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Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma

Diana Miao[1,2], **Claire A. Margolis**[1,2], **Wenhua Gao**[1], **Martin H. Voss**[3,4], **Wei Li**[5], **Dylan J. Martini**[1], **Craig Norton**[1], **Dominick Bossé**[1], **Stephanie M. Wankowicz**[1,2], **Dana Cullen**[6], **Christine Horak**[6], **Megan Wind-Rotolo**[6], **Adam Tracy**[2], **Marios Giannakis**[1,2], **Frank Stephen Hodi**[1], **Charles G. Drake**[7], **Mark W. Ball**[8], **Mohamad E. Allaf**[8], **Alexandra Snyder**[3,*], **Matthew D. Hellmann**[3,4], **Thai Ho**[9], **Robert J. Motzer**[3,4], **Sabina Signoretti**[1], **William G. Kaelin Jr.**[1,10], **Toni K. Choueiri**[1,‡,†], and **Eliezer M. Van Allen**[1,2,‡,†]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02215, USA

[2]Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, MA 02142, USA

[3]Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

[4]Weill Cornell Medical College, New York, NY 10065, USA

[5]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA

[6]Bristol-Myers Squibb, New York, NY 10154, USA

[7]Columbia University Medical Center, New York, NY 10032, USA

[8]James Buchanan Brady Urological Institute and Department of Urology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

[9]Mayo Clinic, Scottsdale, AZ 85259, USA

[10]Howard Hughes Medical Institute, Dana-Farber Cancer Institute, Boston, MA 02215, USA

Abstract

Immune checkpoint inhibitors targeting the programmed cell death-1 receptor (PD-1) improve survival in a subset of patients with clear cell renal cell carcinoma (ccRCC). To identify genomic alterations in ccRCC that correlate with response to anti-PD-1 monotherapy, we performed whole exome sequencing of metastatic ccRCC from 35 patients. We found that clinical benefit was associated with loss-of-function mutations in the *PBRM1* gene (p=0.012), which encodes a

‡Corresponding author. eliezerm_vanallen@dfci.harvard.edu (E.M.V.); toni_choueiri@dfci.harvard.edu (T.K.C.).
*Present address: Adaptive Biotechnologies, Seattle, WA 98102, USA
†These authors contributed equally to this work.

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subunit of a SWI/SNF chromatin remodeling complex (the PBAF subtype). We confirmed this finding in an independent validation cohort of 63 ccRCC patients treated with PD-(L)1 blockade therapy alone or in combination with anti-CTLA-4 therapies (p=0.0071). Gene expression analysis of PBAF-deficient ccRCC cell lines and *PBRM1*-deficient tumors revealed altered transcriptional output in JAK/STAT, hypoxia, and immune signaling pathways. *PBRM1* loss in ccRCC may alter global tumor cell expression profiles to influence responsiveness to immune checkpoint therapy.

Immune checkpoint inhibitors such as nivolumab extend the survival of a subset of patients with metastatic ccRCC (1). Whether specific genomic features of ccRCC are associated with clinical benefit is unclear. In contrast to other human tumor types that respond to immunotherapy, such as non-small cell lung cancer (NSCLC), melanoma, and microsatellite-unstable colorectal adenocarcinoma, ccRCC harbors a low burden of somatic mutations (2–5). Melanoma and NSCLC typically harbor 10 to 400 mutations per megabase (Mb) and these genetic variants can generate tumor-specific antigens (neoantigens) that stimulate a strong anti-tumor immune response (1–4). In contrast, ccRCC harbors an average of only 1.1 mutations/Mb (6, 7) yet it ranks highly among tumor types in terms of immune cytolytic activity (8), immune infiltration score, and T cell infiltration score in the tumor microenvironment (9). These observations led us to hypothesize that distinct molecular mechanisms underlie the immunologically active tumor microenvironment and responsiveness to immune checkpoint therapy in patients with ccRCC.

As part of a prospective clinical trial (10), we first analyzed pre-treatment tumors from 35 patients with metastatic ccRCC on a clinical trial of anti-programmed cell death-1 receptor (anti-PD-1) therapy (nivolumab). Whole exome sequencing (WES) from paired tumor/normal tissue was performed to identify genetic correlates of clinical benefit. To validate the findings, we analyzed an independent cohort of 63 patients with metastatic ccRCC treated with therapies blocking PD-1 (e.g., nivolumab) or its ligand PD-L1 (e.g., atezolizumab) (Fig. 1A and table S1A) (11).

Baseline clinical and demographic features in the discovery cohort have been previously described (10). The subset of patients with complete pre-treatment molecular profiling did not differ substantially in clinical or demographic features from patients whose data did not meet technical quality control standards (fig. S1, A and B, and Supplemental Methods) or from the larger published cohort (10). Given previous evidence suggesting that refined clinical stratifications are necessary to assess clinical benefit from immune checkpoint blockade (12), we defined a composite response endpoint incorporating RECIST (Response Evaluation Criteria In Solid Tumors) (13), radiographic tumor shrinkage, and progression-free survival (PFS) (Fig. 1B and table S1B). Clinical benefit (CB) included patients with complete response (CR) or partial response (PR) by RECIST 1.1 (i.e., tumor shrinkage >30% from baseline) (13) or stable disease (SD) if they had any objective reduction in tumor burden lasting at least 6 months. This modification to include some patients with SD is intended to differentiate those patients with naturally indolent disease (i.e., slow tumor growth not surpassing 20% of baseline tumor size) from those with tumor response to immune checkpoint inhibitors (14). No clinical benefit (NCB) patients experienced progressive disease (PD) by RECIST 1.1 and were discontinued from immunotherapy within

three months. All other patients were termed “intermediate benefit” (IB). One patient in the discovery cohort was classified as CB despite PFS < 6 months because there was continued tumor shrinkage (−67% of baseline tumor size) after an initial period of minor tumor progression, and the patient had overall survival exceeding 32 months (fig. S2, A and B). Consistent with prior observations (1), the dose of nivolumab, patient gender, and baseline PD-L1 immunohistochemical staining from metastatic biopsies did not predict patient overall survival (OS) following initiation of anti-PD-1 therapy (p>0.05 for all; log-rank test) (fig. S3).

Mean exome-wide target coverage in the discovery cohort was 128-fold for tumor sequencing and 91-fold for matched germline sequencing (tables S1A and S2A). Overall nonsynonymous mutation burden was moderate in the discovery cohort (median 82 per exome, range 45–157). The tumors of patients with CB and those with NCB showed similar mutation burdens and intratumoral heterogeneity (Fig. 1, C and D, and table S1, C and D). Mutations and copy number alterations affecting antigen presentation machinery and HLA class I alleles were uncommon and were present in tumors of both CB and NCB patients (fig. S4, A and B).

We next focused our analysis on the mutations most likely to be functionally important. We applied MutSig2CV (15) to identify genes recurrently mutated in the discovery cohort. Of these genes, we limited our search to highly deleterious variants, meaning known hotspot or putative truncating (frameshift insertion or deletion, nonsense mutation, or splice-site) mutations. Of the seven recurrently mutated genes (Fig. 2A and table S1E) (6), *PBRM1* was the only gene in which truncating, or loss-of-function (LOF) (11), mutations were enriched in tumors from patients in the CB vs. NCB group (9/11 vs. 3/13; Fisher’s exact p=0.012, q=0.086, odds ratio for CB=12.93, 95% C.I. 1.54–190.8) (Fig. 2B and table S1F). In this cohort, all truncating *PBRM1* alterations co-occurred with deletion of the non-mutated allele on chromosome 3p (Fig. 2A), resulting in complete LOF of *PBRM1*, and most of the mutations were predicted to be clonal (present in all tumor cells) (table S1F). Prior large-scale sequencing studies have shown that *PBRM1* LOF alterations occur in up to 41% of ccRCC tumors (16) and are commonly clonal events present in all or nearly all tumor cells (17). Patients whose tumors showed biallelic *PBRM1* loss had significantly prolonged OS and PFS compared to patients without *PBRM1* LOF (log-rank p=0.0074 and p=0.029, respectively) (Fig. 2C and fig. S5), and they experienced sustained reductions in tumor burden (Fig. 2D).

To evaluate the reproducibility of this finding, we then examined matched pre-treatment tumor and germline genomic data from an additional 63 patients treated with anti-PD-(L)1 therapy, either alone or in combination with anti-CTLA-4 therapy. Of these 63 patients, *PBRM1* mutation status was derived from WES in 49 and panel sequencing in 14 patients (Fig. 3, A and B, and table S2, A and B) (11). Tumors from CB patients were more likely to harbor truncating alterations in *PBRM1* (17/27 vs. 4/19, Fisher’s exact p=0.0071, odds ratio for CB=6.10, 95% C.I. 1.42–32.64) (Fig. 3, C and D, and table S2C). Although we could not assess copy number alterations in all samples in the validation cohort, the *PBRM1* LOF mutations likely represented biallelic loss, as chromosome 3p deletions are nearly ubiquitous in ccRCC (6). Notably, one of the four NCB patients whose tumor showed a *PBRM1* LOF

mutation also had an alteration in *B2M*, which codes for a protein important in antigen presentation. This provides a potential explanation for the patient's lack of clinical benefit from immune checkpoint blockade therapy despite having a truncating *PBRM1* mutation.

While primary analyses excluded patients with intermediate benefit (IB) due to the unclear effect of immune checkpoint blockade therapy on patient outcomes in this group, the observed trend between *PBRM1* mutation status and clinical benefit persisted with the inclusion of these patients as an intermediate phenotype. In both the discovery and validation cohorts, patients in the IB group had intermediate rates of *PBRM1* LOF (82%, 64%, 23% for CB, IB, NCB in the discovery cohort and 63%, 41%, 21% for CB, IB, NCB in the validation cohort; Fisher-Freeman-Halton Exact p = 0.017 and 0.017). Additionally, while no difference in clinical benefit was observed between treatment-naive and previously-treated patients in the discovery cohort (fig. S2), the progression-free survival benefit conferred by *PBRM1* LOF was more prominent in tumors from previously-treated patients compared to those from patients receiving anti-PD-1 therapy as their first cancer therapy (p=0.009) (fig. S6 and tables S1 and S2).

The *PBRM1* gene codes for BAF180, a subunit of the PBAF subtype of the SWI/SNF chromatin remodeling complex. The PBAF complex suppresses the hypoxia transcriptional signature in $VHL^{-/-}$ ccRCC (18, 19) but its effects on tumor-immune interactions have not been thoroughly studied. To explore the potential impact of this complex on the immunophenotype of ccRCC, we analyzed previously reported whole transcriptome sequencing (RNA-seq) data from A704 ccRCC cell lines with perturbations in the PBAF complex (19). Loss of BAF180 or the related PBAF subunit BRG1, encoded by the gene *SMARCA4*, prevent formation of the intact PBAF complex (19). We performed gene expression analyses of BAF180-null ($A704^{BAF180-/-}$) cell lines vs. PBAF-wildtype ($A704^{BAF180wt}$) cell lines, as well as BRG1-null ($A704^{BAF180wt,\ BRG1-/-}$) cell lines vs. PBAF-wildtype ($A704^{BAF180wt}$) cell lines (Fig. 4A). Differential gene expression analysis showed substantial overlaps (~50%) between the top 100 genes differentially expressed in $A704^{BAF180-/-}$ vs. $A704^{BAF180wt}$ and $A704^{BAF180wt,\ BRG1-/-}$ vs. $A704^{BAF180wt}$ (table S4). This reflects the fact that BAF180 is essential to the PBAF but not the BAF complex, while BRG1 is a required subunit of both. Thus, the BAF180-null and BRG1-null cell lines have some shared characteristics but are also biologically and phenotypically distinct.

Gene set enrichment analysis (GSEA) on 50 "hallmark" gene sets representing major biological processes (20) revealed five gene sets whose expression was significantly enriched in cell lines that were PBAF-deficient. These included genes linked to IL6/JAK-STAT3 signaling, TNF-α signaling via NF-κB, and IL2/STAT5 signaling (Fig. 4A and table S5, A and B). As expected, the hallmark hypoxia gene set was up-regulated in $A704^{BAF180-/-}$ vs. $A704^{BAF180wt}$ cell lines (family-wise error rate - FWER q=0.071) (table S5A) (19). Across the more refined "founder" gene sets describing these five significantly enriched hallmark gene sets, the most strongly enriched gene set in PBAF-deficient cell lines was the KEGG cytokine-cytokine receptor interaction gene set (FWER q=0.0020 for $A704^{BAF180-/-}$ vs. $A704^{BAF180wt}$ and q=0.023 for $A704^{BAF180wt,\ BRG1-/-}$ vs. $A704^{BAF180wt}$) (Fig. 4A and table S5, C to L). This gene set includes both immune-stimulatory (e.g., *IL12, CCL21*) and immune-inhibitory (e.g., *IL10*) genes, but Gene

Ontology term analysis (11) showed that the genes most strongly enriched in PBAF-deficient cell lines were immune-stimulatory (table S6). Previously reported GSEA analysis of untreated ccRCC from The Cancer Genome Atlas (TCGA) and a murine model of *PBRM1* loss also show amplified transcriptional outputs of HIF1 and STAT3, involved in hypoxia response and JAK-STAT signaling respectively, in *PBRM1*-mutant vs. *PBRM1*-wildtype states (18). GSEA analysis of RNA-seq from pre-treatment tumors in the discovery and validation cohorts of this study (n = 18 *PBRM1*-LOF vs. n = 14 *PBRM1*-intact) confirmed increased expression of the hypoxia and IL6/JAK-STAT3 gene sets in the *PBRM1*-LOF tumors (Fig. 4B and tables S7, A and B, and S8). Given JAK-STAT3 pathway gene involvement in the interferon gamma (IFN-γ-) signaling pathway and IFN-γ-dependent cancer immunostimulation (21), differential expression of these genes may impact *PBRM1*-LOF patients' response to anti-PD-(L)1 therapy.

In addition to assessing tumor-intrinsic gene expression with GSEA, we further characterized the quality of the tumor-immune microenvironment in *PBRM1*-LOF vs. *PBRM1*-intact ccRCC in three independent cohorts: TCGA (6), an independent cohort of untreated ccRCC tumors (Sato) (22), and patient tumors from this study (table S8). In all three cohorts, tumors harboring LOF mutations in *PBRM1* showed lower expression of immune inhibitory ligands (e.g., CD276 and BTLA) (23) than those without *PBRM1* mutations. This finding was somewhat unexpected, as high PD-L1 staining is associated with increased responsiveness to anti-PD-1 and anti-PD-L1 agents in other cancer types (24, 25). However, the magnitudes of these differences were small and potentially confounded by differing degrees of tumor-stromal admixture (fig. S7, A to C) (9). We also examined LOF mutations in *VHL*, the most commonly-mutated gene in the TCGA ccRCC cohort. *VHL* mutation status did not correlate with immune-related gene expression (fig. S8), suggesting that observed differences in immune gene expression in the context of *PBRM1* LOF may be specific to the *PBRM1* gene.

In summary, we have shown that patients with metastatic ccRCC harboring truncating mutations in *PBRM1* experienced increased clinical benefit from immune checkpoint therapy. This may be due to distinct immune-related gene expression profiles in *PBRM1*-mutant or PBAF-deficient tumor cells compared to their PBAF-intact counterparts, as shown by RNA-seq analyses in this study, though further in vivo studies will be needed to further explore these findings. Given the high prevalence of *PBRM1* LOF in ccRCC and of SWI/SNF alterations across all cancer types (more than 20%) (26), this finding has important implications as a molecular tool for considering immunotherapy-responsiveness in ccRCC and across cancer types.

In vivo studies of mice harboring tumor clones with inactivation of *PBRM1* – or the related essential PBAF complex components *ARID2* or *BRD7* – show that cells with PBAF loss are more sensitive to T-cell-mediated cytotoxicity compared to their PBAF-intact counterparts (27). This finding lends a mechanistic basis to the results observed here, and helps explain the conflicting results regarding *PBRM1* mutation status as a prognostic variable in ccRCC (in the absence of immunotherapy) in prior studies (28–36). *PBRM1* also previously has been linked to longer PFS with VEGF-targeted therapies (37). The observed interaction between *PBRM1* status, prior treatment (largely with VEGF inhibitors), and response to

immune checkpoint therapy in this study argues for further investigation of patient outcomes from sequential and combination treatment regimens that include anti-PD-(L)1. The relationship between *PBRM1* LOF and clinical benefit from anti-PD-(L)1 therapies in ccRCC, as well as the immunological significance of PBAF loss in other cancer types, merit further preclinical and prospective clinical validation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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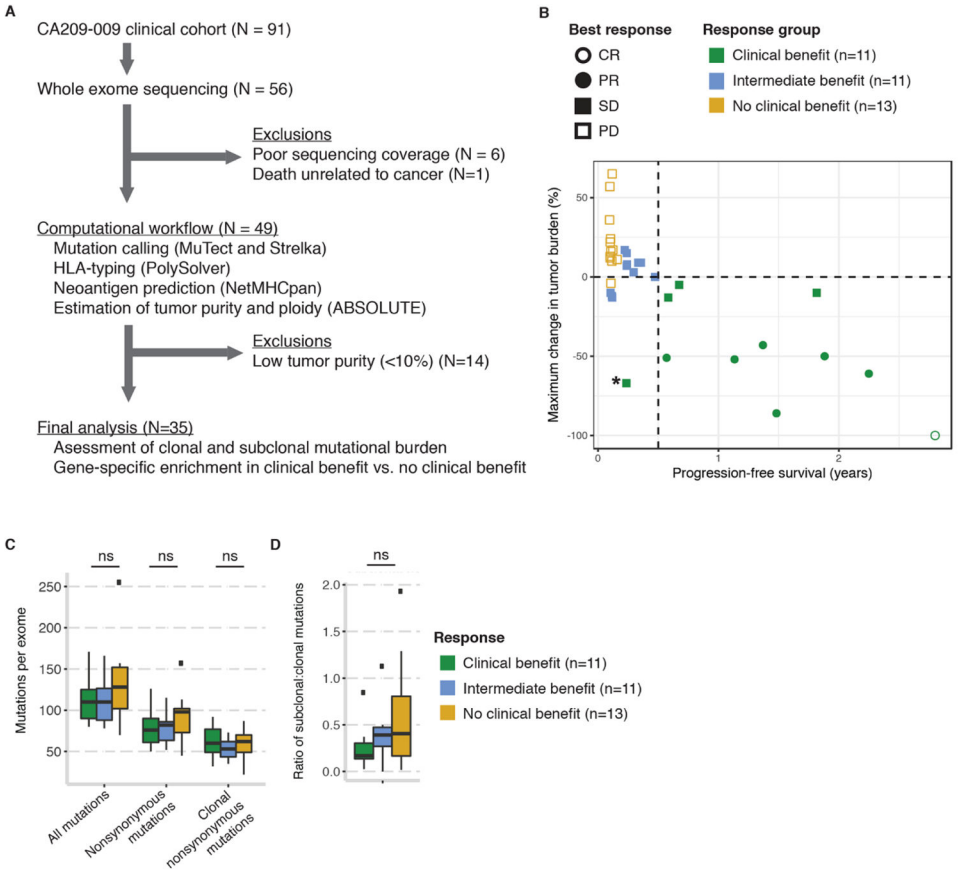

Fig. 1. Cohort consolidation and clinical characteristics of the discovery cohort

(**A**) Sample inclusion/exclusion criteria and computational workflow. (**B**) Clinical stratification by degree of objective change in tumor burden (y-axis) and duration of progression-free survival (x-axis). One patient (RCC_99) not shown due to lack of tumor response data. *Patient RCC_50 was classified as clinical benefit despite PFS<6 months because there was continued tumor shrinkage after an initial period of minor tumor progression (see fig. S2). (**C**) Mutation burden in the discovery cohort by response group. (**D**) Ratio of subclonal to clonal mutations, as estimated by ABSOLUTE, by response group. ns = not significant. Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

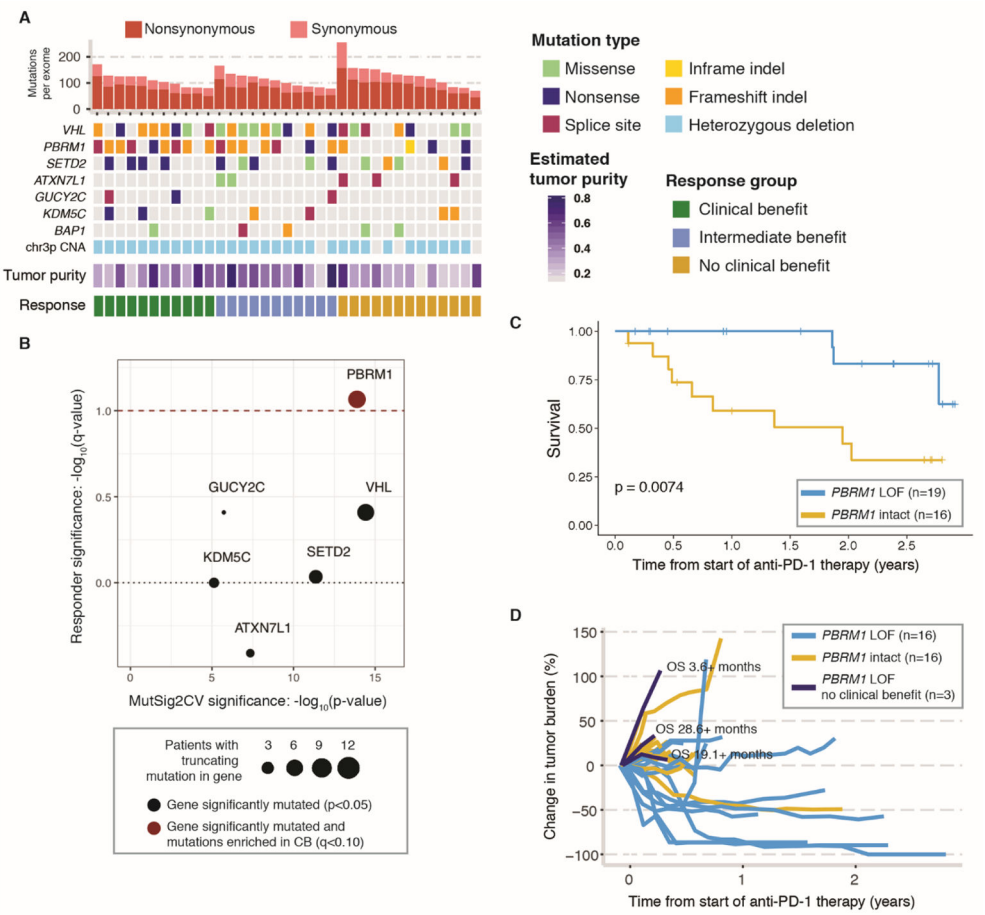

Fig. 2. Analysis of tumor genome features in discovery cohort reveals a correlation between *PBRM1* LOF mutations and clinical benefit from anti-PD-1 therapy

(**A**) Mutations in the discovery cohort. Patients are ordered by response category, with tumor mutation burden in decreasing order within each response category. Shown are the genes that were recurrently mutated at a significant frequency, as assessed by MutSig2CV analysis (table S1E). CNA = copy number alteration. (**B**) Enrichment of truncating mutations in tumors from patients in the CB vs. NCB groups. Red dashed line denotes q<0.1 (Fisher's exact test). Mutations in genes above the black dotted line are enriched in tumors of patients with CB from anti-PD-1 therapy and mutations in genes below the line are enriched in tumors of patients with NCB. (**C**) Kaplan-Meier curve comparing overall survival of patients treated with anti-PD-1 therapy whose tumors did or did not harbor LOF mutations in *PBRM1.* See also fig. S5 for Kaplan-Meier curve comparing progression-free survival of these patients. (**D**) Spider plot showing objective decrease in tumor burden in *PBRM1*-LOF (blue) vs. *PBRM1*-intact (yellow) tumors. Three patients with early progression on anti-PD-1 therapy and truncating mutations in *PBRM1* (dark blue) had long and/or censored OS.

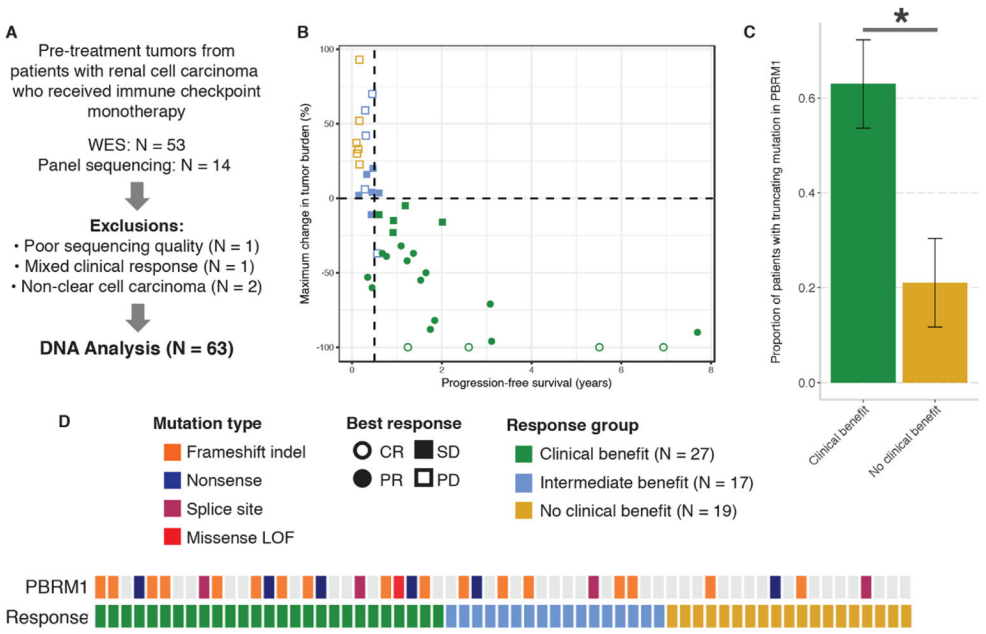

Fig. 3. *PBRM1* LOF mutations correlate with clinical benefit in a validation cohort of ccRCC patients treated with immune checkpoint inhibitors

(**A**) Selection of the validation cohort. (**B**) Clinical outcomes in the validation cohort. Ten patients without post-treatment re-staging scans (eight with clinical PD, two with SD, and one with PR) as well as 14 patients with targeted panel sequencing are not shown. (**C**) Proportion of tumors harboring *PBRM1* LOF mutations in patients in the CB vs. NCB groups. Error bars are S.E. *Fisher's exact $p<0.05$. (**D**) Truncating alterations in *PBRM1* and response to anti-PD-(L)1 therapies by sample. Colored boxes indicate samples with truncating mutations in *PBRM1* while gray denotes samples without *PBRM1* truncating mutations. Missense LOF denotes a missense mutation detected by targeted sequencing that was confirmed to be LOF by PBRM1 immunohistochemistry (see Supplemental Methods).

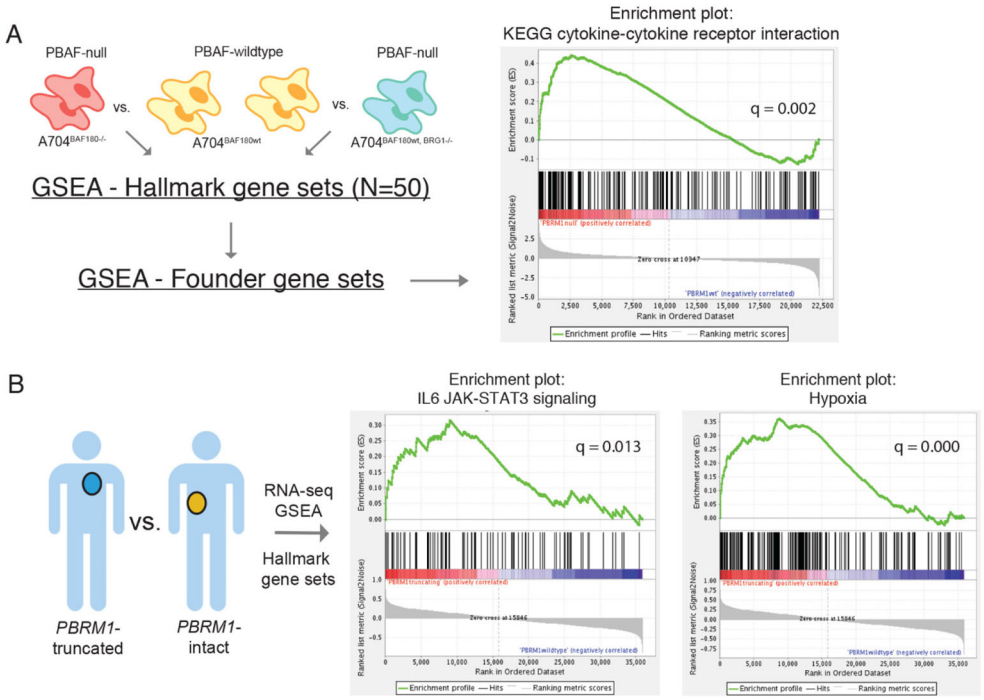

Fig. 4. *PBRM1* mutational status in ccRCC influences immune gene expression

(**A**) GSEA was performed on PBAF-deficient ($A704^{BAF180-/-}$ and $A704^{BAF180wt,\ BRG1-/-}$) vs. PBAF-proficient ($A704^{BAF180wt}$) kidney cancer cell lines using both Hallmark and corresponding Founder gene sets. GSEA enrichment plot shown for the KEGG cytokine-cytokine receptor interaction gene set in $A704^{BAF180-/-}$ vs. $A704^{BAF180wt}$ (*PBRM1* null vs. wildtype). Enrichment plot is similar for $A704^{BAF180wt,\ BRG1-/-}$ vs. $A704^{BAF180wt}$ (*BRG1* null vs. wildtype); see table S4. (**B**) GSEA was also performed on RNA-seq from pre-treatment tumors in the discovery and validation cohorts of this study (n = 18 *PBRM1*-LOF vs. n = 14 *PBRM1*-intact) using the Hallmark gene sets. Enrichment plots show increased expression of the hypoxia and IL6/JAK-STAT3 gene sets in the *PBRM1*-LOF tumors.