Mutations	0.628	0.424	0.931	0.021
Mutation-associated neoantigens (MANAs)	0.599	0.391	0.916	0.018

Association of mutations and MANA with OS

Variable (log scale*)	Hazard Ratio	95% Confidence interval Lower	Upper	P value
Total Somatic Mutations	0.707	0.481	1.038	0.077
Mutation-associated neoantigens (MANAs)	0.676	0.453	1.008	0.055

Fig. 13. Correlation of immune markers with clinical outcome

Trend test to compare CR/PR vs. SD vs. PD

Variable	P value
PD-L1 membranous expression within tumor (>5% vs. <5%)	0.208
PD-L1 expression by TiL and associated macrophages at invasive front (%)	0.043
PD-L1 expression by TiL and associated macrophages on TiLS within tumor (%)	0.024
CD8 density (cells/mm2) within tumor CD8 density (cells/mm2) at invasive front	0.017
CD8 density (cells/mm2) in non-tumor tissue	0.768
	0.348

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

Association of immune markers with response rates

Variable (log scale*)	Odds Ratio	95% Confidence interval Lower	Upper	P value
PD-L1 membranous expression within tumor (>5% vs. <5%)	3.200	0.354	28.945	0.301
PD-L1 expression by TiL and associated macrophages at invasive front (%)	1.075	0.820	1.411	0.600
PD-L1 expression by TiL and associated macrophages on TiLS within tumor (%)	1.139	0.862	1.506	0.361
CD8 density (cells/mm2) within tumor CD8 density (cells/mm2) at invasive front	1.337	0.706	2.531	0.372
CD8 density (cells/mm2) in non-tumor tissue	0.515	0.193	1.374	0.185
	1.219	0.535	2.777	0.637

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

Association of immune markers with PFS

Variable (log scale*)	Hazard Ratio	95% Confidence interval Lower	Upper	P value
PD-L1 membranous expression within tumor (>5% vs. <5%)	0.196	0.025	1.518	0.119
PD-L1 expression by TiL and associated macrophages at invasive front (%)	0.899	0.784	1.031	0.126
PD-L1 expression by TiL and associated macrophages on TiLS within tumor (%)	0.888	0.765	1.031	0.118
CD8 density (cells/mm2) within tumor CD8 density (cells/mm2) at invasive front	0.900	0.794	1.020	0.100
CD8 density (cells/mm2) in non-tumor tissue	1.017	0.607	1.706	0.948
	1.144	0.780	1.677	0.492

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

Association of immune markers with OS

Variable (log scale*)	Hazard Ratio	95% Confidence interval Lower	Upper	P value
PD-L1 membranous expression within tumor (>5% vs. <5%)	0.513	0.062	4.232	0.535
PD-L1 expression by TiL and associated macrophages at invasive front (%)	0.978	0.816	1.173	0.813
PD-L1 expression by TiL and associated macrophages on TiLS within tumor (%)	0.931	0.762	1.138	0.487
CD8 density (cells/mm2) within tumor CD8 density (cells/mm2) at invasive front	0.918	0.813	1.036	0.167
CD8 density (cells/mm2) in non-tumor tissue	0.986	0.502	1.934	0.967
	1.018	0.635	1.631	0.941

* except for PD-L1 Expression on Tumor (>5% vs. <5%), which is represented as a binary variable

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Luis		Diaz	

Residence Information (Select One) ● US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
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Mailing Address of Inventor:

Address 1	6135 Crystal Springs Drive
Address 2	

City	State/Province	Postal Code	Country
Ellicot City	MD	21043	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Bert		Vogelstein	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

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Baltimore	MD	US

Mailing Address of Inventor:

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Address 2	

City	State/Province	Postal Code	Country
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Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kenneth	W.	Kinzler	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

City	Baltimore	State/Province	MD	Country of Residence	US

Mailing Address of Inventor:

Address 1	616 Ponte Villas North		
Address 2			
City	Baltimore	State/Province	MD
Postal Code	21230	Country	US

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Nickolas		Papadopoulos	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Towson	State/Province	MD	Country of Residence	US

Mailing Address of Inventor:

Address 1	606 Horncrest		
Address 2			
City	Towson	State/Province	MD
Postal Code	21204	Country	US

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Dung		Le	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Lutherville	State/Province	MD	Country of Residence	US

Mailing Address of Inventor:

Address 1	12 Laurel Circle		
Address 2			
City	Lutherville	State/Province	MD
Postal Code	21093	Country	US

Inventor 6 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Drew	M.	Pardoll	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Brookville	State/Province	MD	Country of Residence	US

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

Mailing Address of Inventor:

Address 1	19400 James Creek Court		
Address 2			
City	Brookville	**State/Province**	MD
Postal Code	20833	**Country**	US

Inventor 7 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Suzanne	L.	Topalian	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Brookville	**State/Province**	MD	**Country of Residence**	US

Mailing Address of Inventor:

Address 1	19400 James Creek Court		
Address 2			
City	Brookville	**State/Province**	MD
Postal Code	20833	**Country**	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	147364		
Email Address	apsi@fr.com	Add Email	Remove Email

Application Information:

Title of the Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY		
Attorney Docket Number	44807-0114003	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	13	**Suggested Figure for Publication (if any)**	

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	147364		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	15523451	2017-05-01

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
15523451	a 371 of international	PCTUS2015060331	2015-11-12
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCTUS2015060331	Claims benefit of provisional	62190977	2015-07-10
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCTUS2015060331	Claims benefit of provisional	62079357	2014-11-13
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 — Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	The Johns Hopkins University		
Mailing Address Information For Applicant:			
Address 1	3400 North Charles Street		
Address 2			
City	Baltimore	**State/Province**	MD
Country	US	Postal Code	21218
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Cameron Luitjens/			Date (YYYY-MM-DD)	2018-09-27
First Name	Cameron M.	Last Name	Luitjens	Registration Number	58,674

Additional Signature may be generated within this form by selecting the Add button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	44807-0114003
	Application Number	
Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	Checkpoint Blockade and Microsatellite Instability C13241_P13241-03

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number PCT/US15/60331 filed on 11/12/2015.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

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LEGAL NAME OF INVENTOR

Inventor: Luis Diaz Date (Optional): 5-22-2017

Signature: [signature]

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/01 (06-12)
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LEGAL NAME OF INVENTOR

Inventor: Bert Vogelstein Date (Optional): 5.23.17

Signature:

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/01 (06-12)
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Title of Invention	Checkpoint Blockade and Microsatellite Instability C13241_P13241-03

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LEGAL NAME OF INVENTOR

Inventor: Kenneth W. Kinzler Date (Optional): 5/23/2017

Signature: Kenneth W. Kinzler

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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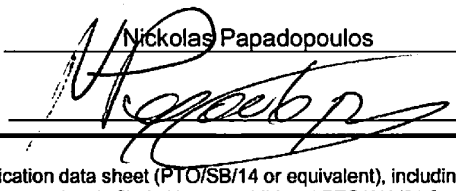
LEGAL NAME OF INVENTOR

Inventor: Nickolas Papadopoulos Date (Optional) : _____

Signature: 

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Title of Invention	Checkpoint Blockade and Microsatellite Instability C13241_P13241-03

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LEGAL NAME OF INVENTOR

Inventor: Dung Le Date (Optional): 5/24/17

Signature:

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

As the below named inventor, I hereby declare that:

This declaration is directed to:

[ ] The attached application, or

[X] United States application or PCT international application number 15/523,451

filed on May 1, 2017.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

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LEGAL NAME OF INVENTOR

Inventor: Drew M. Pardoll Date (Optional): 7/27/2018

Signature:

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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Title of Invention	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

As the below named inventor, I hereby declare that:

This declaration is directed to:

[ ] The attached application, or

[X] United States application or PCT international application number 15/523,451

filed on May 1, 2017.

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LEGAL NAME OF INVENTOR

Inventor: Suzanne L. Topalian Date (Optional): 7/29/2018

Signature:

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

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Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY
First Named Inventor/Applicant Name:	Luis Diaz
Filer:	Cameron Mark Luitjens
Attorney Docket Number:	44807-0114003
Filed as Large Entity	
Filing Fees for **Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)**	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY APPLICATION FILING	1011	1	300	300
UTILITY SEARCH FEE	1111	1	660	660
UTILITY EXAMINATION FEE	1311	1	760	760
REQUEST FOR PRIORITIZED EXAMINATION	1817	1	4000	4000
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	1202	10	100	1000
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**6860**

Electronic Acknowledgement Receipt	
EFS ID:	33851332
Application Number:	16144549
International Application Number:	
Confirmation Number:	4333
Title of Invention:	CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY
First Named Inventor/Applicant Name:	Luis Diaz
Customer Number:	147364
Filer:	Cameron Mark Luitjens/Renee Neuman
Filer Authorized By:	Cameron Mark Luitjens
Attorney Docket Number:	44807-0114003
Receipt Date:	27-SEP-2018
Filing Date:	
Time Stamp:	16:39:31
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$6860
RAM confirmation Number	092818INTEFSW00004013061050
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	TRANS.pdf	109939 038c8fb84e1c4b4fe230c9a11b20656cba6a2463	no	2
Warnings:					
Information:					
2	TrackOne Request	REQ.pdf	149914 21a572c396636dc061034113251239a4da562c27	no	2
Warnings:					
Information:					
3		APPL.pdf	306444 b25a68d0d1120eadc7a33aff539322ed098dcb01	yes	45

Multipart Description/PDF files in .zip description

Document Description	Start	End
Abstract	45	45
Claims	41	44
Specification	1	40

Warnings:					
Information:					
4	Drawings-only black and white line drawings	FIGS.pdf	1260159 bb481635a52eedb338af5fd9c5bd1fabad15f41e	no	13
Warnings:					
Information:					
5	Application Data Sheet	ADS.pdf	1823619 863e23377bf90598b43a1a4ef4be11a5a44167b9	no	10

Warnings:					
Information:					
6	Oath or Declaration filed	DEC.pdf	617902 17f4d7369b2ea7788e5a74189e55f45cfb5068b7	no	7
Warnings:					
Information:					
7	Fee Worksheet (SB06)	fee-info.pdf	40217 e02c85314f969d946a76343a20ff4b89cb009c66	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	4308194	

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<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

FISH.
FISH & RICHARDSON

Street Address
Fish & Richardson P.C.
3200 RBC Plaza
60 South Sixth Street
Minneapolis, MN 55402

Mail Address
P.O. Box 1022
Minneapolis, MN 55440-1022

612 335 5070 main
877 769 7945 fax

September 27, 2018

Attorney Docket No.: 44807-0114003

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Presented for filing is a new continuation patent application for prioritized examination of:

Inventor(s): LUIS DIAZ, BERT VOGELSTEIN, KENNETH W. KINZLER, NICKOLAS PAPADOPOULOS, DUNG LE, DREW M. PARDOLL AND SUZANNE L. TOPALIAN

Title: CHECKPOINT BLOCKADE AND MICROSATELLITE INSTABILITY

Enclosed are the following papers, including those required to receive a filing date under 37 CFR 1.53(b):

	Pages
Specification	40
Claims	4
Abstract	1
Drawing(s)	13
Declaration	7

Enclosures:

— Application Data Sheet, 10 pages.
— Certification and Request for Prioritized Examination (Track I), 1 page

Basic Filing Fee			$300
Search Fee			$660
Examination Fee			$760
Publication fee			$0
Track I processing fee			$140
Track I prioritized examination fee			$4000
Total Claims 30	over 20	**10 x $100**	$1000

Independent Claims 2	over 3	**0 x $460**	$0
Fee for Multiple Dependent claims			$0
Application size fee for each 50 pages over 100 Total Sheets: 58 x .75 - 100/50 = 0x			$0
Total Filing fee			$6860

Please apply any necessary charges or credits to Deposit Account 06-1050, referencing the attorney docket number shown above.

I hereby authorize the USPTO to communicate in response to email communications from the undersigned and practitioners under Customer No. 147364 in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via email. I understand that a copy of these communications will be made of record in the application file. (MPEP 502.03).

If this application is considered to be incomplete, or if a telephone conference would otherwise be helpful, call the undersigned at (612) 335-5070.

Send all correspondence to:

147364
Customer Number

Respectfully submitted,

/Cameron Luitjens/

Cameron M. Luitjens, Ph.D., J.D.
Registration No. 58,674

Enclosures

CVL/jyh
61760402.doc

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 16144549 Document Date: 09/27/2018

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013