There is conflicting data regarding the role of PBAF complex mutations and response to immune checkpoint blockade (ICB) therapy in clear cell renal cell carcinoma (ccRCC) and other solid tumors. We assess the prevalence of PBAF complex mutations from two large cohorts including the pan-cancer TCGA project \( (n = 10,359) \) and the MSK-IMPACT pan-cancer immunotherapy cohort \( (n = 3700) \). Across both cohorts, PBAF complex mutations, predominantly \( PBRM1 \) mutations, are most common in ccRCC. In multivariate models of ccRCC patients treated with ICB \( (n = 189) \), loss-of-function (LOF) mutations in \( PBRM1 \) are not associated with overall survival (OS) \( (HR = 1.24, p = 0.47) \) or time to treatment failure \( (HR = 0.85, p = 0.44) \). In a series of 11 solid tumors \( (n = 2936) \), LOF mutations are not associated with improved OS in a stratified multivariate model \( (HR = 0.9, p = 0.7) \). In a current series of solid tumors treated with ICB, we are unable to demonstrate favorable response to ICB in patients with PBAF complex mutations.
Immune-checkpoint blockade (ICB) therapy has revolutionized the treatment of many malignancies, leading to an extensive search for predictive and prognostic biomarkers. We and others have reported on the association of ICB response with tumor mutation burden (TMB), neoantigen load and clonality, copy number alterations (CNA), microsatellite instability, and human leukocyte antigen zygosity across a variety of cancer types.\(^1\) Additional evidence has linked ICB response to the tumor microenvironment (TME), specifically T cell inflammation\(^2\), and integrated analyses have looked at both TMB and T cell inflammation in joint models.\(^3\) Despite these consistent signals across different studies and cancer types, there is a substantial number of tumors with lower mutation and neoantigen burdens that respond to ICB. Indeed, even studies that link response to TMB or neoantigen burden often have overlap between responders and non-responders.

Recent work from several groups pointed to the association of ICB response and mutations in the SWI/SNF chromatin remodeling complex, more specifically the polybromo and BRG-1 associated factors (PBAF) complex, which includes the genes ARID2, PBRM1, and BRD7 (refs. 6–8). Inactivation of the gene encoded by the PBAF complex was recently found to sensitize melanoma cells to T cell-specific killing.\(^7\) Miao et al.\(^6\) demonstrated that in a series of nearly 100 metastatic clear cell renal cell carcinoma (ccRCC) patients, those harboring loss-of-function (LOF) mutations in PBRM1 had clinical benefit from ICB. They further demonstrated that in other microsatellite stable tumors such as melanoma, lung, bladder, and head and neck cancers, loss of PBAF was also associated with clinical response.\(^8\) Similarly, a recent report validated the association between PBRM1 alterations and ICB response\(^9\) in CheckMate 025, a randomized phase 3 trial of nivolumab versus everolimus which demonstrated a survival benefit for nivolumab in the second- and third-line setting.\(^10\) Further functional and transcriptomic analysis suggested that PBRM1-deficient tumors possessed altered immune signaling pathways. However, in a recent randomized phase II study of metastatic ccRCC, no association was seen between presence of PBRM1 mutations and treatment response to the PD-L1-directed atezolizumab, nor to the combination of atezolizumab plus bevacizumab (\(n = 136\)); there was a favorable effect on treatment response in patients receiving sunitinib (anti-VEGF) on the control arm of the same study (\(n = 72\)).\(^11\) Given the discordant clinical data in PBAF complex loss, as well as its potential impact on the TME, we seek to leverage several large clinical trial data sets with genomic data along with our inhouse ICB-treated patients to explore the effects of PBAF loss on the TME and clinical outcomes. We utilize two large pan-cancer cohorts to determine the frequency of PBAF mutations, and we explore the prognostic significance of PBAF mutations across various solid-tumor malignancies in our Memorial Sloan Kettering Cancer Center (MSKCC) ICB cohort. Finally, we assess the impact of PBRM1 LOF mutations on TME expression programs using a cohort of 594 ccRCC patients with transcriptomic data. We are ultimately unable to demonstrate a favorable response to ICB in patients with PBAF complex mutations and further, gene-expression analysis of PBRM1 mutated metastatic ccRCC patients demonstrate consistent upregulation in hypoxia inducible factor (HIF) signaling and angiogenesis, but inconsistent interferon gamma signaling and other immune response pathways.

**Results**

**Presence of PBAF mutations in TCGA across cancers.** To evaluate the prevalence of PBAF complex mutations, we queried the pan-cancer TCGA atlas (\(n = 10,359\)) and analyzed all three genes in the complex (PBRM1, ARID2, and BRD7). Overall, 7.7% of all tumors possessed any PBAF complex mutation; incidence among malignancies included in the pan-cancer TCGA cohort was highest in ccRCC (KIRC) particularly for PBRM1 mutations, followed by melanoma (SKCM), cholangiocarcinoma (CHOL), stomach (STAD), uterine (UCEC), and bladder (BLCA) cancers (Fig. 1a, b). Additionally, highly mutated tumors were more likely to possess PBAF complex mutations (Fig. 2). Mutations in PBRM1, ARID2, and BRD7 each represented about 3.8% (52.7% LOF), 3.6% (39% LOF), and 1.1% (34.4% LOF), respectively (Supplementary Fig. 1).

**MSK-IMPACT immunotherapy pan-cancer cohort.** We first assessed the incidence of PBRM1 and ARID2 mutations (BRD7 not included in IMPACT) among all patients treated with ICB (\(n = 3700\)). For the MSK-IMPACT cohort, we restricted our analysis to 189 ccRCC patients and 2936 patients treated with immunotherapy comprising 11 other cancer types that had a minimum of 50 patients and 5 PBRM1 or ARID2 mutants. Clinical characteristics of the included cohort, including age, gender and drug class, as well as TMB and fraction genome altered (FGA) vary substantially across cancer types and are included in Table 1. Available PBAF complex mutations included PBRM1 and ARID2, present at 7.4% and 6.5%, respectively, across the pan-cancer cohort; LOF frequencies are 3.9% and 2.3%, respectively. Consistent with the TCGA analysis, PBRM1 mutations were most common in ccRCC patients (46.6%), followed by non-melanoma skin cancer (9%) and melanoma (8%), while ARID2 was most common in melanoma (13%), followed by non-melanoma skin cancer and colorectal cancer (11%) (Supplementary Fig. 2). With the exception of ccRCC, several of the tumors harboring PBAF complex mutations were highly mutated cancer types (Fig. 3a, b). This included both LOF mutations (frameshift and nonsense mutations) as well as missense mutations.

**PBAF mutation and response to immunotherapy in ccRCC.** Given the findings of Miao et al.\(^6\) and Braun et al.\(^9\) with respect to PBRM1 LOF mutations and response to ICB in RCC, we analyzed our cohort of ICB-treated metastatic ccRCC patients (\(n = 189\)) with more detailed clinical annotations including International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) prognostic score (\(n = 180\); Table 2), treatment details and outcomes with therapy, including time-to-treatment failure (TTF). PBRM1 LOF mutations were present in 61 of these 189 patients (32%), and non-LOF mutations were found in 27 patients (14%). Since ARID2 mutations were only present in six patients (4 of them LOF) and might have a distinct effect on outcome, we further analyzed only PBRM1 mutations. There were 57 deaths and 147 treatment failures among these 189 patients, with a median overall survival (OS) of 68.2 months (95% CI 44.4, NA) and median TTF of 8.9 months (95% CI 6.9, 12.42), but no difference for either outcome when comparing patients with PBRM1 LOF to others (Fig. 4). PBRM1 mutation rate was not significantly different in patients who received first line (\(n = 97\), 30% LOF mutations) or second or higher line (\(n = 92, 35\%) ICB or ICB/VEGF combinations. PBRM1 mutations were not associated with TTF in the entire ccRCC cohort (LOF HR 0.73, \(p = 0.11\); non-LOF HR 1.05, \(p = 0.84\)) and not significantly associated with OS (LOF HR = 1.5, \(p = 0.16\); non-LOF HR = 1.05, \(p = 0.91\)) (Table 3). When comparing outcomes with first-line ICB therapy in patients with PBRM1 LOF mutations vs. wild type, no significant differences were seen for TTF (HR = 0.6, \(p = 0.075\)) or OS (HR = 1.7, \(p = 0.29\)); similarly, no differences were seen for those receiving ICB in the second line or higher (TTF HR = 0.87,
Fig. 1 PBAF complex mutations across The Cancer Genome Atlas (TCGA) \( n = 10,359 \). a All PBAF complex mutations as a function of mean tumor burden (left) and loss of function (LOF) only mutations (right). b OncoPrint plot demonstrating loss-of-function vs. non-LOF PBAF complex mutations across the TCGA.

Fig. 2 Mutation burden in PBAF complex altered tumors. Tumor mutation burden (TMB) of PBAF complex mutated tumors in the TCGA plotted against TMB ratio of mutated tumors vs. wild type.
11 cancer types in a Cox model stratified by gender. LOF and non-LOF mutations were not significantly associated with TTF (LOF HR = 0.85, 95% CI 0.57, 1.28, p = 0.44; non-LOF HR = 1.22, 95% CI 0.77, 1.94, p = 0.4). Similarly, in the model for OS adjusted for IMDC risk score and line of therapy (significant predictors of OS), PBRM1 was not significant (LOF HR = 1.24, 95% CI 0.69, 2.25, p = 0.47; non-LOF HR = 0.88, 95% CI 0.36, 2.14, p = 0.78).

**PBAF complex mutation and ICB outcomes in other cancer types.** To assess the impact of PBAF complex mutations in non-RCC cohorts treated with ICB profiles with MSK-IMPACT, we restricted our analysis to 11 tumor types with at least 50 patients and at least 5 patients with PBRM1 or ARID2 mutations (n = 2936). These included bladder, colorectal, non-small-cell lung, esophageagastic, endometrial, non-melanoma skin, hematobiliary, head and neck cancers, melanoma, glioma, and cancer of unknown primary. Overall prevalence was 4.9% for PBRM1 (2% of them LOF) and 6.7% for ARID2 (3% of them LOF). PBRM1 mutations were not significantly associated with OS in a cohort of 11 cancer types in a Cox model stratified by gender (LOF HR = 0.95, 95% CI 0.61, 1.5, p = 0.77; non-LOF HR = 1.25, 95% CI 0.81, 1.95, p = 0.30), and remained insignificant after adjusting for TMB and total CNA (LOF HR = 1.2, 95% CI 0.81, 1.81, p = 0.37; non-LOF HR = 1.32, 95% CI 0.92, 1.9, p = 0.13) (Supplementary Table 1). Results were similar when combining PBRM1 and ARID2; LOF HR = 0.85, 95% CI 0.65, 1.1, p = 0.25 unadjusted and HR = 1.1, 95% CI 0.83, 1.45, p = 0.52 adjusted. Given the higher frequency of ARID2 mutations in non-RCC cohorts, we combined PBRM1 and ARID2 LOF and non-LOF mutations for individual subtype analysis, which was significant in non-small-cell lung cancers (Fig. 6a, Supplementary Table 2). When univariately examining LOF mutations in PBRM1 and ARID2 as well as LOF in PBRM1 alone, they remained significantly associated with adverse OS in non-small-cell lung cancer (Fig. 6b, c). In individual cancer types, PBRM1 was correlated with worse OS in non-small-cell lung cancers (n = 983; HR 2.91, p < 0.001) after adjusting for TMB and total CNA (Supplementary Table 3). A significant correlation with adverse OS was also seen in bladder cancer (n = 245; HR 11.85, p < 0.001); however, only three PBRM1 mutants comprised this group. ARID2 was not significant in either cancer type.

**PBRM1 mutations and the TME.** Previous work by our group and others suggested that PBRM1 loss was associated with further hypoxic signaling and angiogenic expression11, 12. This was further bolstered by the association with improved response of PBRM1 mutated tumors to VEGF blockade therapies13–15. We utilized transcriptomic data from three independent cohorts to analyze the impact of PBRM1 LOF mutations on transcriptional pathway enrichment. These included COMPARZ16, a phase 3 randomized trial comparing the efficacy and safety of pazopanib and sunitinib as first-line therapy (n = 352 [targeted exome and whole-genome RNA microarray]), McDermott et al.4, a randomized phase 2 study of atezolizumab alone or combined with bevacizumab versus sunitinib in treatment-naïve metastatic renal cell carcinoma (n = 201 [whole exome + RNASeq]), and Miao et al.8, which analyzed a cohort of approximately 100 metastatic ccRCC patients to identify genomic alterations correlating to response to ICB (n = 41 [whole exome + RNASeq]). All three cohorts demonstrated higher hypoxia pathway enrichment in PBRM1 mutated samples with GSEA p value as 0.002, 0.008, and 0.002, respectively. In the COMPARZ and McDermott et al. cohorts, we observed downregulation of interferon alpha and gamma response genes. With respect to interferon gamma response or JAK/STAT signaling, we were able to validate higher expression in the Miao et al. cohort (as previously reported) but we found lower expression in both the COMPARZ and McDermott et al. cohorts. We further performed immune deconvolution using ssGSEA focusing on immune and angiogenic gene signatures. We consistently observed significantly higher angiogenic gene expression in PBRM1 mutated tumors in the COMPARZ and McDermott et al. data sets, p = 0.0004 and 0.005, respectively, and a similar trend in Miao et al. cohort (Fig. 7a). Further, immunohistochemical (IHC) staining from the COMPARZ and McDermott cohort demonstrates significantly higher CD31-positive staining in PBRM1 mutated tumors, implying higher
degrees of tumor angiogenesis in PBRM1 mutated tumors (Fig. 7b). IHC studies from the two cohorts also reveal higher PD-L1-negative and lower PD-L1-positive staining tumors in PBRM1 mutated tumors compared to wild type (Fig. 7b) and no difference in CD8 positivity between PBRM1 mutant and wild-type tumors (Supplementary Fig. 3). Immune deconvolution of bulk expression data failed to find any specific immune enrichment patterns across the three cohorts when stratified by PBRM1 mutation status (Supplementary Fig. 4).

**Discussion**

The identification of genomic biomarkers for ICB therapy remains an evolving field. While several studies seem to validate tumor mutation and neoantigen burden, along with mismatch repair mutations1–3, numerous other studies have relied on single gene mutations with relatively small cohorts and often not correcting for potential confounding factors such as TMB or microsatellite instability/mismatch repair status. Our analysis of over 3000 patients treated with ICB did not find an association with PBAF complex loss and ICB response in both univariate or multivariate tests. Specifically, we found PBAF complex mutations to be most common in ccRCC tumors which were dominated by PBRM1 mutations; interrogation of this cohort of ICB-treated ccRCC patients failed to reveal an association between PBRM1/ARID2 mutations and overall survival or time-to-treat failure. Furthermore, analysis of both the McDermott et al. and our recently analyzed COMPARZ cohort showed unchanged or lower IFNγ signaling in the PBRM1 mutants. 3p loss (which encompasses four commonly mutated genes: VHL, PBRM1, SETD2, BAP1) is a ubiquitous, pathognomonic event in ccRCC, occurring in upwards of 90% of tumors. Preclinical data support the notion that VHL (the most commonly mutated gene in clear cell RCC) and PBRM1 co-occur; although VHL is the initial driver event in the pathogenesis of clear cell RCC, genetic deletion of VHL in mice is insufficient to initiate kidney tumors12. After loss of VHL, loss of additional 3p21 tumor suppressor genes (PBRM1) further activates HIF1/STAT3 signaling in mouse kidney and positions mTORC1 activation as the third preferred driver event.
**PBRM1** encodes for the protein BAFl80, a subunit of the PBAF subtype SWI/SNF chromatin remodeling complex. The PBAF complex regulates access to DNA bound to histones by transiently altering the nucleosome structure so that the DNA may be accessed by the cell's transcriptional machinery. Mutation of **PBRM1** leads to the integration of an altered BAFl80 subunit and thereby alters the normal activity of the PBAF complex.

The PBAF complex has been reported to control the immune system by modulating immune recruitment and T cell activation through the IFNα signaling and JAK/STAT pathway. IFNα serves as a critical cytokine for tumor immunity and its signaling axis is mediated through STAT3 and STAT5 signal transduction. Notably, STAT3 is part of an important intrinsic pathway for inflammation by inducing genes that lead to the production of IL-6, 10, 11, 17, 23, CXCL12, and COX-2 (ref. 19). The activation of STAT3 is normally a transient process activated by numerous cytokines, including IL-6 (ref. 20), IFNγ (ref. 21), and TNFα (ref. 22), which is strictly controlled to prevent unscheduled gene regulation. The pathway of activation is initiated through JAK phosphorylation whereby STAT3 becomes phosphorylated and combines into dimers to enter the nucleus via importin alpha5/NI1 (ref. 24). Once in the nucleus, STAT3 induces transcription of genes involved in cell survival and proliferation.

However, unbridled activation of STAT3 is oncogenic and has been reported in a variety of tumors, including renal cancers, and can go unchecked due to mutations in negative regulatory mechanisms. Growing evidence supports the role of **PBRM1** (via PBAF) as one of these negative regulators of STAT3 and in turn acts to downregulate the transcription of proliferative and interferon stimulated genes (ISG). In 2011, Verela et al. showed that **PBRM1** knockout enhanced proliferation and migration of kidney cancer cell lines. Later, Pan et al. showed through RNA-sequencing that among **PBRM1** and interferon stimulated genes (ISG). In 2011, Verela et al. showed that **PBRM1** in ccRCC: overall survival and time to treatment failure (TTF) in ccRCC patients across MSK-IMPACT.

**Fig. 4 Survival and time to treatment failure in PBRM1 mutated MSKCC ccRCC patients.** Kaplan–Meier curves demonstrating overall survival (median 68.2 months; 95% CI 44.4, NA) and time-to-treatment failure (TTF) (median 8.9 months; 95% CI 6.9, 12.42) in clear cell RCC patients across MSK-IMPACT.
Table 3 Univariate and multivariate regression models of ICB and combination therapy response in PBRM1 mutated ccRCC patients in MSKCC cohort (n = 189).

| Variable | Time-to-treatment failure | Overall survival |
|----------|---------------------------|------------------|
|          | Univariate analysis       | Multivariate model | Univariate analysis | Multivariate model |
|          | HR (95% CI) | P value | HR | 95% CI | P value | HR | 95% CI | P value |
| PBRM1 wild type | 1 | Ref. | 1 | 0.85 | 0.57 | 1.28 | 0.44 |
| LOF | 0.73 (0.5-1.07) | 0.112 | 1.5 (0.85-2.66) | 0.161 |
| Non-LOF | 1.05 (0.67-1.65) | 0.838 | 1.05 (0.46-2.4) | 0.914 |
| TMB | 0.94 (0.89-0.99) | 0.029 | 0.99 (0.9-1.1) | 0.778 |
| Age at treatment | 1.29 (0-610) | 0.936 | 1.31 (0.56-6.68) | 0.96 |
| Genome doubled | 1.08 (0.74-1.59) | 0.695 | 1.56 (0.89-2.74) | 0.115 |
| Fraction CNA | 1.46 (0.79-2.71) | 0.227 | 2.04 (0.78-5.35) | 0.144 |
| CTLA-4 status | 0.84 (0.155) | 0.874 | 0.98 (0.19) | 0.78 |
| No | 1 | 1 | 1 | 1 |
| Yes | 1.33 (0.9-1.99) | 0.211 | 0.74 (0.15-4.4) | <0.001 |
| IMDC risk | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| 2 | 1 | 1 | 1 | 1 |
| 3 | 1.35 (0.84-2.17) | 0.003 | 3 (1.59-5.66) | 4.12 |
| Drug class | 3.46 (0.38-0.76) | <0.001 | 3.46 (0.38-0.76) | <0.001 |
| IO | 1 | 1 | 1 | 1 |
| IO-IO | 1 (0.66-1.54) | 1 | 0.54 (0.27-1.09) | 0.46 (0.24-0.87) |
| IO-VEGF | 0.93 (0.38-0.8) | <0.001 | 0.93 (0.38-0.8) | <0.001 |
| Line of therapy | 0.211 | 0.81 (0.39-0.59) | 0.702 |
| >1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 |
| BAP1 | 0.842 | 0.96 (0.64-1.44) | 0.96 (0.64-1.44) | 0.96 (0.64-1.44) |
| No | 1 | 1 | 1 | 1 |
| Yes | 0.96 (0.64-1.44) | 0.48 | 1.14 (0.59-2.21) | 0.71 |
| SETD2 | 0.88 (0.62-1.26) | 0.88 (0.62-1.26) | 0.9 (0.51-1.59) | 0.9 (0.51-1.59) |

P values derived from Cox proportional hazards model. For multivariate model IO and IO/IO were combined into one category. Multivariate model is based on 180 patients with available risk score.
with more favorable TTF ($p = 0.01$, median 12.0 months for PBRM1 mutants versus 6.9 months for wild-type tumors)\textsuperscript{35}.

Finally, we have shown in the COMPARZ cohort that PBRM1 mutations are associated with both higher angiogenesis expression and response to anti-VEGF therapy. This parallels the findings of the sunitinib arm of the McDermott et al. cohort, wherein sunitinib efficacy was enriched in highly angiogenic tumors and coincided with PBRM1 mutant tumors, demonstrating improved progression free survival and objective response rates in tumors with high angiogenic gene signature.

Braun et al.\textsuperscript{9} recently reported on the association between PBRM1 alterations and ICB response in CheckMate 025, a large
randomized phase 3 trial of nivolumab versus everolimus in advanced renal cell carcinoma. The validation study demonstrated a modest, albeit significant, mutation effect on improved response and survival in nivolumab-treated patients, none in subjects on the everolimus arm. Intriguingly, this effect was observed in patients who received prior antiangiogenic therapy; previous studies of PBRM1 mutations in the first-line setting had negative results, and PBRM1 alterations have also been associated with benefit from antiangiogenic therapies. It is noteworthy to mention that among nivolumab-treated patients, a higher proportion of responders (15 of 38) harbored truncating PBRM1 mutations, which was statistically significant but numerically similar to the non-responders (16 of 74). This lack of effect in the first-line setting may explain the lack of response of the atezolizumab arm in the first-line setting may explain the lack of response of the atezolizumab arm in the first-line setting. In this study, the Pan-cancer TCGA cohort showed a significantly higher angiogenic gene expression compared to the previous studies of PBRM1 mutations in the first-line setting. The Fisher's exact test was performed for the comparison of the angiogenic gene expression between the two groups. The p-values from the Fisher's exact test for the comparison of the angiogenic gene expression between the two groups are reported. The non-parametric Wilcoxon rank-sum test was used to compare the angiogenic gene expression between the two groups, and the p-values are reported. The results from the Wilcoxon rank-sum test were adjusted for multiple comparisons using the Bonferroni correction.

Methods

Cohort selection. TCGA: Pan-cancer TCGA cohort (n = 10,359 from 31 cancer subtypes) data, including ARID2, BRD7, and PBRM1 mutations, mutation count as well as FGA (which is an estimate proportion of the tumor genome affected by copy number gains and losses), were queried and downloaded from MSKCC ChioPortal (chiportal.mskcc.org). Specifically, FGA is calculated as the length of segments with log2 CNA value larger than 0.2, divided by the length of all segments measured. Among various mutation types, the truncating mutation (putative driver) is treated as a LOF mutation and the remainder treated as non-LOF. MSK ICB Cohort: After receiving institutional review board (IRB) approval at MSKCC, institutional pharmacy records were used to identify patients who had received at least one dose of immunotherapy at MSKCC for metastatic cancer, and these were then cross-referenced with patients who had MSK-IMPACT testing done in the context of routine clinical care. Informed consent was obtained from all patients prior to MSK-IMPACT testing. For MSK pan-cancer cohort we selected all patients treated with immunotherapy at MSKCC between 2010 and 2018 who had their tumor profiled with MSK-IMPACT targeted sequencing platform (n = 3700 from 51 cancer subtypes). After limiting non-renal cell carcinoma patients to 11 cancer types with at least 50 patients and 5 PBRM1 or ARID2 mutations, the pan-cancer cohort was comprised of 2936 patients.

Details of tissue processing and next-generation sequencing and analysis were previously described. Patients enrolled in ongoing clinical trials for which study outcomes have not been reported were removed, as were a small proportion of patients with either localized disease treated in the neoadjuvant setting or localized.
disease. Other preceding or concurrent non-ICB treatments were not recorded or accounted for in the analysis.

**IMPACT mutational profiling.** The MSK-IMPACT assay was performed on DNA extracted from formalin-fixed, paraffin-embedded primary tumor samples as previously published. The total number of somatic mutations identified was normalized to the exomic coverage of the respective MSK-IMPACT panel in megabases. Importantly, concurrent sequencing of germline DNA from peripheral blood was performed for all samples to identify somatic tumor mutations. For each histology, we subsequently identified cases in the top twentieth percentile of TMB and determined the log-rank 

**Statistical methods.** OS was defined as time from the start of immunotherapy until death or last date of follow-up. For patients who received multiple courses of ICB, the first treatment was used for analysis. Patients were censored at the date of most recently attended appointment at MSKCC if death was not recorded in the electronic medical record. For the ccRC cohort, TTF was defined as time from the start of immunotherapy until treatment discontinuation for any reason. The Kaplan–Meier method was used to estimated TTF and OS curves. Effect of the mutations was tested using univariate and multivariate Cox proportional hazard regression. For the analysis of pan-cancer cohort, the Cox model was stratified by the cancer type. In analysis of ccRC cohort all variables with p < 0.05 univariately were included in the multivariable model. Wilcoxon rank-sum test implemented as R wilcox.test function was used for testing the difference of deconvolved immune features between mutant and wild-type groups. All analyses were performed in the R platform v3.6.1.

**Data availability.** Sequencing data from MSK-IMPACT and TCGA cohorts were obtained from MSKCC cBioPortal (http://cbioportal.org) and the GDC data portal (https://gdc.cancer.gov/about-data/publications/pancanatlas), respectively. The expression data used for the transcriptomic analyses were obtained from the original publications and are publicly available (McDermott et al.38 cohort: European Genome-Phenome Archive (EGA) at accession number EGASA0001002928, Miao et al.39 cohort: dbGap at accession number phs001565.v1.p1). Trial data sets for COMPARZ38 are available on the link to Data Access Committee [novartis.databeharing@novartis.com] at the EGA repository under the accession code EGADB0001001930. All remaining relevant data are available in the article, Supplementary Information, or from the corresponding author upon reasonable request.

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A.A.H., M.H.V., T.A.C., and R.J.M. conceived and supervised the project. A.A.H. and K.A. wrote the manuscript with input from all authors. I.O., J.F., F.K., R.G.D., and E.R. were involved in formal data analysis and supervised the methodology of the project. D.H., K.A.B., Y.G., and S.M.K. curated the data. A.B., J.H., and C.J.F. provided data needed for analysis. All authors proofread the manuscript.

Competing interests
A.A.H. corresponding author, certifies that all conflicts of interest, including specific financial interests, relationships, and affiliations relevant to the subject matter or materials discussed in the manuscript are the following: T.A.C. is a paid consultant for Bristol-Myers Squibb and Illumina, reports receiving commercial research grants from Astra-Zeneca, Illumina, Pfizer, and BMS, and holds ownership interest (including patents) in Gritstone. M.H.V. reports honoraria from Novartis; consulting/advisory role for Alexion Pharmaceuticals, Bayer, Calithera Biosciences, Corvus Pharmaceuticals, Exelixis, Eisai, GlaxoSmithKline, Natra, Novartis, and Pfizer; research funding from Pfizer, Bristol-Myers Squibb, and Genentech/Roche; and travel, accommodations, and expenses from Eisai, Novartis, and Takeda. R.J.M. reports grants and personal fees from Pfizer, Novartis, Eisai, Genentech/Roche; personal fees from Exelixis, Merck, Lilly, and Incyte; and grants from BMS outside the submitted work. The remaining authors declare no conflict of interest.

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