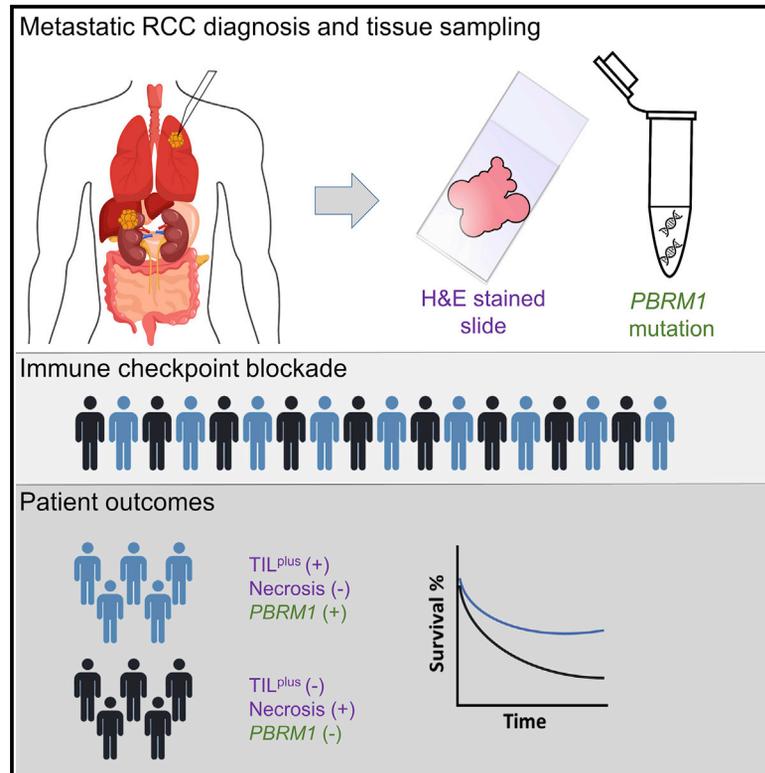**Report**

Combinatorial biomarker for predicting outcomes to anti-PD-1 therapy in patients with metastatic clear cell renal cell carcinoma

Graphical abstract

Authors

Julie Stein Deutsch, Evan J. Lipson, Ludmila Danilova, ..., Sabina Signoretti, Michael Atkins, Janis M. Taube

Correspondence

jtaube1@jhmi.edu

In brief

Deutsch et al. show that H&E slide scores of immune response and necrosis predict outcomes following immune checkpoint blockade in renal cell carcinoma. When combined with *PBRM1* mutational status, patients are further stratified. The influence of tumoral necrosis on infiltrating immune cells has broad translational relevance for tissue-based biomarker development.

Highlights

- H&E score of immune cells in pre-immunotherapy tumors associates with improved OS

- Necrosis in pre-treatment specimens attenuates the beneficial effect of TIL[plus]

- TIL[plus] and necrosis can be combined with *PBRM1* status to improve OS stratification

- H&E slides used in routine, clinical care can be used for biomarker development

CelPress

Report

Combinatorial biomarker for predicting outcomes to anti-PD-1 therapy in patients with metastatic clear cell renal cell carcinoma

Julie Stein Deutsch,[1] Evan J. Lipson,[2,5] Ludmila Danilova,[2,5] Suzanne L. Topalian,[4,5] Jaroslaw Jedrych,[1,3] Ezra Baraban,[1] Yasser Ged,[2] Nirmish Singla,[2] Toni K. Choueiri,[6] Saurabh Gupta,[7] Robert J. Motzer,[8] David McDermott,[9] Sabina Signoretti,[10] Michael Atkins,[11] and Janis M. Taube[1,2,3,5,12,*]

[1]Department of Pathology, Johns Hopkins University, Baltimore, MD 21287, USA
[2]Department of Oncology, Johns Hopkins University, Baltimore, MD 21287, USA
[3]Department of Dermatology, Johns Hopkins University, Baltimore, MD 21287, USA
[4]Department of Surgery, Johns Hopkins University, Baltimore, MD, USA
[5]The Bloomberg~Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University, Baltimore, MD 21287, USA
[6]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA
[7]Bristol-Myers Squibb, Princeton, NJ, USA
[8]Department of Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[9]Department of Hematology/Oncology, Beth Israel Deaconess Medical Center, Boston, MA, USA
[10]Department of Pathology, Dana-Farber Cancer Institute, Boston, MA, USA
[11]Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, USA
[12]Lead contact
*Correspondence: jtaube1@jhmi.edu
https://doi.org/10.1016/j.xcrm.2023.100947

SUMMARY

With a rapidly developing immunotherapeutic landscape for patients with metastatic clear cell renal cell carcinoma, biomarkers of efficacy are highly desirable to guide treatment strategy. Hematoxylin and eosin (H&E)-stained slides are inexpensive and widely available in pathology laboratories, including in resource-poor settings. Here, H&E scoring of tumor-infiltrating immune cells (TIL^{plus}) in pre-treatment tumor specimens using light microscopy is associated with improved overall survival (OS) in three independent cohorts of patients receiving immune checkpoint blockade. Necrosis score alone does not associate with OS; however, necrosis modifies the predictive effect of TIL^{plus}, a finding that has broad translational relevance for tissue-based biomarker development. *PBRM1* mutational status is combined with H&E scores to further refine outcome predictions (OS, p = 0.007, and objective response, p = 0.04). These findings bring H&E assessment to the fore for biomarker development in future prospective, randomized trials, and emerging multi-omics classifiers.

INTRODUCTION

The therapeutic landscape for patients with metastatic clear cell renal cell carcinoma (mccRCC) is rapidly evolving, and includes regimens with immunotherapeutic and antiangiogenic agents, either alone or in combination.[1,2] First-line treatment options include combination anti-PD-1 + anti-CTLA4 blockade and anti-PD-1 + TKI, with an option for anti-PD-1 monotherapy, among others, in patients who cannot tolerate or who have progressed on other regimens.[2] With a number of therapeutic options now available, there is an unmet need for biomarkers that can help match a patient to the regimen most likely to result in clinical benefit. Such pairings would ideally also help minimize unnecessary therapeutic exposure and associated toxicities.

FDA-approved pre-treatment tumor biomarkers such as PD-L1 immunohistochemistry (IHC) and tumor mutational burden (TMB) that associate with response to anti-PD-(L)1-based therapies in non-small cell lung carcinoma (NSCLC) and other cancers are not predictive in patients with mccRCC.[3,4] Mutations in

PBRM1 have also been explored as prognostic and predictive biomarkers. *PBRM1* is a member of the SWI/SNF chromatin remodeling complex, and mutations in this gene are seen in ~40% of patients with RCC.[5] Initial reports suggested that loss of function in *PBRM1* led to an enhanced interferon-γ (IFN-γ) gene expression profile and higher cytotoxic T cell activity when compared with controls.[6] However, a large follow-up study that tested the association between *PBRM1* mutation and transcriptomic data from 594 patients with ccRCC failed to provide additional support for those findings.[5] When deployed as a solitary biomarker, *PBRM1* mutational status appears to be modestly associated, at best, with survival after immune checkpoint blockade (ICB).[7,8]

In other tumor types, multimodality and multiplex biomarkers have been shown to have higher predictive value for response to ICB than unidimensional markers.[9] To that end, the combination of gene expression signatures with TMB and PD-L1 IHC was explored in patients with mccRCC receiving ICB, but this strategy failed to validate in independent cohorts.[10,11] Multiplex
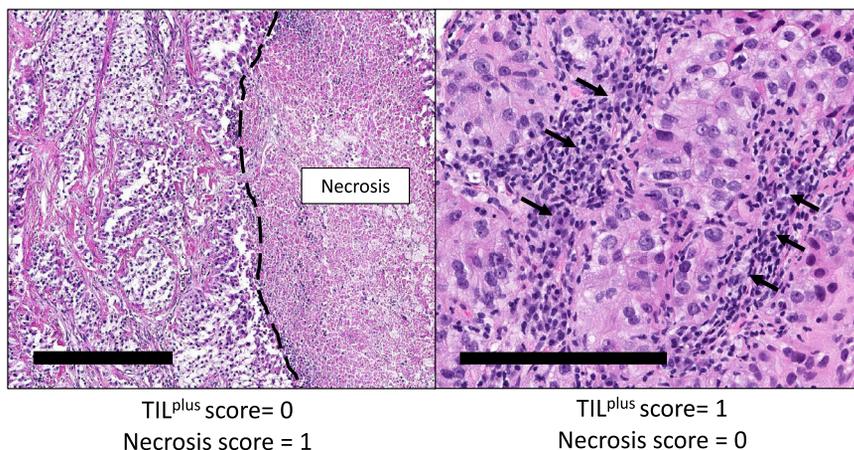
Figure 1. Examples of TIL^{plus} and necrosis scoring in patients with metastatic ccRCC treated with anti-PD-1

Left: photomicrograph of a pre-treatment specimen with TIL^{plus} = 0, i.e., no evidence of pre-existing immune response to tumor, with a substantial amount (≥10% of surface area) of necrosis (demarcated by dashed line). Scale bar, 400 μm. Right: sample TIL^{plus} = 1 with evidence of infiltrating immune cells (black arrows) and no necrosis present. Scale bar, 200 μm. H&E staining, both panels.

TIL^{plus} score= 0
Necrosis score = 1

TIL^{plus} score= 1
Necrosis score = 0

immunofluorescence (mIF)/IHC platforms that allow the simultaneous assessment of multiple markers on one slide at single-cell resolution have perhaps shown the most biomarker potential to date in mccRCC.[12,13] These technologies are able to quantify co-expression of multiple markers by single cells, and spatial relationships between different cell types. They are also currently expensive, time-consuming, require specialized expertise, and typically analyze only ∼1% of the tumor microenvironment (TME) area available on the slide.

Histopathologic diagnosis assessed on an H&E-stained slide is the "gold standard" for diagnosing and staging cancer in medical practices worldwide. This staining modality, which has been in clinical use for well a century, is a part of routine surgical pathology workflows. As such, it is a pre-existing resource that is present for almost all patients and has largely been overlooked as a possible source of biomarker information for ICB. Features readily observed on H&E include the presence of immune cell populations such as tumor-infiltrating lymphocytes (TILs), macrophages, plasma cells, and neutrophils (when present), among others. Necrosis is also readily identified on H&E slides and, in contrast to mIF, features such as TILs and necrosis are easily identified across whole slides (100% of the TME captured for evaluation) in meaningfully sized cohorts.

We hypothesized that infiltrating immune cells would serve as a positive prognostic feature for outcomes following anti-PD-1-based therapy in mccRCC, and that necrosis would adversely influence outcomes. To that end, we examined pre-treatment metastatic tumor specimens from patients with RCC that had been stained with H&E to determine the biomarker potential of these features as well as to assess the potential interplay between them. We also examined *PBRM1* loss of function in this context, integrating H&E assessment of immune infiltrate and necrosis with *PBRM1* mutation status, to determine if there was added value in the development of a combinatorial biomarker for patient outcomes following anti-PD-1-based therapy.

RESULTS

Tumor specimens, patients, and treatments received
Across the 3 cohorts, 201 tumor specimens were assessed for study eligibility, and 136 met inclusion criteria. The associated

CONSORT participant flow diagrams are shown in Figure S1. Specifically, we studied 63 pre-treatment biopsies from patients receiving nivolumab monotherapy as first- or later-line therapy on CheckMate 009, i.e., the discovery cohort; 58 biopsies from patients receiving later-line nivolumab (n = 19) or everolimus (n = 39) on CheckMate 025 (validation cohort); and 15 biopsies from treatment-naive patients receiving nivolumab plus ipilimumab on CheckMate 214 (extension cohort). Each biopsy specimen studied corresponds to an individual patient. Additional clinical details for each of these cohorts are provided in Table S1.

TIL^{plus} and necrosis scores on pre-treatment biopsies and association with clinical outcomes
In the discovery cohort, patient specimens were scored for TIL^{plus} and necrosis (Figure 1). Speciments with a TIL^{plus} score of 1 were associated with improved overall survival (OS) compared with those with a TIL^{plus} score of 0 (median OS not reached vs. 16.4 months, log rank test p = 0.008; Figure 2A). These findings were then examined in an independent cohort receiving anti-PD-1, with validation (median OS 40.0 vs. 4.4 months, log rank test p < 0.0001). Next, we tested whether the presence of TIL^{plus} also correlated with OS in patients treated with anti-PD-1 + anti-CTLA-4, and we observed a similar trend, although the sample size for patients with metastatic lesions that had been biopsied was limited. We also tested for an association with PFS in each trial and treatment setting (Figure S2). A meta-analysis of all patients across the three cohorts, comparing those with or without TIL^{plus}, is shown in Figure 2B (hazard ratio [HR], 95% confidence interval [CI]: 0.42 [0.23–0.74], median OS 47.9 vs. 16.0 months, respectively, log rank test p = 0.0006; and HR, 95% CI: 0.67 [0.42–1.07] median PFS 7.5 vs. 2.7 months, respectively, log rank test, p = 0.07). TIL^{plus} score also associated with objective response, p = 0.02. Notably, in the cohort of patients treated with everolimus (control arm), the presence of a pre-existing immune infiltrate did not associate with OS or objective response, indicating that our findings are specific to patients treated with ICB, Figure 2C.

We next tested whether the presence of pre-treatment tumor necrosis correlated with OS. The presence of geographic necrosis as a single feature did not associate with OS; however, it modified the beneficial effect of having an immune infiltrate when one was present. Patients whose tumors had substantial
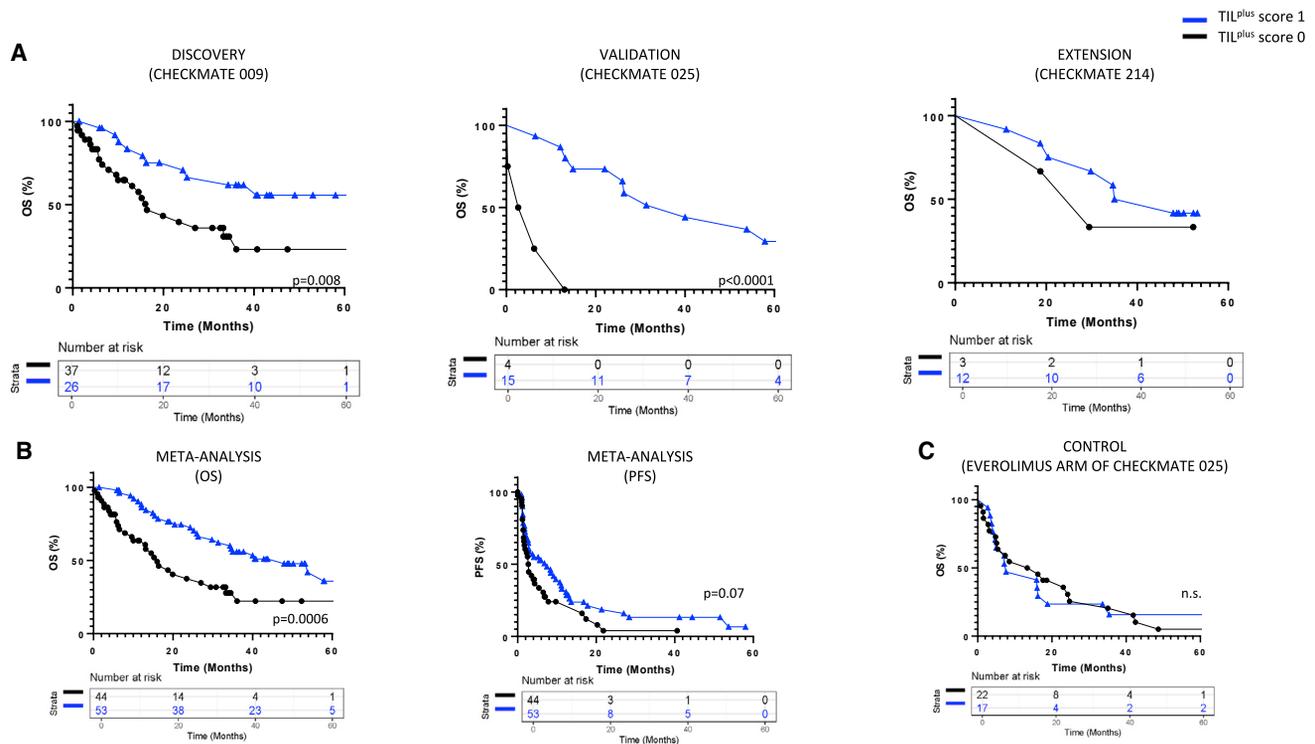
Figure 2. Pathologic scoring of H&E slides from metastatic specimens biopsied within 1 year of anti-PD-1 treatment initiation associates with OS

(A) Left: in the discovery cohort of n = 63 pre-treatment tumor biopsies from metastases (CheckMate 009), OS was significantly increased in patients with a TIL^{plus} score of 1 as compared with 0 (log rank test, p = 0.008). Middle: these same findings are validated in an independent cohort of pre-treatment specimens from metastases in patients with ccRCC treated with anti-PD-1 therapy (CheckMate 025) (p < 0.0001). Right: the findings were then extended into the pre-treatment specimens from metastases in patients with ccRCC treated with combination anti-PD-1/CTLA-4 blockade (CheckMate 214), although the analysis was limited due to the small number of pre-treatment biopsies available from metastatic lesions.

(B) Meta-analysis from the three trial cohorts (p = 0.0006).

(C) Notably, there was no significant association between TIL^{plus} score and OS in patients treated with the mTOR inhibitor everolimus on the control arm of CheckMate 025.

necrosis had inferior OS when compared with patients with the same TIL^{plus} score, but whose tumors lacked necrosis, Figure 3A. This finding was first observed in the discovery cohort and then validated in the CheckMate 025 cohort, Figure 3B. There were no patients receiving dual PD-1/CTLA-4 blockade who had substantial necrosis in their pre-treatment specimens, precluding analysis. Similar to what was seen when TIL^{plus} scores were tested alone, combining TIL^{plus} and necrosis scores was not predictive of outcomes for patients receiving everolimus, Figure 3C. Prior systemic therapy in comparison to no prior treatment did not affect TIL^{plus} or necrosis scores.

Combining *PBRM1* loss-of-function mutation with H&E scoring of immune cell infiltrates (TIL^{plus}) and necrosis provides a stronger predictor of OS and objective response after anti-PD-1 therapy

PBRM1 mutations have previously been shown to associate with response to anti-PD-1 treatment.[7] *PBRM1* loss-of-function mutation status was available for 48.5% (47/97) of the patients with evaluable H&E-stained pre-treatment tumor specimens; these patients did not differ substantially in baseline characteristics

from the remainder of the cohort (Table S2). *PBRM1* mutation status (mutant vs. wild type) in this subset correlated with OS (HR, 95% CI: 0.33 [0.15–0.74], log rank test, p = 0.02). An association between *PBRM1* mutation and TIL^{plus} was not observed.

Given the relatively small sample sizes of patients in each cohort having genomic data available for review, we performed a meta-analysis examining whether the triple combination of H&E-assessed TIL^{plus} and necrosis and *PBRM1* mutation had increased precision in predicting OS after anti-PD-1-based therapy. The combination of H&E scoring with *PBRM1* mutation status stratified patients into three groups, with patients having all three positive factors, i.e., TIL^{plus} score of 1, necrosis score of 0, and the presence of a *PBRM1* mutation, demonstrating the best OS, Figure 4. Patients with two of the three features demonstrated an intermediate survival, while those with only one of the three features demonstrated the worst survival (median survival not reached, 33.3 months, and 23.4 months for the three groups, log rank test p = 0.007). The combination of H&E scoring with *PBRM1* mutation status resulted in an improved HR when comparing patients with all three features present vs. only one feature present (HR, 95% CI: 0.18 [0.04–0.78], log rank test,
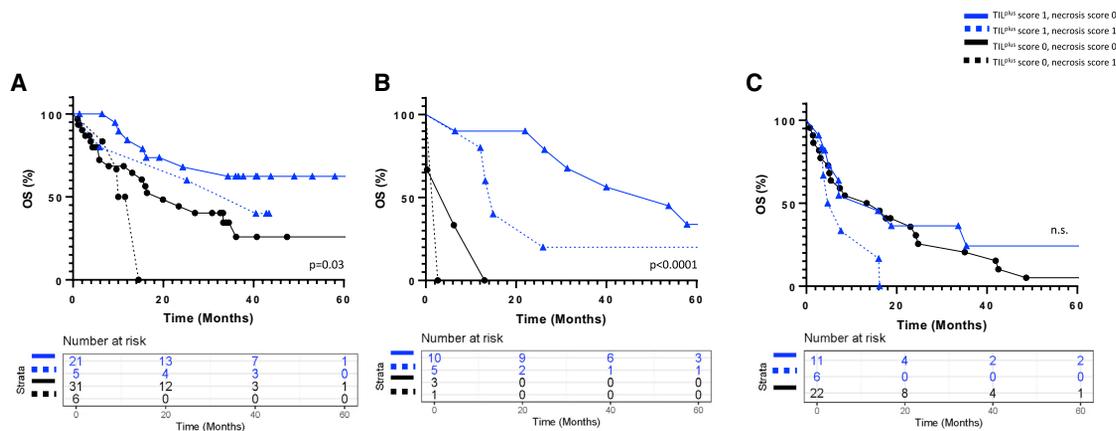
Figure 3. Pathologic scoring of H&E slides in pre-treatment specimens from patients receiving nivolumab in CheckMate 009 and CheckMate 025 and everolimus in CheckMate 025

(A) In the n = 63 pre-treatment mCCRCC specimens from CheckMate 009, OS was significantly increased in patients with a TIL^{plus} score of 1 (blue line) as compared with 0 (black line). When the presence of substantial necrosis (>10% of tumor surface area on slide) was taken into account (dashed lines), patients showed inferior OS than their counterparts with the same immune infiltrate score but who lacked necrosis (solid lines) (log rank test, p = 0.03).

(B) Similar findings were observed in metastases from n = 19 patients from an independent cohort, CheckMate 025, who received anti-PD-1, p < 0.0001.

(C) In contrast, these findings did not predict patient outcomes for n = 39 patients receiving everolimus from CheckMate 025. This relationship could not be tested in patients receiving anti-PD-1 + CTLA-4 dual blockade, as none of the specimens contained geographic necrosis.

p = 0.004), showing the benefit of the combinatorial biomarker over H&E scores or *PBRM1* mutation status alone. This association was not dependent on whether anti-PD-1 was received as first- or later-line therapy for mccRCC, Figure S3. The combinatorial score was also associated with objective response to therapy (p = 0.04). Finally, TIL^{plus} and PD-L1 IHC were also tested for combinatorial value and showed potential utility in stratifying patient outcomes after anti-PD-1-based therapy, Figure S4.

A PubMed search identified 38 publications across tumor types that utilized multimodality biomarker approaches to predict patient outcomes following anti-PD-1-based therapies (Figure 5; Table S3). None used H&E features as a part of their characterization, further underscoring the underutilization of H&E-based insights in biomarker discovery for anti-PD-1 therapies.

DISCUSSION

H&E-stained slides are only now starting to be characterized in the context of immunotherapy. To date, they have been utilized to assess pathologic response to neoadjuvant treatment in the definitive resection specimen, or in on-treatment biopsies for patients with advanced unresectable disease.[14–17] Here, we show in independent cohorts that markers derived from pre-treatment H&E-stained tumor tissue sections can be used to predict survival in patients treated with anti-PD-1-based therapies using a routine surgical pathology workflow. Furthermore, our data demonstrate the utility of a combinatorial biomarker including H&E findings and *PBRM1* mutation status for further stratification of patients with mccRCC. This approach could be used to identify patients who would be likely to respond to anti-PD-1 monotherapy, sparing unnecessary exposure to a second agent and enhancing risk/benefit calculations when making that decision.[18] Along the same lines, it also facilitates the identification of a group of patients less likely to respond to anti-PD-1-based

therapies (i.e., tumors with necrosis and no TIL^{plus}), allowing for enrichment of a population for clinical trials exploring alternate therapeutic regimens.

One of the most widely explored tissue-based biomarkers for prognosis and response to ICB is CD8$^+$ cytotoxic T cell infiltration into tumors. However, CD8$^+$ T cell density as a prognostic and predictive biomarker is not as tightly linked to outcomes for patients with ccRCC as is observed for many other tumor types.[19] Some studies in mccRCC have found increased CD8$^+$ TIL density in specific tumor regions at baseline associated with improved patient outcomes following anti-PD-1 therapy,[11–13] while others failed to show an association.[20] Numerous studies in ccRCC have also shown that the CD8$^+$ T cells, when present, are often dysfunctional.[21–24] Furthermore, CD8 staining provides an evaluation of only a subset of immune cells, and it is recognized that macrophages and B cells may also associate with survival benefit following anti-PD-1 therapy.[25–27] The H&E assessment performed herein includes evaluation of all lymphocyte subsets and associated macrophages, plasma cells, and other immune cells allowing for a more holistic assessment of immune infiltration.

Tumor necrosis is also a feature that can be readily assessed on H&E-stained slides. In patients with localized ccRCC, the presence of tumoral necrosis has prognostic significance,[28] but the potential significance of ccRCC necrosis as a predictive factor for immunotherapy outcomes has not previously been characterized. Here, the presence of necrosis alone in pre-treatment metastatic specimens did not associate with OS, which is in keeping with a previous study in melanoma patients treated with anti-PD-1.[17] However, when necrosis and immune cell scores were combined, patient outcomes after immunotherapy could be stratified. Namely, patients whose tumors had necrosis and lacked TIL^{plus} had the worst median OS. For patients with TIL^{plus}, necrosis attenuated the otherwise favorable survival

Figure 4. Combining TIL^{plus} and necrosis scores on H&E with *PBRM1* mutation status in pre-treatment biopsies from metastases improves OS prediction after anti-PD-1

Patients with score = 3 (TIL^{plus}, no or minimal necrosis, and *PBRM1* mutation) showed significantly improved survival when compared with those with only a single feature (score = 1). There were no patients that had a combinatorial score of zero. A statistically distinct intermediate prognostic group was also identified for patients whose tumors harbored any two of these features (score = 2) log rank test, p < 0.0001). Patient level data are provided for H&E TIL^{plus} and necrosis score and *PBRM1* mutation status. Information regarding objective response status and the trial cohort is also provided (A, CheckMate 009; B, CheckMate 025; C, CheckMate 214). CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

benefit of the infiltrate. In preclinical studies, tumoral necrosis has been shown to lead to the release of intracellular potassium ions into the extracellular space, resulting in restrained T cell effector function.[29] The clinical/translational representation for this immunometabolic finding, consistent with the findings reported herein, has broad relevance for biomarker development. As a general rule, necrotic tissue is summarily excluded from tissue-based analyses, given the inherent difficulties of characterizing protein, RNA, and DNA in these regions. This exclusion typically takes place without additional notation or characterization. Our findings suggest that the necrosis itself may have inherent biomarker value and should be captured and analyzed as a key descriptor of the TME.

Unidimensional predictive markers are the current mainstay in clinical oncology. More recently, pre-treatment multimodality biomarkers have been explored in different tumor types, and there have been reports of improved biomarker performance when multiple modalities are employed, e.g., in patients with melanoma tested for an IFN-γ gene signature and TMB.[30] We reviewed the literature for multimodality biomarker approaches in studies of patients with ccRCC receiving anti-PD-1-based regimens and found strategies that combined whole exome

sequencing, RNA expression profiling, and IHC, although some studies failed to validate in additional cohorts, or there was unclear additive benefit beyond the single biomarkers tested.[10] Here, we highlight the utility of combining H&E scoring with genomic testing for *PBRM1* mutations in identifying patients either most or least likely to benefit from anti-PD-1, as well as a group with an intermediate long-term outcome. We also found that TIL^{plus} scoring and PD-L1 IHC may have additive value, suggesting that H&E-stained slides combined with IHC merits additional exploration as another combinatorial biomarker strategy.

PBRM1 mutations have been associated with increased immune-related gene expression signatures as well as CD8⁺ T cell infiltration in some studies, and with lower IFN-γ and JAK/STAT3 expression in others.[7,20,31,32] We did not demonstrate a clear interaction between the presence of TIL^{plus} and *PBRM1* mutations, which would have been anticipated if there was indeed a functional relationship between infiltrating immune cells and this somatic genetic alteration with regard to immunotherapy response. While the functional significance of *PBRM1* mutations in the pre-treatment TME remains somewhat unclear, the lack of interaction of this feature with TIL^{plus} allowed for added biomarker value when combined with H&E slide scoring.
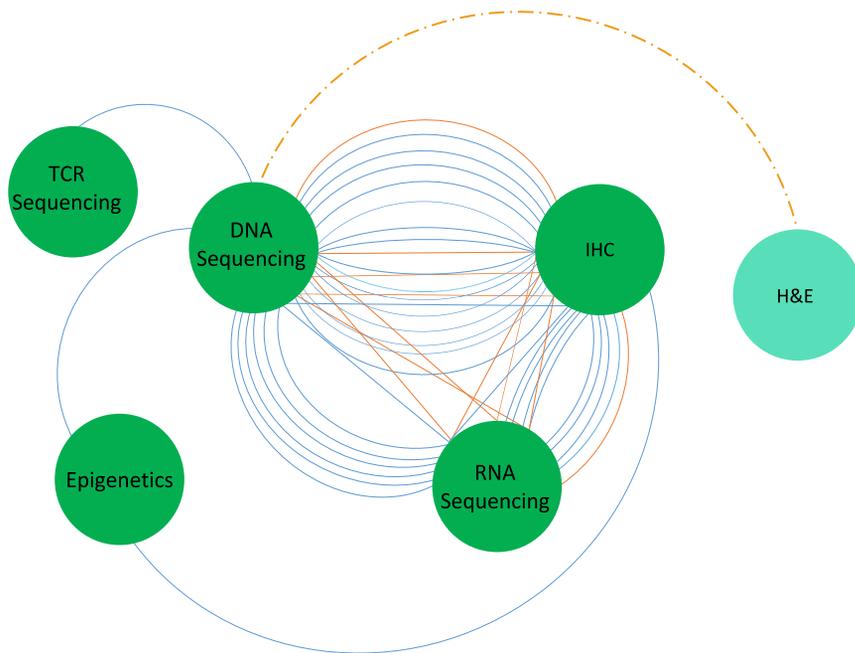
Figure 5. Multimodality biomarker studies identified by literature review
Each line in this network represents a study that tested multimodality biomarkers in pre-treatment tissue specimens for an association with patient outcomes following anti-PD-(L)1-based therapy. Each individual solid, curved line represents a single study combining the modalities shown in the green node. Each triangle indicates studies that combined three modalities (DNA sequencing, RNA sequencing, and IHC). The orange lines show studies that were conducted using specimens from patients with RCC. The blue lines show studies conducted in other tumor types, including NSCLC, melanoma, urothelial carcinoma, gastric/gastroesophageal carcinoma, Merkel cell carcinoma, triple-negative breast cancer, and head and neck squamous cell carcinoma. The dotted orange line represents this study. The 38 studies identified upon literature review and included in the network diagram are listed in Table S3.

One limitation of this study is that these results are based on retrospective analyses of specimens from prospective clinical trials. Our results suggest that H&E-based markers should be included as a part of future prospective clinical trials to further assess their ability to predict patient outcomes, both independently and in combination with other assay modalities. Another limitation of this study is that the genomic component focused on *PBRM1* as a singular gene. Additional somatic genomic alterations such as 9p21.3 deletions, which have also been associated with worse OS following ICB in patients with mccRCC,[20] could be tested for potential added value to the combinatorial biomarker presented. Tumor specimens should also be studied from patients receiving novel anti-PD-1-based combination treatment regimens, e.g., anti-PD-1 plus VEGF inhibitor +/− anti-CTLA-4, as well as in the adjuvant and neoadjuvant settings.[2,33,34] Additional future explorations could include the application of machine learning and deep learning to automate H&E-based tissue analyses and potentially discover new complex markers predicting treatment outcomes.

In summary, there is currently an unmet need for robust predictive biomarkers for patients with mccRCC receiving immunotherapy that can be readily deployed across clinical settings. Numerous unidimensional and multidimensional biomarkers have been proposed, but they have not validated across multiple cohorts. mIF has shown promise as a high-tech approach to biomarker development in this setting, but is expensive, requires specialized equipment, and is not yet standardized for clinical use, even among leading academic institutions.[13,35] As shown here, TIL[plus] and necrosis scoring predicted outcomes in independent patient cohorts and does not require expensive equipment or specialized expertise, positioning H&E as a "modality" for biomarker development as well as for potential future clinical use in resource-poor settings.

Limitations of the study

Our results are limited by the proportion of patients in each of the individual cohorts that had *PBRM1* mutation status available. Larger cohorts of patients will also be required to explore optimal thresholds of TIL[plus] and percent necrosis from H&E slides for biomarker development. Continued biomarker development will take place in future prospective, randomized trials.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Clinicopathologic features of cohorts
- METHOD DETAILS
  - Scoring of H&E stained slides
  - Genomic studies, PD-L1 IHC, and combinatorial scores
  - Literature review
  - H&E scoring system development
  - Reproducibility study
- QUANTIFICATION AND STATISTICAL ANALYSIS
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2023.100947.

Cell Reports Medicine
Report

AUTHOR CONTRIBUTIONS

Conceptualization, J.S.D. and J.M.T.; methodology, J.S.D., E.J.L., L.D., S.L.T., S.S., T.K.C., M.B.A., and J.M.T.; data collection, curation, and statistical analysis, J.S.D., L.D., J.J., E.B., T.K.C., R.J.M., S.G., D.M., M.B.A., and J.M.T.; resources, Y.G., N.S., T.K.C., R.J.M., D.M., and S.S.; funding acquisition, S.L.T. and J.M.T.; study supervision, J.S.D. and J.M.T. All authors contributed to writing the manuscript. All authors reviewed and approved the final manuscript.

DECLARATION OF INTERESTS

Dr. Taube reports grants and consulting from Bristol-Myers Squibb and Akoya Biosciences, consulting for Merck, AstraZeneca, Genentech, GlaxoSmithKline, Regeneron, Lunaphore, and Compugen outside the submitted work. Dr. Deutsch and Dr. Taube report an institutional patent filed on machine learning for scoring pathologic response to immunotherapy. Dr. Topalian reports research grants from Bristol Myers Squibb; personal fees from AstraZeneca and Immunocore; and personal fees and stock options from Five Prime Therapeutics and Dragonfly Therapeutics. Dr. Topalian's spouse has financial relationships with the following entities: Amgen, Bristol Myers Squibb, Compugen, DNAtrix, Dracen Pharmaceuticals, Enara Bio, Immunomic Therapeutics, Janssen Pharmaceuticals, ManaT Bio, RAPT Therapeutics, Tizona LLC, Trieza Therapeutics, TRex Bio Ltd, and WindMIL. Dr. Lipson receives institutional research grant funding from Bristol-Myers Squibb, Merck, and Regeneron, and during the past 2 years, has consulted for Bristol-Myers Squibb, Eisai, Genentech, Instil Bio, MacroGenics, Merck, Natera, Nektar Therapeutics, Odonate, OncoSec, Pfizer, Rain Therapeutics, Regeneron, and Sanofi Genzyme. Dr. Choueiri reports institutional and personal, paid and/or unpaid support for research, advisory boards, consultancy, and honoraria from AstraZeneca, Aravive, Aveo, Bayer, Bristol Myers-Squibb, Calithera, Circle Pharma, Eisai, EMD Serono, Exelixis, GlaxoSmithKline, IQVA, Infinity, Ipsen, Jansen, Kanaph, Lilly, Merck, Nikang, Nuscan, Novartis, Pfizer, Roche, Sanofi/Aventis, Surface Oncology, Takeda, Tempest, Up-To-Date, CME events (Peerview, OncLive, MJH, and others), outside the submitted work. Institutional patents filed on molecular alterations and immunotherapy response/toxicity, and ctDNA. Equity: Tempest, Pionyr, Osel, Precede Bio. CureResponse. Committees: NCCN, GU Steering Committee, ASCO/ESMO, ACCRU, KidneyCan. Medical writing and editorial assistance support may have been funded by Communications companies in part. No speaker's bureau. Mentored several non-US citizens on research projects with potential funding (in part) from non-US sources/Foreign Components. The institution (Dana-Farber Cancer Institute) may have received additional independent funding of drug companies or/and royalties potentially involved in research around the subject matter. Dr. Atkins reports personal support for, advisory boards and consultancy from Bristol Myers Squibb, Merck, Eisai, Aveo, Pfizer, Werewolf, Fathom, Pyxis Oncology, PACT, Elpis, X4Pharma, ValoHealth, ScholarRock, Surface, Takeda, Simcha, Genentech-Roche, Exelixis, Iovance, COTA, Idera, Agenus, Asher Bio, Neoleukin, AstraZeneca, Calithera, SeaGen, Sanofi, SAB Bio, OncoRena, Pliant Therapeutics, Up-To-Date, and GlaxoSmithKline. Institutional Research support from BMS, Merck, and Pfizer and Stock or Stock options from Werewolf, Pyxis

Oncology, and Elpis outside the submitted work. Dr. Signoretti reports receiving commercial research grants from Bristol-Myers Squibb, AstraZeneca, Exelixis, and Novartis; is a consultant/advisory board member for Merck, AstraZeneca, Bristol-Myers Squibb, CRISPR Therapeutics AG, AACR, and NCI; and receives royalties from Biogenex. Saurabh Gupta is a full-time employee of Bristol-Myers Squibb and owns BMS stock. Dr. Motzer reports clinical trial support (institutional) from BMS for this manuscript; advisory board fees from AstraZeneca, AVEO, Eisai, EMD Serono, Exelixis, Genentech/Roche, Incyte, Lilly Oncology, Merck, Novartis, and Pfizer; and fees (institutional) for coordinating PI from AVEO, BMS, Eisai, Exelixis, Genentech/Roche, Merck, and Pfizer. Dr. Ged reports advisory roles and has received honoraria from Aveo, Bristol Myers Squibb, BostonGene, and Exelixis. Drs. Baraban, Singla, Jedrych, and Danilova declare no competing interests.

REFERENCES

1. Motzer, R.J., Jonasch, E., Boyle, S., Carlo, M.I., Manley, B., Agarwal, N., Alva, A., Beckermann, K., Choueiri, T.K., Costello, B.A., et al. (2020). NCCN guidelines insights: kidney cancer, version 1.2021. J. Natl. Compr. Canc. Netw. *18*, 1160–1170.

2. Rini, B.I., Battle, D., Figlin, R.A., George, D.J., Hammers, H., Hutson, T., Jonasch, E., Joseph, R.W., McDermott, D.F., Motzer, R.J., et al. (2019). The society for immunotherapy of cancer consensus statement on immunotherapy for the treatment of advanced renal cell carcinoma (RCC). J. Immunother. Cancer *7*, 354.

3. Motzer, R.J., Escudier, B., McDermott, D.F., George, S., Hammers, H.J., Srinivas, S., Tykodi, S.S., Sosman, J.A., Procopio, G., Plimack, E.R., et al. (2015). Nivolumab versus everolimus in advanced renal cell carcinoma. N. Engl. J. Med. *373*, 1803–1813.

4. Reck, M., Rodríguez-Abreu, D., Robinson, A.G., Hui, R., Csőszi, T., Fülöp, A., Gottfried, M., Peled, N., Tafreshi, A., Cuffe, S., et al. (2016). Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. N. Engl. J. Med. *375*, 1823–1833.

5. Hakimi, A.A., Attalla, K., DiNatale, R.G., Ostrovnaya, I., Flynn, J., Blum, K.A., Ged, Y., Hoen, D., Kendall, S.M., Reznik, E., et al. (2020). A pan-cancer analysis of PBAF complex mutations and their association with immunotherapy response. Nat. Commun. *11*, 4168.

6. Pan, D., Kobayashi, A., Jiang, P., Ferrari de Andrade, L., Tay, R.E., Luoma, A.M., Tsoucas, D., Qiu, X., Lim, K., Rao, P., et al. (2018). A major chromatin regulator determines resistance of tumor cells to T cell–mediated killing. Science *359*, 770–775.

7. Miao, D., Margolis, C.A., Gao, W., Voss, M.H., Li, W., Martini, D.J., Norton, C., Bossé, D., Wankowicz, S.M., Cullen, D., et al. (2018). Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. Science *359*, 801–806.

8. Braun, D.A., Ishii, Y., Walsh, A.M., Van Allen, E.M., Wu, C.J., Shukla, S.A., and Choueiri, T.K. (2019). Clinical validation of PBRM1 alterations as a marker of immune checkpoint inhibitor response in renal cell carcinoma. JAMA Oncol. *5*, 1631–1633.

9. Lu, S., Stein, J.E., Rimm, D.L., Wang, D.W., Bell, J.M., Johnson, D.B., Sosman, J.A., Schalper, K.A., Anders, R.A., Wang, H., et al. (2019). Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: a systematic review and meta-analysis. JAMA Oncol. *5*, 1195–1204.

10. Banchereau, R., Leng, N., Zill, O., Sokol, E., Liu, G., Pavlick, D., Maund, S., Liu, L.F., Kadel, E., Baldwin, N., et al. (2021). Molecular determinants of response to PD-L1 blockade across tumor types. Nat. Commun. *12*, 3969.

11. Motzer, R.J., Robbins, P.B., Powles, T., Albiges, L., Haanen, J.B., Larkin, J., Mu, X.J., Ching, K.A., Uemura, M., Pal, S.K., et al. (2020). Avelumab

plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. Nat. Med. *26*, 1733–1741.

12. Ficial, M., Jegede, O.A., Sant'Angelo, M., Hou, Y., Flaifel, A., Pignon, J.C., Braun, D.A., Wind-Rotolo, M., Sticco-Ivins, M.A., Catalano, P.J., et al. (2021). Expression of T-cell exhaustion molecules and human endogenous retroviruses as predictive biomarkers for response to nivolumab in metastatic clear cell renal cell carcinoma. Clin. Cancer Res. *27*, 1371–1380.

13. Pignon, J.C., Jegede, O., Shukla, S.A., Braun, D.A., Horak, C.E., Wind-Rotolo, M., Ishii, Y., Catalano, P.J., Grosha, J., Flaifel, A., et al. (2019). irRECIST for the evaluation of candidate biomarkers of response to nivolumab in metastatic clear cell renal cell carcinoma: analysis of a phase II prospective clinical trial. Clin. Cancer Res. *25*, 2174–2184.

14. Tetzlaff, M.T., Messina, J.L., Stein, J.E., Xu, X., Amaria, R.N., Blank, C.U., van de Wiel, B.A., Ferguson, P.M., Rawson, R.V., Ross, M.I., et al. (2018). Pathological assessment of resection specimens after neoadjuvant therapy for metastatic melanoma. Ann. Oncol. *29*, 1861–1868.

15. Cottrell, T.R., Thompson, E.D., Forde, P.M., Stein, J.E., Duffield, A.S., Anagnostou, V., Rekhtman, N., Anders, R.A., Cuda, J.D., Illei, P.B., et al. (2018). Pathologic features of response to neoadjuvant anti-PD-1 in resected non-small cell lung carcinoma: a proposal for quantitative immune-related pathologic response criteria (irPRC). Ann. Oncol. *29*, 1853–1860.

16. Stein, J.E., Lipson, E.J., Cottrell, T.R., Forde, P.M., Anders, R.A., Cimino-Mathews, A., Thompson, E.D., Allaf, M.E., Yarchoan, M., Feliciano, J., et al. (2020). Pan-tumor pathologic scoring of response to PD-(L)1 blockade. Clin. Cancer Res. *26*, 545–551.

17. Stein, J.E., Soni, A., Danilova, L., Cottrell, T.R., Gajewski, T.F., Hodi, F.S., Bhatia, S., Urba, W.J., Sharfman, W.H., Wind-Rotolo, M., et al. (2019). Major pathologic response on biopsy (MPR bx) in patients with advanced melanoma treated with anti-PD-1: evidence for an early, on-therapy biomarker of response. Ann. Oncol. *30*, 589–596.

18. Xu, W., Atkins, M.B., and McDermott, D.F. (2020). Checkpoint inhibitor immunotherapy in kidney cancer. Nat. Rev. Urol. *17*, 137–150.

19. Fridman, W.H., Pagès, F., Sautès-Fridman, C., and Galon, J. (2012). The immune contexture in human tumours: impact on clinical outcome. Nat. Rev. Cancer *12*, 298–306.

20. Braun, D.A., Hou, Y., Bakouny, Z., Ficial, M., Sant' Angelo, M., Forman, J., Ross-Macdonald, P., Berger, A.C., Jegede, O.A., Elagina, L., et al. (2020). Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. Nat. Med. *26*, 909–918.

21. Menard, L.C., Fischer, P., Kakrecha, B., Linsley, P.S., Wambre, E., Liu, M.C., Rust, B.J., Lee, D., Penhallow, B., Manjarrez Orduno, N., and Nadler, S.G. (2018). Renal cell carcinoma (RCC) tumors display large expansion of double positive (DP) CD4+CD8+ T cells with expression of exhaustion markers. Front. Immunol. *9*, 2728.

22. van Asten, S.D., de Groot, R., van Loenen, M.M., Castenmiller, S.M., de Jong, J., Monkhorst, K., Haanen, J.B.A.G., Amsen, D., Bex, A., Spaapen, R.M., and Wolkers, M.C. (2021). T cells expanded from renal cell carcinoma display tumor-specific CD137 expression but lack significant IFN-γ, TNF-α or IL-2 production. OncoImmunology *10*, 1860482.

23. Granier, C., Dariane, C., Combe, P., Verkarre, V., Urien, S., Badoual, C., Roussel, H., Mandavit, M., Ravel, P., Sibony, M., et al. (2017). Tim-3 expression on tumor-infiltrating PD-1+CD8+ T cells correlates with poor clinical outcome in renal cell carcinoma. Cancer Res. *77*, 1075–1082.

24. Nakano, O., Sato, M., Naito, Y., Suzuki, K., Orikasa, S., Aizawa, M., Suzuki, Y., Shintaku, I., Nagura, H., and Ohtani, H. (2001). Proliferative activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathologic demonstration of antitumor immunity. Cancer Res. *61*, 5132–5136.

25. Anagnostou, V., Bruhm, D.C., Niknafs, N., White, J.R., Shao, X.M., Sidhom, J.W., Stein, J., Tsai, H.L., Wang, H., Belcaid, Z., et al. (2020). Integra-

tive tumor and immune cell multi-omic analyses predict response to immune checkpoint blockade in melanoma. Cell Rep. Med. *1*, 100139.

26. Herbst, R.S., Soria, J.C., Kowanetz, M., Fine, G.D., Hamid, O., Gordon, M.S., Sosman, J.A., McDermott, D.F., Powderly, J.D., Gettinger, S.N., et al. (2014). Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature (London) *515*, 563–567.

27. Liu, Y., Zugazagoitia, J., Ahmed, F.S., Henick, B.S., Gettinger, S.N., Herbst, R.S., Schalper, K.A., and Rimm, D.L. (2020). Immune cell PD-L1 colocalizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy. Clin. Cancer Res. *26*, 970–977.

28. Zhang, L., Zha, Z., Qu, W., Zhao, H., Yuan, J., Feng, Y., and Wu, B. (2018). Tumor necrosis as a prognostic variable for the clinical outcome in patients with renal cell carcinoma: a systematic review and meta-analysis. BMC Cancer *18*, 870.

29. Eil, R., Vodnala, S.K., Clever, D., Klebanoff, C.A., Sukumar, M., Pan, J.H., Palmer, D.C., Gros, A., Yamamoto, T.N., Patel, S.J., et al. (2016). Ionic immune suppression within the tumour microenvironment limits T cell effector function. Nature (London) *537*, 539–543.

30. Rozeman, E.A., Hoefsmit, E.P., Reijers, I.L.M., Saw, R.P.M., Versluis, J.M., Krijgsman, O., Dimitriadis, P., Sikorska, K., van de Wiel, B.A., Eriksson, H., et al. (2021). Survival and biomarker analyses from the OpACIN-neo and OpACIN neoadjuvant immunotherapy trials in stage III melanoma. Nat. Med. *27*, 256–263.

31. Liu, X.D., Kong, W., Peterson, C.B., McGrail, D.J., Hoang, A., Zhang, X., Lam, T., Pilie, P.G., Zhu, H., Beckermann, K.E., et al. (2020). PBRM1 loss defines a nonimmunogenic tumor phenotype associated with checkpoint inhibitor resistance in renal carcinoma. Nat. Commun. *11*, 2135.

32. Zimmer, K., Kocher, F., Untergasser, G., Puccini, A., Xiu, J., Wolf, D., Spizzo, G., Goldberg, R.M., Grothey, A., Shields, A.F., et al. (2021). Identification and prognostic impact of PBRM1 mutations in biliary tract cancers: results of a comprehensive molecular profiling study. J. Clin. Oncol. *39*, 4022.

33. Choueiri, T.K., Albiges, L., Powles, T., Scheffold, C., Wang, F., and Motzer, R.J. (2020). A phase III study (COSMIC-313) of cabozantinib (C) in combination with nivolumab (N) and ipilimumab (I) in patients (pts) with previously untreated advanced renal cell carcinoma (aRCC) of intermediate or poor risk. Jco *38*, TPS767.

34. Serzan, M.T., and Atkins, M.B. (2021). Current and emerging therapies for first line treatment of metastatic clear cell renal cell carcinoma. J. Cancer Metastasis Treat. *7*, 39.

35. Berry, S., Giraldo, N.A., Green, B.F., Cottrell, T.R., Stein, J.E., Engle, E.L., Xu, H., Ogurtsova, A., Roberts, C., Wang, D., et al. (2021). Analysis of multispectral imaging with the AstroPath platform informs efficacy of PD-1 blockade. Science *372*, eaba2609.

36. McShane, L.M., Altman, D.G., Sauerbrei, W., Taube, S.E., Gion, M., and Clark, G.M.; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics (2005). Reporting recommendations for tumor marker prognostic studies (REMARK). J. Natl. Cancer Inst. *97*, 1180–1184.

37. Choueiri, T.K., Fishman, M.N., Escudier, B., McDermott, D.F., Drake, C.G., Kluger, H., Stadler, W.M., Perez-Gracia, J.L., McNeel, D.G., Curti, B., et al. (2016). Immunomodulatory activity of nivolumab in metastatic renal cell carcinoma. Clin. Cancer Res. *22*, 5461–5471.

38. Motzer, R.J., Tannir, N.M., McDermott, D.F., Arén Frontera, O., Melichar, B., Choueiri, T.K., Plimack, E.R., Barthélémy, P., Porta, C., George, S., et al. (2018). Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N. Engl. J. Med. *378*, 1277–1290.

39. Thompson, R.H., Gillett, M.D., Cheville, J.C., Lohse, C.M., Dong, H., Webster, W.S., Krejci, K.G., Lobo, J.R., Sengupta, S., Chen, L., et al. (2004). Costimulatory B7-H1 in renal cell carcinoma patients: indicator of tumor aggressiveness and potential therapeutic target. Proc. Natl. Acad. Sci. USA *101*, 17174–17179.

40. Taube, J.M., Anders, R.A., Young, G.D., Xu, H., Sharma, R., McMiller, T.L., Chen, S., Klein, A.P., Pardoll, D.M., Topalian, S.L., and Chen, L. (2012). Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci. Transl. Med. *4*, 127ra37.

41. Zigeuner, R., Hutterer, G., Chromecki, T., Imamovic, A., Kampel-Kettner, K., Rehak, P., Langner, C., and Pummer, K. (2010). External validation of the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score for clear-cell renal cell carcinoma in a single European centre applying routine pathology. Eur. Urol. *57*, 102–109.

42. Frank, I., Blute, M.L., Cheville, J.C., Lohse, C.M., Weaver, A.L., and Zincke, H. (2002). An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy based on tumor stage, size, grade and necrosis: the SSIGN score. J. Urol. *168*, 2395–2400.

43. College of American Pathologists (2018). Protocol for the Examination of Specimens from Patients with Invasive Carcinoma of the Breast.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Tumor tissue from participants in the CheckMate 009 clinical trial	CheckMate 009	https://www.clinicaltrials.gov/ct2/show/NCT01358721
Tumor tissue from participants in the CheckMate 025 clinical trial	CheckMate 025	https://clinicaltrials.gov/ct2/show/NCT01668784
Tumor tissue from participants in the CheckMate 214 clinical trial	CheckMate 214	https://clinicaltrials.gov/ct2/show/NCT02231749
Deposited data		
H&E whole slide scans with example TIL[plus] and necrosis scores	De-identified archival specimens	https://digital.pathology.johnshopkins.edu/imageSets/5879
Software and algorithms		
GraphPad Prism	Dotmatics	https://www.graphpad.com/scientific-software/prism/
R	R Core Team	https://www.r-project.org/

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Janis M. Taube, MD (jtaube1@jhmi.edu).

Materials availability
The study did not generate new unique reagents.

Data and code availability
- All data reported in this paper and any additional information required to reanalyze the data will be shared by the lead contact upon request. This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All patients signed written informed consent prior to having any study procedures performed. This study was approved by the Johns Hopkins Institutional Review Board and adheres to the REMARK criteria for biomarker discovery.[36]

Clinicopathologic features of cohorts
The discovery cohort consisted of tumor specimens from previously-treated or treatment-naïve patients with mccRCC who received various nivolumab (anti-PD-1) monotherapy regimens on a multi-institutional biomarker study, CheckMate 009 (NCT01358721).[37] In this study, prospectively-collected, image-guided core needle biopsies of ccRCC metastases that had not been exposed to previous radiation therapy were required prior to treatment initiation. The second cohort consisted of patients with mccRCC whose tumors progressed on antiangiogenic therapy and were randomized to receive either nivolumab or everolimus on CheckMate 025 (NCT01668784).[3] The third cohort consisted of treatment-naïve patients with advanced or metastatic ccRCC who received four doses of nivolumab plus ipilimumab (anti-CTLA-4) followed by nivolumab monotherapy, on CheckMate 214 (NCT02231749).[38] To align with the discovery cohort, only patients with tissue obtained from a metastatic site within 1 year of ICB initiation were examined in the other two cohorts.

For all three cohorts, specimens were excluded if a diagnosis of ccRCC could not be confirmed by a board-certified pathologist (JMT) and/or available tumor biopsy tissue had <2 mm^2 surface area or exhibited poor tissue integrity. Information on objective response by RECIST v1.1, OS, and risk assessment was also collected (see Table S1 for additional demographic and clinicopathologic details). A subset of patients had multiple pre-treatment biopsies available. For these patients, the largest biopsy was analyzed.

METHOD DETAILS

Scoring of H&E stained slides

H&E stained slides were reviewed by a board-certified pathologist blinded to patient outcome, for the presence of immune infiltrates and necrosis, Figure 1. The mononuclear immune infiltrate, including tumor infiltrating lymphocytes, macrophages, plasma cells, and other associated immune cells, termed TIL[plus], was scored as "0" if no immune infiltrate was identified interfacing with tumor, or "1" for the presence of immune infiltrate involving tumor. This could include TIL[plus] tightly cuffing tumor nests and/or infiltrating between tumor cells. Necrosis in any part of the TME was scored as "0" if none or focal (≤10% of surface area involved by necrosis), or "1" if substantial, geographic necrosis (>10% surface area).

Genomic studies, PD-L1 IHC, and combinatorial scores

PBRM1 loss-of-function mutations were determined by whole exome sequencing as previously reported.[7,8] Similarly, previously reported PD-L1 IHC scores were obtained for these cohorts.[3,15] A combinatorial score was developed that included the TIL[plus] score, the necrosis score, and whether a *PBRM1* mutation was detected. A score that combined TIL[plus] and PD-L1 IHC expression was also tested.

Literature review

We performed a PubMed search from inception through August 2021 to identify multimodality biomarker studies using pre-treatment biospecimens for predicting clinical outcomes following anti-PD-1-based therapies, with the following search syntax: tumor mutational burden OR mutational load OR mutational density OR gene expression profiling OR gene signature OR mRNA OR multiplex immunofluorescence OR multiplex immunohistochemistry AND anti-PD-1 OR anti-PD-L1 OR nivolumab OR pembrolizumab OR atezolizumab OR durvalumab OR BMS-936558 OR BMS-936559 OR MK-3475 OR MPDL3280A OR MEDI4736 OR MSB0010718C. Separate searches using the following syntax were also deployed: (hematoxylin) AND (anti-PD-1 OR anti-PD-L1 OR nivolumab OR pembrolizumab OR atezolizumab OR durvalumab OR BMS-936558 OR BMS-936559 OR MK-3475 OR MPDL3280A OR MEDI4736 OR MSB0010718C); (pathology) AND (tumor mutational burden OR mutational load OR mutational density OR gene expression profiling OR gene signature OR mRNA OR multiplex immunofluorescence OR multiplex immunohistochemistry) AND (anti-PD-1 OR anti-PD-L1 OR nivolumab OR pembrolizumab OR atezolizumab OR durvalumab OR BMS-936558 OR BMS-936559 OR MK-3475 OR MPDL3280A OR MEDI4736 OR MSB0010718C). The searches were limited to human studies with English translation available. As the focus was on multi-modality biomarkers, studies combining multiple, distinct analytes detected using the same 'modality' were not included, e.g. a combination of PD-L1 and CD8 by IHC did not qualify as 'multimodality'. Studies with no anti-PD-(L)1-treated populations, studies without or with only limited comparisons to clinical outcomes, studies investigating blood-based profiling and/or hematologic malignancies, and studies with <15 patients were excluded.

H&E scoring system development

TIL[plus] score

In the CAP surgical pathology protocol for melanoma, TIL scores are recorded as a part of staging. In this system, TIL are graded as absent, non-brisk, or brisk, *i.e.* a 3-tiered system. Foundational studies related to immunoactive molecule expression such as PD-L1(B7-H1) expression in RCC (and melanoma) used a 4-tiered system, e.g., 0=absent, 1=focal, 2=moderate, 3=marked.[39,40] Given that we were studying immune checkpoint inhibitors, we started with such a 4-tiered system. Notably, there were no cases that qualified as score '3', i.e. marked infiltration by TIL per the aforementioned criteria, in the discovery cohort. Additionally, there was no clear advantage to distinguishing between a focal (score '1') and a moderate (score '2') infiltrate in the discovery cohort (Figure S5A), and thus TIL[plus] was reduced to a binary score of simply TIL[plus] present (score '1') vs. absent (score '0').

Necrosis score

The assessment of necrosis on routine pathology in RCC evaluates necrosis simply as present or absent,[41,42] while the CAP cancer pathology protocol sheets for breast carcinoma and sarcoma include three categories. For breast cancer, necrosis is scored as not identified; present, focal (small foci or single cell necrosis), i.e. ≤10% present; or central (extensive "comedo" necrosis).[43] We tried this three-tiered scoring system to determine if there was any benefit in this setting, but did not find a difference in the discovery cohort between cases that had no necrosis and those with small foci of necrosis ≤10% (Figure S5B), thus leading to the final binary categories of ≤ 10% or >10%.

Slide scans of specimens with scores

Example whole slide scans of 5 cases showing representative features for TIL[plus] = 0 or 1 and/or necrosis = 0 or 1 can be viewed at: https://digital.pathology.johnshopkins.edu/imageSets/5879.

Reproducibility study

Two pathologists (J.J. and E.B.) who were not involved in the development of the scoring system were trained on 5 cases. Each pathologist then independently scored 25 cases for TIL[plus] and necrosis. The resultant Kappa coefficient was 0.8, which represents substantial agreement.

QUANTIFICATION AND STATISTICAL ANALYSIS

The Kaplan–Meier estimator was used to perform survival analysis to associate the biomarker scores with patient outcomes, using the log-rank test to determine statistical significance. Fisher's exact test was used to assess differences in scores by gender, as well as to assess any potential differences in scores between patients who received prior systemic therapy (e.g., VEGF-targeted therapies, cytotoxic chemotherapy). Ages of patients were compared using Mann-Whitney test. Fisher's exact test was used to associate TIL^{plus} and the combinatorial score with objective response. All tests were two-sided, and P values of ≤ 0.05 were considered significant. Analyses were performed in GraphPad and R.

ADDITIONAL RESOURCES

Further information relevant to the clinical trial CheckMate 009 (NCT01358721), CheckMate 025 (NCT01668784), CheckMate 214 (NCT02231749) can be found at https://www.clinicaltrials.gov/ct2/show/NCT01358721, https://clinicaltrials.gov/ct2/show/NCT01668784, and https://clinicaltrials.gov/ct2/show/NCT02231749, respectively.