Cancer Immunology Research

Immunopathologic Stratification of Colorectal Cancer for Checkpoint Blockade Immunotherapy

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Abstract

Mismatch-repair deficiency in solid tumors predicts their response to PD-1 blockade. Based on this principle, pembrolizumab is approved as standard of care for patients with unresectable or metastatic microsatellite instability–high (MSI-H) cancer. Despite this success, a large majority of metastatic colorectal cancer patients are not MSI-H and do not benefit from checkpoint blockade treatment. Predictive biomarkers to develop personalized medicines and guide clinical trials are needed for these patients. We, therefore, asked whether immunohistologic stratification of metastatic colorectal cancer based on primary tumor PD-L1 expression associated with the presence or absence of extracellular mucin defines a subset of metastatic colorectal cancer patients who exhibit a preexisting antitumor immune response and who could potentially benefit from the checkpoint blockade. To address this, we studied 26 advanced metastatic colorectal cancer patients treated with pembrolizumab (NCT01876511). To stratify patients, incorporation of histopathologic characteristics (percentage of extracellular mucin) and PD-L1 expression at the invasive front were used to generate a composite score, the CPM (composite PD-L1 and mucin) score, which discriminated patients who exhibited clinical benefit (complete, partial, or stable disease) from those patients with progressive disease. When validated in larger cohorts, the CPM score in combination with MSI testing may guide immunotherapy interventions for colorectal cancer patient treatment.

Introduction

Oncologic precision medicine involves screening for and selecting therapies based on an individual's tumor-specific biomarkers to optimize clinical outcomes and minimize adverse events. The use of mismatch-repair (MMR) deficiency as a predictive biomarker of colorectal cancer response to PD-1 blockade was first reported by Le and colleagues in 2015 (1) and confirmed in 2017 (2). MMR deficiency, leading to accumulation of nonsynonymous mutations, predicts the response of solid tumors to PD-1 blockade, and based on this principle, the FDA granted accelerated approval to pembrolizumab for adult and pediatric patients with unresectable or metastatic microsatellite instability–high (MSI-H) tumors. This is the first example of a tissue-agnostic FDA approval of a treatment based on a patient's tumor biomarker status rather than on tumor histology. Despite this success, a large majority of patients do not benefit from checkpoint inhibitors (3). Multiple genomic and immunologic factors may potentially contribute to anti–PD-1's efficacy in subsets of patients with melanoma or non–small cell lung cancer, among other cancers (4). Therefore, predictive biomarkers to develop personalized medicines and guide clinical trial development are an urgent unmet need.

Four elements have taken the limelight in the search for biomarkers: (i) PD-L1 expression in the tumor microenvironment; (ii) the presence of abundant T-cell infiltrates and surrogate transcriptional signatures of IFNγ function; (iii) estimations of

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tumor mutational burden (TMB); and (iv) studies on the composition of the gut microbiome, all contributing in identifying baseline (pretreatment) immune-related biomarkers to predict clinical outcome of immunotherapy (5–8). Integration of PD-L1 expression and TMB was proposed to better identify patients who will benefit from checkpoint inhibition (9, 10). However, each biomarker by itself may not be able to accurately delineate patients who benefit from immunotherapy (11). We, therefore, focused our study on the tumor immune microenvironment (TiME) of metastatic colorectal cancer (mCRC) using primary colon tumor specimens from mCRC patients treated with pembrolizumab (NCT01876511) and compared tumor specimens of patients who exhibited clinical benefit [CB: complete response (CR), partial response (PR), and stable disease (SD)] with patients developing progressive disease (PD). The objective was to understand the nature of the immunohistopathologic components of the TiME that associated with the CB of these patients and ultimately delineate a population of immunoreactive colorectal cancer potentially suitable for immune interventions.

Materials and Methods

Clinical trial and patient selection

Patients with previously treated mCRC were selected from six centers (Johns Hopkins University, Providence Portland Medical Center, Stanford University, Ohio State University, Abramson Cancer Center at University of Pennsylvania, and NCI) for this phase II study (NCT01876511) using pembrolizumab (anti–PD-1). To be eligible for participation in this study, patients had to be at least 18 years of age and 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 colorectal cancer must have received at least two prior cancer therapies, and patients with other cancer types must have received at least one prior cancer therapy. Patients with untreated brain metastases, a history of human immunodeficiency virus, hepatitis B, hepatitis C, clinically significant ascites/effusions, or autoimmune disease were excluded. A total of 86 patients with treatment-refractory progressive, advanced, MMR-deficient (MMRd) cancers were recruited in three cohorts, cohort A for the MSI^+ mCRC, cohort B for the microsatellite stable (MSS) mCRC, and cohort C for the MSI^+ non-colorectal cancer (1). Additional longitudinal data from 11 colorectal cancer and 7 non-colorectal cancer patients with MMRd cancers from our previous report were included (1, 2).

For study enrollment, MMR deficiency was determined at each participating institution by IHC for MMR proteins or by PCR-based tests for MSI. When sufficient tissue was available, MSI in DNA purified from the tumor was assessed with an MSI Analysis System (Promega). Our analysis utilized samples obtained from cohorts A and B of the trial, colorectal cancer–only cases, and specimens included in this article were those with sufficient material available and had corresponding clinically annotated data. We segregated patients according to their CB to checkpoint inhibition. Groups were composed of CR/PR/SD patients who were deemed to have CB versus the PD patients. This study was approved by the Institutional Review Board of Johns Hopkins University and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The patients described in this study provided written informed consent, and tumor colon tissues were obtained in accordance with the Health Insurance Portability and Accountability Act (detailed in refs. 1, 2).

Histopathology, IHC, and image analysis

Formalin-fixed paraffin-embedded (FFPE) tissue sections from resected colon tumors obtained at diagnosis were stained with a hematoxylin and eosin (H&E) combination. Extracellular mucin pools were defined as the collection of mucin not associated with malignant epithelial cells and quantified as the percentage of the tumor surface area replaced by extracellular mucin pools (12). Digital quantification of tumor and mucin areas was performed utilizing the HALO image analysis platform from Indica Labs (Supplementary Fig. S1). FFPE colon tumor tissue sections were also stained for CD8 (clone C8144B, Cell Marque) and PD-L1 (clone 5H1) as previously reported (13, 14). For $CD8^+$ T-cell density quantification, 90% of the tumor cellular area was annotated, and we selectively included the tumor area that contained malignant epithelial cells and excluded extracellular mucinous areas. Digital quantification was performed utilizing the HALO image analysis. PD-L1 was scored at the invasive front, which is the region where the tumor tissue juxtaposes the normal colonic tissue (13). We assessed interobserver agreement by using independent readings from two pathologists (R.A. Anders and E.D. Thompson) blinded to the outcomes of patients. Correlation of the scoring between the two pathologists was tested by determining the Spearman rank correlation coefficients. Statistical comparisons of the percentage of mucin detected or PD-L1 expression between patients who had CB and PD were performed using the nonparametric Mann–Whitney test. All the analyses were performed using the software R version 3.5.1 (The R Foundation for Statistical Computing).

Composite PD-L1 mucin score

Logistic regression was applied to build a composite score combining PD-L1 and mucin (CPM score) to distinguish patients who did and did not benefit from pembrolizumab treatment based on the data from 26 patients (16 CR/PR/SD and 10 PD). According to this model, we calculated the CPM score as the average of the percentage of mucin detected and PD-L1 expression ($CPM = [\%\ PD\text{-}L1 + \%\ extracellular\ mucin]/2$). The performance of the CPM score in distinguishing CB versus PD was assessed using receiver operating characteristic (ROC) analysis. The area under the ROC curve (AUC) for CPM score, a measure of how well it distinguishes the two groups, was compared with PD-L1 alone and mucin alone using the DeLong test. The classification tree method, based on recursive partitioning that minimizes the misclassification error, was used to determine the cutoff threshold of the CPM score in classifying patients into CB versus PD. All the analyses were performed using the software R version 3.5.1 (The R Foundation for Statistical Computing).

Results

We first sought to compare the baseline histopathologic characteristics of mCRC patients with CB (CR/PR/SD) upon treatment with checkpoint inhibition with the features of PD patients. Demographics of patients used in this study are

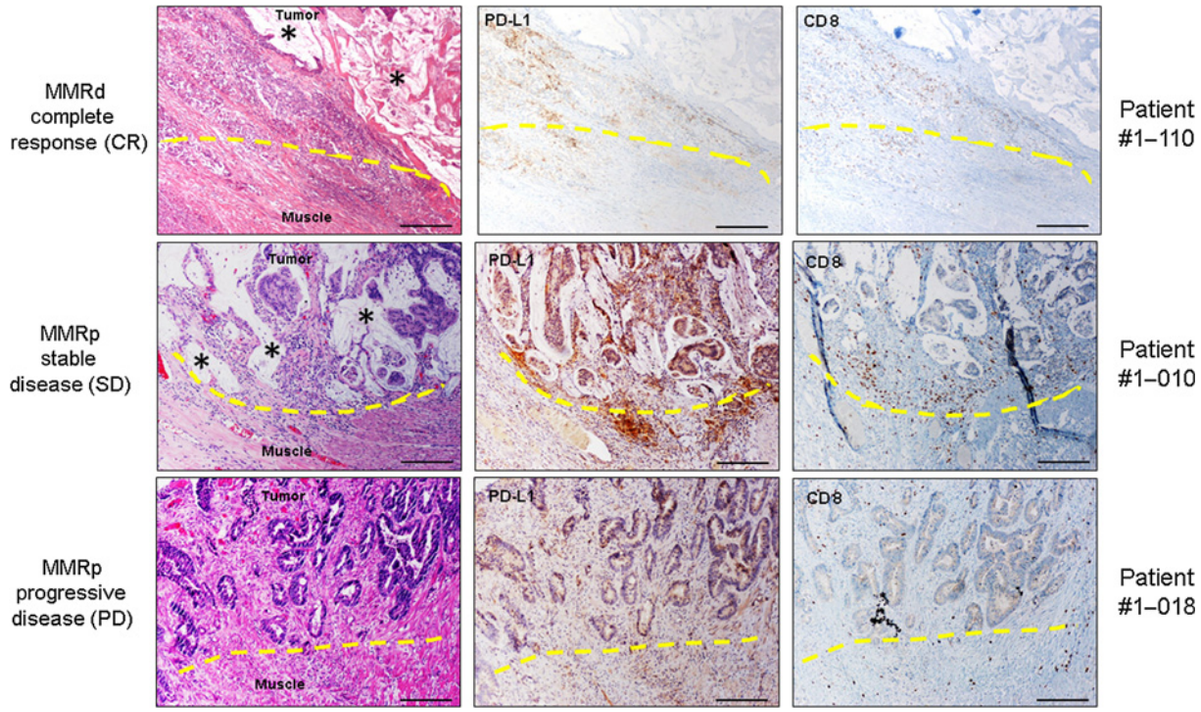

Figure 1.

Representative H&E, PD-L1 IHC, and CD8$^+$ T-cell IHC of baseline colon tumor specimens of patients treated with pembrolizumab (anti–PD-1). Characteristic patterns of mucinous component, PD-L1 expression at the invasive front (IF), and CD8$^+$ T-cell infiltration in samples from patients exhibiting CB (CR/PR/SD) versus PD. Patient #1-110, MMRd with CR; patient #1-010, MMR-proficient (MMRp) with SD; and patient #1-018, MMRp with PD. Asterisks represent extracellular mucin pools in tumor area and dashed yellow lines mark IF region. Images at 10× magnification; scale bar, 1 millimeter.

described in Supplementary Table S1. With this approach, the two main distinctive features noted in the analyzed pretreatment colon specimens of patients were the presence of a mucinous component and PD-L1 positivity at the invasive front (Fig. 1; Supplementary Fig. S1). Overall, mCRC patients who exhibited CB had tumors that contained higher percentages of mucin covering the tumor area and PD-L1 expression at the invasive front (Fig. 2). In contrast, patients who experienced PD neither developed mucinous features nor exhibited high percentages of PD-L1 staining (Fig. 2). These data suggested that large mucinous areas could result from tumor tissue destruction by an advancing field of immune cells, leaving behind extracellular mucin pools.

This phenomenon has been observed in the setting of chemotherapy, and the current recommendation by the College of American Pathologists is to regard extracellular mucin as a type of treatment response and not as residual tumor (12, 15, 16). However, we could not rule out the possibility of the tumor tissue actively secreting mucin. We, therefore, devised a composite PD-L1/mucin (CPM) score that integrated expression of PD-L1 at the invasive front with the detection of extracellular mucinous areas. The weighting factors for mucin and PD-L1 were almost identical (0.106 and 0.130, respectively) according to the logistic model estimates. Therefore, we calculated the CPM score as the average of the percentage of mucin detected and PD-L1 expression to predict CB (Fig. 3; Supplementary Table S2). With this approach, the AUC for the CPM score was 0.994, and therefore higher than the AUC for the PD-L1 alone and mucin alone [AUC = 0.787 (PD-L1 vs. CPM, $P = 0.018$) and 0.882 (mucin vs. CPM, $P = 0.057$), respectively], indicating the good performance of the CPM score in distinguishing CB versus PD (Fig. 4).

The cutoff value of the CPM score was determined to be 14% (see Materials and Methods section). Among the 16 patients who achieved CB, 15 (94%) had CPM scored greater than or equal to 14%; all PD patients had a CPM score lower than 14% (Fig. 3). Note that due to a limited set of data available extracted from the clinical trial, any level between 10.5 and 17.5 as cutoff threshold would lead to the same classification accuracy.

In our analysis, two blinded pathologists analyzed biopsy slides from 16 CB patients and 10 PD patients. Scoring between the two pathologists correlated, with r values of 0.99 and 0.97, respectively (Supplementary Fig. S2). Although significantly different between CB and PD patients, mucin and PD-L1 scores, individually ($P = 0.0004$ and 0.0120, respectively, CB vs. PD patients), did not clearly segregate CB and PD patients (Fig. 2). On the contrary, we demonstrated that 15 of 16 CB patients had a CPM score >14% (CB vs. PD, $P < 0.0001$; Fig. 3). We also found that two MMR-proficient (MMRp) mCRC with SD (patients 1-010 and 1-040) had a CPM score >14% (Fig. 3). None of the PD patients had a CPM score >14% (Fig. 3).

Although prior observations indicate that the presence of CD8$^+$ tumor-infiltrating lymphocytes in tumor biopsy samples is associated with improved survival in colorectal cancer patients (17), in our study, CD8$^+$ T-cell densities in mCRC patients with CB and PD were not statistically different, and our analysis did not separate patients according to their response pattern once an outlier patient was removed from the data set (patient 1-052

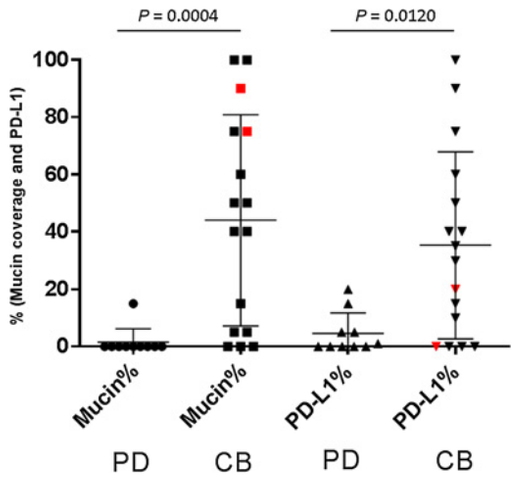

Figure 2.
Individual extracellular mucin quantification and PD-L1 scoring at invasive front (IF) of pretreatment resected colon tumor specimens. PD, $n = 10$, CB: CR/PR/SD, $n = 16$. Two MMR-proficient colorectal cancer patients with SD are indicated in red. Mean +standard deviation; two-sided nonparametric Mann–Whitney test; statistical significance when $P < 0.05$.

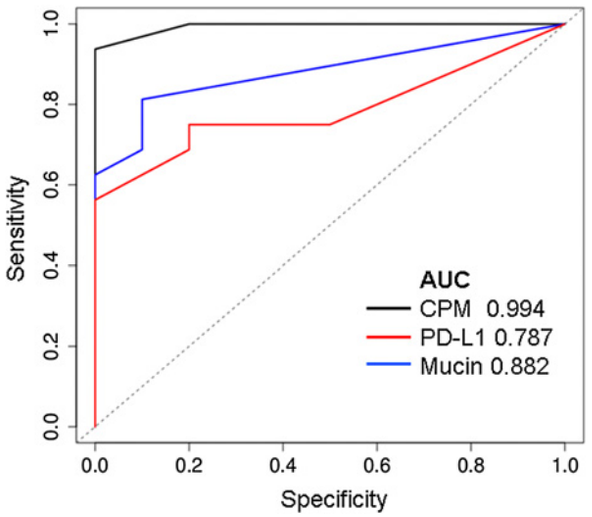

Figure 4.
ROC curves demonstrate the performance of the CPM score to stratify CB (CR/PR/SD) versus PD patients. AUC is shown for the CPM score, percentage of PD-L1 expression, and percentage of mucin area. The DeLong test was used to compare PD-L1 alone versus CPM ($P = 0.018$) and mucin alone versus CPM ($P = 0.057$). The dotted diagonal line represents an ROC curve of a classifier based on pure chance.

with intratumoral CD8$^+$ T-cell density = 3,025 cells/mm^2 vs. an average of 282 CD8$^+$ cells/mm^2 for the rest of the cohort; Supplementary Table S3; Supplementary Figs. S3 and S4), demonstrating that CD8$^+$ T cells are often excluded from tumor areas where large extracellular mucin areas replace tumor tissue. We, therefore, propose that CD8$^+$ T-cell counts in these tissue areas may underestimate the extent of the endogenous intratumoral immune response. Spearman correlations between individual components of our composite score (percentage of extracellular mucin and percentage of PD-L1 expression) and their respective correlation with the corresponding intratumoral CD8$^+$ T-cell densities did not show an association, suggesting that the selected immunopathologic features, extracellular mucin and PD-L1, are largely independent, thus complementary, to each other (Supplementary Figs. S5 and S6).

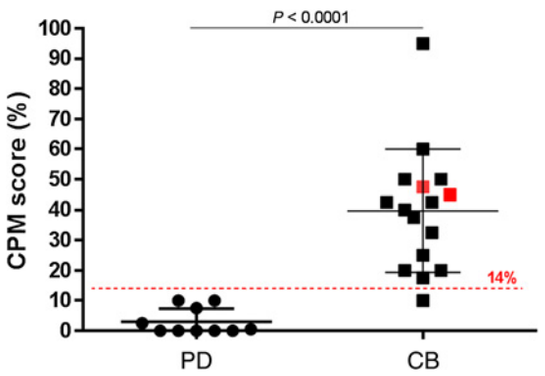

Figure 3.
Composite PD-L1/mucin (CPM) score segregates CB from PD patients. The CPM score integrates expression of PD-L1 at the invasive front and the detection of acellular mucinous areas in colon tumor specimens. The cutoff value of the CPM score was determined to be 14% (red dashed line). Two MMRp colorectal cancer patients with SD are indicated in red. PD, $n = 10$, CB: CR/PR/SD, $n = 16$. Mean ± standard deviation; two-sided nonparametric Mann–Whitney test; statistical significance when $P < 0.05$.

Discussion

We herein proposed a complementary score that integrates the expression of PD-L1 at the invasive front region of tumors in combination with the detection of extracellular mucinous areas. With this approach, we demonstrated that MMRd and MMRp mCRC patients with CB from checkpoint blockade exhibited significantly higher CPM scores than mCRC patients with PD. Given that preexisting immunologic features of both the host and the tumor may contribute to how patients will respond with immunotherapy (5, 10), we believe that reporting mucinous features along with PD-L1 expression in routine pathology practices may delineate a subset of mCRC patients who might benefit from checkpoint blockade–based immunotherapies. The role of the adaptive immune response in controlling the growth and recurrence of human tumors has now been documented in the setting of multiple cancers (18). Thus, identifying baseline immune-related biomarkers to select patients and predict clinical outcome to immunotherapy is of the essence.

Although immune-checkpoint blockade, which activates the endogenous immune system against cancer, has led to breakthroughs for a variety of malignancies, clinical responses are limited to a subgroup of patients (~12%; ref. 3). The type, density, and location of immune cells within colorectal tumor samples have been found to be better at predicting survival of patients than the histopathologic methods currently used to stage colorectal cancer (17). Adaptive immune cell infiltration was observed to have a prognostic value superior to the classic

extension and invasion tumor criteria (19). The "Immunoscore" quantifying the density of $CD3^+$ and $CD8^+$ T cells in the tumor center and its invasive margin was proposed as a novel immune classification in colorectal tumors (17). Accumulating exceptions (such as lack of response to treatment in some patients, the incomplete correlation between PD-L1 expression and clinical effectiveness of PD-1 blockade, refs. 5, 20, 21; and the counter examples in renal cell carcinoma in which the presence of T cells is generally associated with poor outcome, ref. 22) indicate that a more comprehensive profiling of local immune cells and their function is warranted. In our study, by pairing the clinical response data with an interrogation of the TiME of samples served as an inestimable window into the TiME of mCRC patients, which is critically important to identify relevant biomarkers independent of MSI status. We conclude that mCRC patients who exhibited CB to anti–PD-1 treatment displayed consistent immunopathologic features that could be quantified; i.e., the percentage of PD-L1 expression at the tumor invasive front and the corresponding amount of extracellular mucin present in the specimens. This composite score can be readily calculated using existing FDA-approved IHC tests for PD-L1 expression, which use different anti–PD-L1 clones (4), and H&E staining to estimate extracellular mucin content.

Both parameters used in this study were valid regardless of the MSI status of patients. Two MMRp mCRC patients, who observed long-term SD, had a CPM score >14%. Mucinous adenocarcinoma is part of the World Health Organization classification of colorectal carcinoma. Traditionally, the grading of mucinous and signet ring carcinomas, which were previously invariably graded as G3/high grade, is dependent on the MSI status (23). Interestingly, a previous article reported an inverse association of tumor CD274 (PD-L1) expression with tumor MSI status and the extent of extracellular mucin (24). The focus of our study was to justify that the CPM combination score was better than mucin or PD-L1 alone for discriminating response versus no response in colorectal cancer patients. Although $CD8^+$ T-cell infiltration has been clearly established as a prognostic factor in the case of colorectal cancer and is associated with better progression-free survival and overall survival (17), it has not been shown to be a predictive marker that can guide patient selection to receive immuno-oncology therapy. Our observations on a limited number of patients enrolled in the clinical trial NCT01876511 will be prospectively validated in our follow-up clinical trial, in which two cohorts of MMRp mCRC patients are enrolled based on their positive or negative CPM score and subsequently assigned to receive combination immunotherapy (NCT03642067). Combined with MSI testing to select MMRd colorectal cancer for checkpoint blockade treatment, this approach has the potential to open up immunotherapy to a broader population of colorectal cancer patients by including MSS colorectal cancer patients who are currently not captured by current molecular biomarkers. Such is the case of the outlier patient (#1-010) characterized by a CPM score >14% and who exhibited SD for more than 3 years after initiation of anti–PD-1 therapy (25). MSS stage IV colorectal cancer patients and their caregivers have a sense of urgency that is not currently reflected in clinical trials, and we believe that our data could contribute to addressing this unmet need.

Disclosure of Potential Conflicts of Interest

N.J. Llosa reports receiving a commercial research grant from and is a consultant/advisory member for Bristol-Myers Squibb. E.M. Jaffee reports receiving commercial research grants from Aduro Biotech and Bristol-Myers Squibb and is a consultant/advisory board member for CSTONE, DragonFly, and Genocea. C.L. Sears reports receiving a commercial research grant from Bristol-Myers Squibb. D.T. Le reports receiving commercial research grants from Merck and Bristol-Myers Squibb, has received speakers bureau honoraria from Merck, and is a consultant/advisory board member for Merck and Bristol-Myers Squibb. L.A. Diaz reports receiving a commercial research grant from Merck, has ownership interest (including patents) in PGDx, Amgen, Thrive, Neophor, and Jounce, and is a consultant/advisory board member for PGDx, Merck, Neophore, and Jounce. D.M. Pardoll reports receiving commercial research grants from Bristol-Myers Squibb, Compugen, and AstraZeneca, has ownership interest (including patents) in DNAtrix, Dracen Pharma, Five Prime Therapeutics, Potenza, Tizona, Trieza, Aduro Biotech, Ervaxx, and WindMil, and is a consultant/advisory board member for Aduro Biotech, Amgen, Merck, Rock Springs Capitol, Tizona, Bayer, Camden Partners, Dynavax, Ervaxx, Five Prime Thera, FLX Bio, Immunomic, and Janssen. F. Housseau reports receiving other commercial research support from Bristol-Meyers Squibb. No potential conflicts of interest were disclosed by the other authors.

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Other (pathology review): E.D. Thompson

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References

1. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015; 372:2509–20.

2. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357:409–13.

3. Haslam A, Prasad V. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. JAMA Netw Open 2019;2:e192535.
4. Nishino M, Ramaiya NH, Hatabu H, Hodi FS. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. Nat Rev Clin Oncol 2017;14:655–68.
5. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res 2014;20:5064–74.
6. Hellmann MD, Callahan MK, Awad MM, Calvo E, Ascierto PA, Atmaca A, et al. Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. Cancer Cell 2018;33:853–61.e4.
7. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 2016;44:698–711.
8. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018;359:97–103.
9. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. J Clin Oncol 2018;36:633–41.
10. Lu S, Stein JE, Rimm DL, Wang DW, Bell JM, Johnson DB, et al. Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: a systematic review and meta-analysis. JAMA Oncol 2019 Jul 18 [Epub ahead of print].
11. Signorelli D, Giannatempo P, Grazia G, Aiello MM, Bertolini F, Mirabile A, et al. Patients selection for immunotherapy in solid tumors: overcome the naive vision of a single biomarker. Biomed Res Int 2019;2019:9056417.
12. Shia J, McManus M, Guillem JG, Leibold T, Zhou Q, Tang LH, et al. Significance of acellular mucin pools in rectal carcinoma after neoadjuvant chemoradiotherapy. Am J Surg Pathol 2011;35:127–34.
13. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov 2015;5:43–51.
14. Sunshine JC, Nguyen PL, Kaunitz GJ, Cottrell TR, Berry S, Esandrio J, et al. PD-L1 expression in melanoma: a quantitative immunohistochemical antibody comparison. Clin Cancer Res 2017;23:4938–44.
15. Bhatti AB, Akbar A, Khattak S, Kazmi AS, Jamshed A, Syed AA. Impact of acellular mucin pools on survival in patients with complete pathological response to neoadjuvant treatment in rectal cancer. Int J Surg 2014;12:1123–6.
16. Reggiani Bonetti L, Lionti S, Domati F, Barresi V. Do pathological variables have prognostic significance in rectal adenocarcinoma treated with neoadjuvant chemoradiotherapy and surgery? World J Gastroenterol 2017;23:1412–23.
17. Pages F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 2018;391:2128–39.
18. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565–70.
19. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–4.
20. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366:2443–54.
21. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 2014;32:1020–30.
22. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res 2007;13:1757–61.
23. Aust DE. [WHO classification 2010 for the lower gastrointestinal tract: what is new?]. Pathologe 2011;32Suppl 2:326–31.
24. Masugi Y, Nishihara R, Yang J, Mima K, da Silva A, Shi Y, et al. Tumour CD274 (PD-L1) expression and T cells in colorectal cancer. Gut 2017;66:1463–73.
25. Smith KN, Llosa NJ, Cottrell TR, Siegel N, Fan H, Suri P, et al. Persistent mutant oncogene specific T cells in two patients benefitting from anti-PD-1. J Immunother Cancer 2019;7:40.