Mammalian SWI/SNF Complex Genomic Alterations and Immune Checkpoint Blockade in Solid Tumors

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ABSTRACT

Prior data have variably implicated the inactivation of the mammalian SWItch/Sucrose Non-Fermentable (mSWI/SNF) complex with increased tumor sensitivity to immune checkpoint inhibitors (ICI). Herein, we examined the association between mSWI/SNF variants and clinical outcomes to ICIs. We correlated somatic loss-of-function (LOF) variants in a predefined set of mSWI/SNF genes (*ARID1A, ARID1B, SMARCA4, SMARCB1, PBRM1*, and *ARID2*) with clinical outcomes in patients with cancer treated with systemic ICIs. We identified 676 patients from Dana-Farber Cancer Institute (DFCI, Boston, MA) and 848 patients from a publicly available database from Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY) who met the inclusion criteria. Multivariable analyses were conducted and adjusted for available baseline factors and tumor mutational burden. Median follow-up was 19.6 (17.6–22.0) months and 28.0 (25.0–29.0) months for the DFCI and MSKCC cohorts, respectively. Seven solid tumor subtypes were examined. In the DFCI cohort, LOF variants of mSWI/SNF did not predict improved overall survival (OS), time-to-treatment failure (TTF), or disease control rate. Only patients with renal cell carcinoma with mSWI/SNF LOF showed significantly improved OS and TTF with adjusted HRs (95% confidence interval) of 0.33 (0.16–0.7) and 0.49 (0.27–0.88), respectively, and this was mostly driven by *PRBM1*. In the MSKCC cohort, where only OS was captured, LOF mSWI/SNF did not correlate with improved outcomes across any tumor subtype. We did not find a consistent association between mSWI/SNF LOF variants and improved clinical outcomes to ICIs, suggesting that mSWI/SNF variants should not be considered as biomarkers of response to ICIs.

Introduction

Since their introduction into clinical practice, immune checkpoint inhibitors (ICI), namely anti–PD-1/PD-L1 and anti–CTLA-4, have proven to be an effective antineoplastic drug class across several cancer subtypes (1–15). Despite the durable responses achieved with ICIs, the majority of patients do not respond, and universal determinants of clinical benefit are still lacking (16, 17). Therefore, there remains an unmet need to develop predictive biomarkers essential to improve patient benefit, reduce the risk of toxicity, and advance combination strategies.

Over the past few years, evidence from both preclinical and clinical settings has implicated the mammalian SWItch/Sucrose Non-Fermentable (mSWI/SNF) complex as one of the players impacting response to ICIs. The mSWI/SNF complex is one of the key factors regulating gene expression and thus plays an important role in cell division, cell differentiation, and DNA replication (18, 19). This complex exists in three large macromolecular complexes: BRG1/BRM–associated factor (BAF), polybromo-associated BAF (PBAF), and noncanonical BAF (ncBAF) complexes, which are collectively composed of the protein products of 29 genes (19). Whole-exome sequencing efforts have shown that 20% of human cancers harbor mutations in at least one the 29 genes involved in this complex (20). Notably, a wide range of mutation frequencies exists among the different mSWI/SNF genes, with significant variation across different cancer subtypes. These mutational patterns may be partially explained by the cell type–specific expression and function of the various subunits across cancers (20, 21). For example, *SMARCA4* genomic alterations (GA) are relatively common in non–small cell lung cancers (NSCLC, 11%; refs. 22, 23), *ARID1A* in ovarian clear-cell carcinomas (∼50%; ref. 24) and urothelial carcinoma (29%; ref. 25), *PBRM1* in renal cell carcinoma (RCC, 40%; ref. 26), and *ARID2* in melanoma (7%; ref. 27).

Preclinical evidence has associated mSWI/SNF inactivation with increased $CD8^+$ T-cell infiltration, enhanced sensitivity to

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T-cell–mediated cytotoxicity, and improved tumor response to ICIs in some cancer types (28, 29). A study has shown that *ARID1A*-deficient tumors in syngeneic mice demonstrate significantly increased tumor-infiltrating lymphocytes and improved response to ICIs compared with controls (29). Interestingly, analysis of The Cancer Genome Atlas (TCGA) data has linked decreased expression of multiple mSWI/SNF genes (*SMARCA4, PBRM1, ARID1A, ARID2*) with increased mRNA levels of stimulated 3′ antisense retroviral coding sequences (SPARCS), a subclass of endogenous retroviruses that triggers innate immunity (30).

Clinically, several studies have assessed genetic correlates of response to checkpoint blockade, with a focus on the mSWI/SNF complex (Supplementary Table S1; refs. 31–41). A salient feature of these studies is the variability of evidence in relation to whether loss of the mSWI/SNF correlates with clinical benefit in patients treated with ICIs across different lines of ICI therapy. For example, in RCC, loss-of-function (LOF) mutations in *PBRM1* in the post tyrosine-kinase inhibitor (TKI) setting associates with clinical benefit to the single-agent PD-1 inhibitor, nivolumab (40). This was later validated in a more comprehensive analysis of a randomized controlled trial of patients with RCC treated with nivolumab versus everolimus (32). In contrast, two other clinical trials in the frontline RCC setting did not find a significant association between *PBRM1* alterations and improved clinical outcomes in patients treated with either PD-L1–based combination therapies or atezolizumab alone (8, 33, 39).

Nearly all prior studies on the mSWI/SNF complex have focused on individual tumor types without studying pan-cancer relationships (40). These studies were also very heterogenous overall in their assessments, including the examined tumor histologies, the considered line of ICI therapy, the reported patient outcomes, as well as the assessed mSWI/SNF GAs. Herein, in two independent cohorts, we systematically assessed the association between LOF variants in mSWI/SNF subunits and outcomes of patients with cancer treated with ICIs, using extensive clinical phenotyping and rigorous statistical analyses across multiple cancer types.

Materials and Methods

Study design and patient cohorts

We tested our hypothesis in two independent cohorts: Dana-Farber Cancer Institute (DFCI, Boston MA) cohort of 676 patients and an external cohort from Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY) of 848 patients (42). We included patients with solid tumor histologies, where ICIs were FDA approved for therapy, who received at least one dose of an anti–PD-1/PD-L1 or anti–CTLA-4 agent in the metastatic setting, and who had next-generation targeted sequencing (NGS) of their tumor tissue performed (as described below). Included tumor histologies were melanoma, NSCLC, RCC, urothelial carcinoma, colorectal adenocarcinoma, esophagogastric adenocarcinoma (EGC), head and neck squamous cell carcinoma (HNSCC), cancer of unknown primary (CUP), and small-cell lung cancer (SCLC). The number of patients per each tumor histology is detailed in **Table 1**. Patients were excluded if they were lost to follow-up, had no measurable disease, or had clinical deterioration within one week of the first ICI dose. Tumors with missense mutations were excluded from the analysis, as we could not confidently assess functional outcomes of these mutations. The patient studies were conducted in accordance with the ethical guidelines of the Declaration of Helsinki. This study was performed after approval by the Institutional Review Board (IRB) of DFCI (Boston, MA), and informed written consent was obtained from each subject or each subject's guardian. The MSKCC data were de-identified and publicly available. For the DFCI cohort, tissue collected encompassed primary and metastatic tumors from core biopsies and/or surgical resections. In addition to tumor tissue, patients in the MSKCC cohort also had matched normal or blood collected. Tissue specimens were collected

Table 1. Baseline clinical characteristics of the overall population.

	DFCI cohort		MSKCC cohort	
	***N* (median)**	**% (range)**	***N* (median)**	**% (range)**
Age at ICI	64	21–90	NA	NA
Tumor type				
Colorectal adenocarcinoma	35	5.2	63	7.4
EGC	66	9.8	59	7.0
HNSCC	31	4.6	68	8.0
Melanoma	86	12.7	192	22.6
Non–small cell lung carcinoma	334	49.4	255	30.1
RCC	68	10.1	118	13.9
Urothelial carcinoma	56	8.3	93	11.0
Site of specimen sequenced				
Metastatic	347	51.3	NA	NA
Primary	329	48.7	NA	NA
ICI type				
Combination	212	31.4	152	17.9
Single	464	68.6	696	82.1
ICI class				
Anti–PD-1/PD-L1	589	87.1	636	75.0
Anti–CTLA-4	12	1.8	60	7.1
Anti–PD-1/PD-L1 + anti–CTLA-4	75	11.1	152	17.9
Number of prior lines				
0	329	48.7	NA	NA
1	220	32.5	NA	NA
≥2	127	18.7	NA	NA

between 2009–2018 for DFCI and between 2013–2017 for MSKCC and were stored as formalin-fixed paraffin-embedded tissue. DNA was extracted from blood after collection and was stored at −20°C if not proceeding directly to the library preparation.

Data collection

The clinical variables assessed included gender, primary tumor site, age at initiation of ICI therapy, type and class of ICI-based regimen for both cohorts. Additional clinical variables available for the DFCI cohort included lines of therapy prior to ICIs and site of lesion subjected to targeted sequencing. Patients were excluded if they were lost to follow-up, had no measurable disease, or had clinical deterioration within one week of the first ICI dose. Microsatellite instability (MSI) analysis for the DFCI colorectal adenocarcinoma cohort was determined using IHC.

Genomic analysis

Details of the tissue collection, DNA extraction, and tumor targeted sequencing using the Oncopanel/PROFILE and MSK-IMPACT for DFCI and MSKCC cohorts, respectively, were described previously in detail (panel of genes assessed in Supplementary Table S2; refs. 43–47). Briefly, for the DFCI cohort, core biopsy and/or surgical resection specimens were reviewed by Brigham and Women's Hospital (BWH, Boston, MA) board-certified pathologists to confirm the diagnosis, histologic subtype, tumor grade, and stage. Tumor regions consisting of at least 20% tumor cells were macrodissected from unstained slides, and DNA was isolated using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's instructions. DNA quantification was performed by Nanodrop and Pico-Green assays. Targeted gene sequencing was performed using an institutional analytic platform, Oncopanel, that is certified for clinical use and patient reporting under the Clinical Laboratory Improvement Amendments (CLIA) Act. A total of 200 ng genomic DNA from each sample was subject to targeted exon capture and sequencing using one of two versions of the Oncopanel assay (V2-V3) developed at BWH (Boston, MA). The Oncopanel gene panel includes capture probes for 275–447 cancer-associated genes, as well as intronic portions of 60 genes for rearrangement detection (44). Targeted capture was performed using a solution-phase Agilent SureSelect hybrid capture kit and custom bait-sets. Sequencing libraries were prepared from captured DNA, as described in detail elsewhere (44). Paired-end sequencing was performed on an Illumina HiSeq 2500. Reads were demultiplexed using Picard tools (http://picard.sourceforge.net) and aligned to human reference genome b37 using the Burrows-Wheeler Aligner (48; http://bio-bwa.sourceforge.net/bwa.shtml).

For the MSKCC cohort, a hematoxylin and eosin (H&E)-stained slide was reviewed by a molecular pathology fellow and annotated for relevant specimen information including tumor type, tumor purity, and whether macrodissection of the indicated tumor region was necessary prior to nucleic extraction. Genomic DNA extraction was performed on the Chemagic STAR instrument (Hamilton) from formalin-fixed, paraffin-embedded (FFPE) tumors and patient-matched normal blood using the Chemagen Magnetic Bead Technology (PerkinElmer). DNA samples were normalized to yield 50–250 ng input and diluted in 55 μL on the Biomek FXP Laboratory Automation Workstation (Beckman Coulter) prior to shearing on the Covaris instrument (46). Sequencing libraries were prepared using the KAPA HTP Protocol (KapaBiosystems) and the Biomek FX System (Beckman Coulter) through several enzymatic steps, including end repair, A-base addition, ligation of Illumina sequence adaptors, followed by PCR amplification and clean-up. Tumors and matched normal were combined in pools of 24–36 libraries for multiplexed captures using custom-designed biotinylated probes (Nimblegen). Captured DNA fragments were sequenced on an Illumina HiSeq2500 as paired-end 100-base pair reads. Reads were demultiplexed using BCL2FASTQ version 1.8.3 (Illumina) and aligned to human reference genome b37 using the Burrows-Wheeler Aligner (48; http://bio-bwa.sourceforge.net/bwa.shtml).

For both cohorts, low-quality reads and duplicates were filtered and eliminated using Picard. We focused our mutational and copy number variation (CNV) analyses on the six genes most commonly altered (reported in at least 5% or more in a tumor type) within the mSWI/SNF complex: *PBRM1, ARID2, ARID1A, ARID1B, SMARCA4*, or *SMARCB1* (20). Tumor mutation burden (TMB) was defined as the number of exonic, nonsynonymous base substitutions, and indel mutations per megabase of genome examined.

Variant assessment

For the DFCI cohort, as the Oncopanel analysis was performed on tumor samples only without germline analysis, we excluded variants that were observed at a frequency ≥0.1% in the Exome Aggregation Consortium (ExAC) database (49), as they were considered likely germline variants (50). For the MSKCC cohort, germline variants were eliminated through the use of patient-matched blood DNA (46). Single-nucleotide variants (SNV) and small insertions/deletions (indels) were analyzed using MuTect v.1 0.27200 and annotated using Oncotator. LOF variants were defined as nonsense mutations, frameshift insertions or deletions, splice-site variants affecting consensus nucleotides, or homozygous deletions. Tumors with missense mutations were excluded from the analysis as we could not confidently assess functional outcomes of these mutations, consistent with prior studies of NGS (32, 51, 52). CNVs were identified using a custom R-based tool (VisCap-Cancer; ref. 53) that compares read-depth at all genomic regions assayed among different samples. For both cohorts, we focused on homozygous deletion CNVs in this analysis, and we excluded heterozygous deletions, as the latter are associated with background noise in the Oncopanel and MSK-IMPACT sequencing platforms (44, 53).

Statistical analysis

The clinical outcomes included overall survival (OS) for both cohorts. For the DFCI cohort, more clinical granularity in outcomes was obtained, including time-to-treatment failure (TTF), overall response rate (ORR), and disease control rate (DCR). OS was calculated from the date of ICI initiation to the date of death. Alive patients were censored at the date of last follow-up. TTF was calculated from the start date of ICI therapy to the start date of the next treatment or death. Patients alive and not started on next line were censored at the date of last follow-up. Response was investigator-assessed. ORR was defined as complete response (CR) or partial response (PR). DCR was defined as CR, PR, or stable disease for more than 8 weeks.

The association between GAs and other patient and disease characteristics was evaluated using the Fisher exact test for categorical variables and Kruskal–Wallis test for continuous variables. The distributions of OS and TTF were estimated with the Kaplan–Meier method along with 95% confidence intervals (95% CI), and their associations with GAs were examined with the Wald χ^2 test from the Cox regression, adjusted for lines of therapy prior to ICI for the DFCI cohort, and type of ICI (single vs. combination) for the MSKCC cohort. For cohorts with significant associations, we further adjusted for

TMB as a continuous variable when possible. The effects of GAs on ORR and DCR were presented as ORs estimated from logistic regression models, adjusted for prior lines of therapy and TMB in the DFCI cohort. All comparisons were conducted separately for each tumor histology. No multiple comparison adjustment was made given the exploratory nature. Statistical analyses were performed using SAS version 9.4 (SAS Institute). Two-sided *P* values are reported.

Results

Study population and patient characteristics

Of 17,046 patients with tumor sequencing at Dana-Farber Cancer Institute (Boston, MA), we identified 676 patients who received at least one dose of ICI in the metastatic setting between June 2013 and January 2019 and met the eligibility criteria for this study (Materials and Methods; Supplementary Fig. S1; Supplementary Table S3). We excluded two tumor types (SCLC and CUP) from the analysis because of the small sample size ($N = 2$ and $N = 6$, respectively). From the MSKCC cohort, we identified 848 patients who fit the inclusion criteria of this study across the seven included tumor types (Supplementary Table S4).

Median follow-up was 19.6 months (95% CI: 17.6–22.0) and 28.0 months (95% CI: 25.0–29.0) for the DFCI and MSKCC cohorts, respectively (Supplementary Table S5). The most prevalent tumors in the two cohorts, respectively, were NSCLC (DFCI: $N = 334$, 49.4%; MSKCC: $N = 255$, 30.1%), melanoma (DFCI: $N = 86$, 12.7%; MSKCC: $N = 192$, 22.6%), and RCC (DFCI: $N = 68$, 10.1%; MSKCC: $N = 118$, 13.9%). Median age at first dose of ICI was 64 (range: 21–90) years for the DFCI cohort. The most common age group in the MSKCC cohort was 61–70 years ($N = 269$; 31.7%). The majority of the cohort received anti–PD-1– or anti–PD-L1–based therapy (DFCI: $N = 589$, 87.1%; MSKCC: $N = 636$, 75.0%), most commonly as single agents (DFCI: $N = 464$, 68.6%; MSKCC: $N = 696$, 82.1%; **Table 1**; Supplementary Tables S3 and S4).

Spectrum of GAs within the mSWI/SNF complex

The most commonly altered genes in the DFCI and MSKCC cohorts (pan-cancer), respectively, were *ARID1A* (DFCI: $N = 89$, 13%; MSKCC: $N = 77$, 9%) and *PBRM1* (DFCI: $N = 70$, 10%; MSKCC: $N = 59$, 7%; **Fig. 1A**; Supplementary Tables S6–S10). Among the different tumor subtypes, RCC tumors harbored the highest rate of any LOF mSWI/SNF (DFCI: $N = 38$, 56%; MSKCC: $N = 48$, 41%). BAF complex, defined as *ARID1A* or *ARID1B*, LOF variants were more prevalent in colorectal adenocarcinoma (DFCI: $N = 16$, 46%; MSKCC: $N = 13$, 21%) and urothelial carcinoma (DFCI: $N = 22$, 39%; MSKCC: $N = 23$, 25%), whereas PBAF complex, defined as *PBRM1* or *ARID2*,

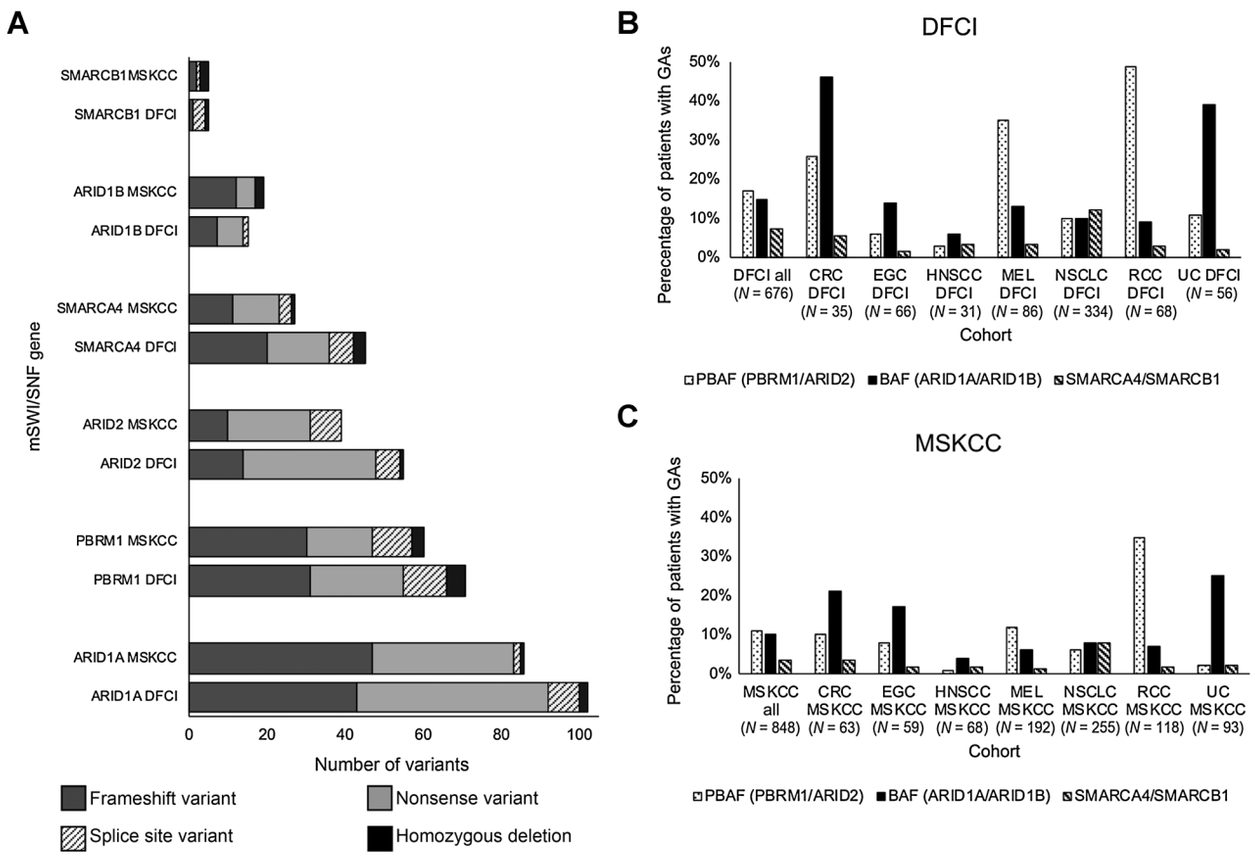

Figure 1.

Spectrum of GAs in the mSWI/SNF complex across tumor histologies. **A,** Frequency and variant types detected across the 6 mSWI/SNF complex genes in the DFCI ($N = 676$) and MSKCC ($N = 848$) cohorts. Number of patients per each gene is indicated. **B,** Frequency of detected mSWI/SNF subcomplex mutations across tumor histologies in the DFCI cohort. Number of patients for each tumor type indicated. **C,** Frequency of detected mSWI/SNF subcomplex mutations across tumor histologies in the MSKCC cohort. Number of patients for each tumor type indicated. CRC, colorectal cancer; MEL, melanoma; UC, urothelial carcinoma.

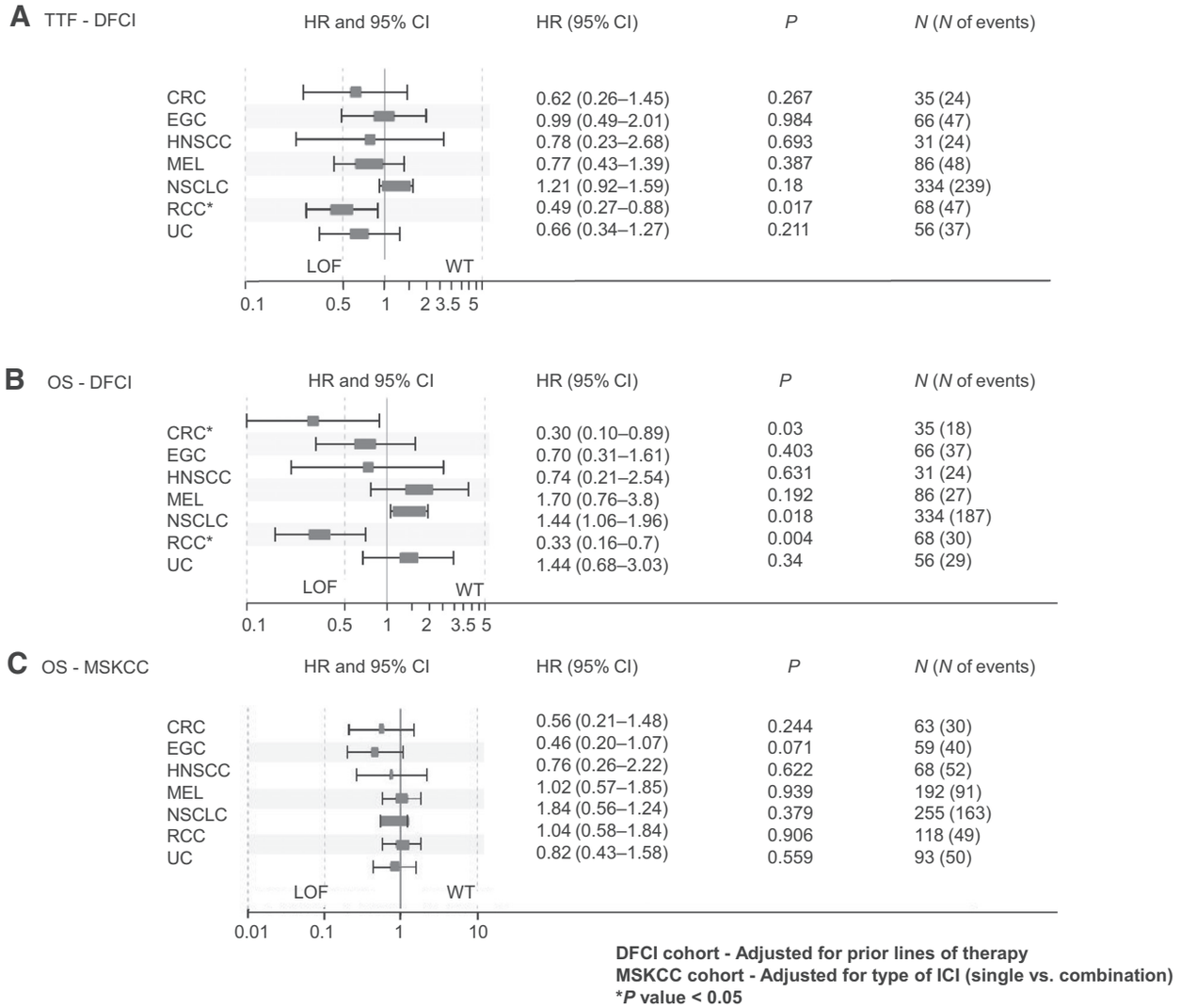

Figure 2.

Analysis of survival outcomes in patients with LOF mSWI/SNF and WT across different tumor histologies. **A,** Adjusted HR for TTF in the DFCI cohort ($N = 676$). **B,** Adjusted HRs for OS in the DFCI cohort. **C,** Adjusted HRs for OS in the MSKCC cohort ($N = 848$). χ^2 Wald statistic. Cohort adjustments indicated. Error bars, SD. CRC, colorectal cancer; MEL, melanoma; UC, urothelial carcinoma.

LOF variants were most abundant in RCC (DFCI: $N = 33$, 49%; MSKCC: $N = 41$, 35%; **Fig. 1B** and **C**; Supplementary Tables S6–S10). *PBRM1* was the predominantly altered PBAF complex gene in RCC ($N = 33/33$ and $N = 39/41$ in the DFCI and MSKCC cohorts, respectively). Among the seven histologies examined, all tumors, with the exception of HNSCC and EGC, harboring LOF variants within the mSWI/SNF complex had significantly higher TMB compared with wild-type (WT) in both cohorts ($P < 0.05$; Supplementary Fig. S2; Supplementary Table S11).

Treatment outcomes of patients with LOF GAs in mSWI/SNF genes

Multivariable analysis of the DFCI cohort showed significantly improved OS and DCR in patients with colorectal adenocarcinoma and RCC whose tumors carried LOF variants in the mSWI/SNF complex. Conversely, patients with NSCLC showed worse OS in the LOF group (**Figs. 2–3**; Supplementary Fig. S3; **Table 2**). OS and TTF outcomes for the overall DFCI cohort across histologies are summarized in Supplementary Table S5.

To explore whether the observed associations were driven by individual members of the mSWI/SNF complex, we analyzed clinical outcomes associated with GAs in the PBAF and BAF complexes separately (Supplementary Figs. S4 and S5; Supplementary Tables S12 and S13). In the colorectal adenocarcinoma DFCI cohort, the adjusted OS HR was 0.30 (0.10–0.89), favoring LOF GAs in mSWI/SNF genes ($P = 0.03$). In terms of response, significantly higher ORR and DCR were associated with the presence of a LOF variant in any of the mSWI/SNF genes (ORR: LOF = 6/18, 33% vs. WT = 0/17,

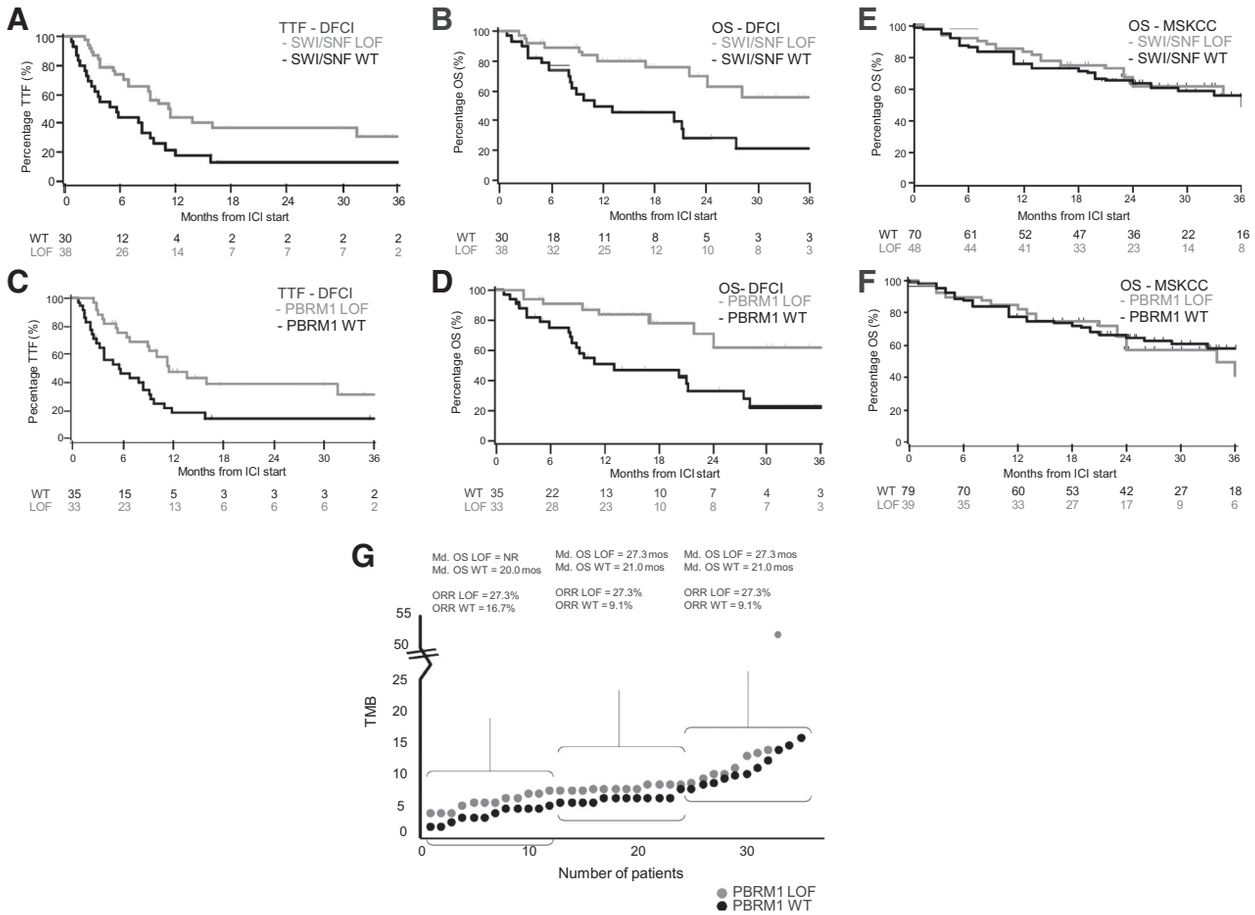

Figure 3.

Clinical outcomes in the RCC cohort. **A,** TTF in patients with LOF mSWI/SNF complex and WT genes in the DFCI cohort. **B,** OS in patients with LOF mSWI/SNF complex and WT genes in the DFCI cohort. **C,** TTF in patients with LOF and WT *PBRM1* in the DFCI cohort. **D,** OS in patients with LOF and WT *PBRM1* in the DFCI cohort. **E,** OS in patients with LOF mSWI/SNF complex and WT genes in the MSKCC cohort. **F,** OS in patients with LOF and WT *PBRM1* in the MSKCC cohort. **G,** OS and ORR across TMB tertiles in *PBRM1* LOF versus WT genes in the DFCI cohort. Md., median; NR, not reached; mos, months. Number of patients indicated below each graph (**A–F**). OS and TTF estimated with the Kaplan–Meier method along with 95% CI. χ^2 Wald statistic. *P*-value threshold for significance was 0.05.

0%; DCR: LOF = 11/18, 61% vs. WT = 4/17, 24%; $P = 0.019$ and 0.041, respectively). We observed similar trends for OS, TTF, ORR, and DCR for both PBAF and BAF complexes separately (Supplementary Figs. S4–S6; Supplementary Tables S12 and S13). TMB was significantly higher in the mSWI/SNF LOF cohort compared with the WT cohort, but the small sample size of the colorectal adenocarcinoma cohort precluded TMB from being included into the multivariable analysis. A positive correlation was observed between MSI-status and mutational status of the mSWI/SNF genes in the colorectal adenocarcinoma cohort. Of 18 patients with LOF mSWI/SNF, 16 (88.9%) had MSI-high tumors versus only 1 of 15 (6.7%) patients within the WT group (Supplementary Table S14).

Among patients with RCC in the DFCI cohort, the mutant cohort showed significantly improved TTF and OS, with adjusted HRs of 0.49 (0.27–0.88) and 0.33 (0.16–0.7), respectively, compared with the WT cohort (**Fig. 2**). Median TTF for the mutant cohort was 11.3 (6.9–31.6) months, and the OS was not reached (22 months–NR) compared with median TTF of 5.6 (2.6–9.2) months and median OS of 10.9 (8–21.4) months for the WT cohort (**Fig. 3A** and **B**). A significantly higher DCR was observed for patients with LOF variants in the RCC cohort (LOF = 27/38, 71% vs. WT = 12/30, 40%, $P = 0.014$) compared with patients with WT variants (**Table 2**). After further adjusting for TMB, the OS benefit was maintained [adjusted HR 0.41 (0.18–0.91), $P = 0.029$], and the adjusted HR for TTF was 0.70 (0.37–1.33, $P = 0.278$). The adjusted ORs for DCR and ORR was 2.86 (0.91–8.99) and 1.81 (0.49–6.64), respectively ($P = 0.073$, 0.371), after adjusting for TMB and prior lines of therapy.

To further explore these associations, we analyzed *PBRM1* LOF variants and clinical outcomes in RCC, given that *PBRM1* is the most frequently altered mSWI/SNF gene in RCC (33/38; **Fig. 3C** and **D**; Supplementary Table S10). Median TTF for *PBRM1* mutant and WT cohorts were 11.5 (6.9–NR) months and 5.6 (2.9–8.4), respectively. Median OS for *PBRM1* mutant was not reached (22–NR) compared with a median OS of 13.1 (8.3–27.5) months for the WT. We observed similar associations in terms of TTF, OS, and DCR for the mutant cohort compared with the WT cohort, as well after correcting for TMB as a continuous variable (Supplementary Table S15). We then compared OS and ORR in *PBRM1* LOF vs. WT across tertiles of TMB (**Fig. 3G**). It was consistent that *PBRM1* LOF was associated with better clinical outcomes compared with WT. However, meaningful statistical

Table 2. Antitumor activity in the LOF and WT mSWI/SNF cohorts.

A: ORR in the LOF and WT mSWI/SNF cohorts

	WT			LOF mSWI/SNF			
	Total	**N**	**ORR (%)**	**Total**	**N**	**ORR (%)**	***P***
Colorectal adenocarcinoma[a]	**17**	**0**	**0%**	**18**	**6**	**33.0%**	**0.019**
EGC	53	9	17%	13	4	31.0%	0.267
HNSCC	27	3	11%	4	2	50.0%	0.112
Melanoma	47	17	36%	39	13	33.0%	0.824
Non–small cell lung carcinoma	230	60	26%	104	22	21.0%	0.41
RCC	30	5	17%	38	12	32.0%	0.259
Urothelial carcinoma	27	6	22%	29	7	24.0%	0.99

B: DCR in the LOF and WT mSWI/SNF cohorts

	WT			LOF mSWI/SNF			
	Total	***N***	**DCR (%)**	**Total**	***N***	**DCR (%)**	***P***
Colorectal adenocarcinoma[a]	**17**	**4**	**24%**	**18**	**11**	**61%**	**0.0409**
EGC	53	16	30%	13	7	54%	0.1921
HNSCC	27	6	22%	4	2	50%	0.2683
Melanoma	47	26	55%	39	24	62%	0.6619
Non–small cell lung carcinoma	230	126	55%	104	52	50%	0.4775
RCC[a]	**30**	**12**	**40%**	**38**	**27**	**71%**	**0.0139**
Urothelial carcinoma	27	10	37%	29	13	45%	0.5965

Note: Bold font signifies $P < 0.05$.
[a]$P < 0.05$.

comparisons could not be performed given the small sample size in each group. We obtained mirroring results when the analysis was restricted to patients with metastatic RCC treated with single-agent ICIs at DFCI ($N = 28$), supporting the notion that *PBRM1* LOF results in improved outcomes, but multivariate analysis could not be performed given the small cohort size (Supplementary Table S16). In contrast, patients in the NSCLC DFCI cohort with LOF variants within the mSWI/SNF showed significantly worse OS compared with WT adjusted HR = 1.44 (1.06–1.96), with no significant difference in TTF [adjusted HR of 1.21 (0.92–1.59)] or ORR. After adjusting further for TMB, mSWI/SNF LOF tumor variants portended worse OS and TTF in the NSCLC cohort [adjusted HRs of 1.57 (1.13–2.17) and 1.38 (1.03–1.85), respectively] (Supplementary Table S17). Patients in the DFCI melanoma, HNSCC, EGC, and urothelial carcinoma cohorts did not show any association between mutational status of the mSWI/SNF complex or the individual subcomplexes PBAF and BAF, and either OS, TTF, or ORR (**Fig. 3**; Supplementary Figs. S3–S5).

In the MSKCC cohort ($N = 848$ patients), we did not detect any significant associations across all tumor subtypes between LOF variants in the mSWI/SNF genes explored and OS after adjusting for the type of ICI administered (**Fig. 3**; Supplementary Fig. S7). For this analysis, we could not adjust for other clinical variables, such as lines of therapy prior to ICIs because these data were not available. Similarly, TTF and response data were not available in the MSKCC cohort.

Discussion

In this study, we evaluated the effect of GAs of mSWI/SNF complex genes in patients treated with ICIs. Analysis of 676 and 848 solid tumors from patients treated with ICIs at DFCI and MSKCC, respectively, did not support the notion that loss of the mSWI/SNF complex is a pan-cancer biomarker of clinical benefit from ICIs.

Findings from the colorectal adenocarcinoma cohort at DFCI highlight an association between LOF mSWI/SNF complex genes and MSI status, where 88.9% of patients with LOF mSWI/SNF had MSI-high tumors by IHC. This is in line with previous literature correlating loss of *ARID1A* with mismatch-repair deficiency in endometrial, ovarian, and colorectal carcinomas (29, 54, 55). However, this creates uncertainty on whether the observed benefit is driven by the MSI status or by loss of the mSWI/SNF complex genes because it is well established in the literature that mismatch-repair deficiency increases sensitivity to immune checkpoint blockers (56, 57). An OS benefit in mSWI/SNF LOF variants was not observed in the MSKCC cohort, challenging the validity of this association.

In the DFCI RCC cohort, a consistent OS, TTF, and DCR benefit was observed in the mSWI/SNF LOF compared with WT at DFCI. The OS benefit was maintained after correcting for TMB, and these associations were mostly driven by *PBRM1* LOF variants. Conversely, no association was observed between mSWI/SNF LOF and OS benefit in the MSKCC cohort. This further adds to the variability in published literature. Some studies have suggested that patients with RCC may benefit from ICIs when their tumors carry LOF mSWI/SNF complex variants because this creates an immune-responsive milieu, with increased expression of immune-stimulatory gene sets related to IL6-JAK-STAT3 signaling and TNFα signaling via NF-κB, increased sensitization to T-cell–mediated killing, and enhanced accessibility to IFNγ-inducible genes (28, 40, 41). In contrast, others have reported that *PBRM1* loss reduces IFNγ-STAT1 activity and promotes resistance to immunotherapy in RCC (58). One of the possible causes of these variable results in our study may be related to the different patient populations or to the different factors taken into consideration in the two independent cohorts. We corrected for prior lines of therapy in the DFCI cohort and for type of ICI in the MSKCC cohort. Another explanation could be due to the fragility of the correlation between

PBRM1 LOF and clinical benefit to immunotherapy, which can be explored through prospective clinical trials (59). Finally, the evidence supporting *PBRM1* LOF as a prognostic biomarker of benefit from ICIs is derived from patients receiving ICIs in the post-VEGF-TKI–targeted therapy setting (32, 40). Given that data on prior lines of therapy from the MSKCC cohort were not available, this may partially explain the variable results.

Although no significant associations were demonstrated between mSWI/SNF complex LOF and TTF or ORR in the NSCLC cohort, these patients showed an inferior OS and TTF compared with patients whose tumors carried the WT mSWI/SNF genes in the DFCI cohort. Consistent with published data, *SMARCA4* was the most frequently mutated member of the mSWI/SNF complex in NSCLC at a rate of 12% (22). *SMARCA4*-deficient lung adenocarcinomas are associated with poor prognosis and response to platinum-based therapies, not ICIs (60, 61). This may explain the worse outcomes of these patients. No significant association was observed between mSWI/SNF complex mutations and OS in the MSKCC cohort, which may be attributed to the different patient populations.

The question of correlating GAs with clinical outcomes is crucial to address in the field of immunotherapy, given the variable response rates with ICIs and the substantial toxicity these agents may have. Although this study did not provide a pan-cancer biomarker predicting response to checkpoint inhibitors, it provides evidence that LOF of the mSWI/SNF complex in patients receiving an ICI-based therapy may not be a key player driving the response. Despite previous promising results in individual cancer cohorts (32, 37, 40, 41), in our study, LOF variants of the mSWI/SNF complex genes were not associated with improved clinical outcomes in patients with solid cancer treated with ICIs.

Our study has several limitations, given that it is retrospective in nature. First, this is a relatively select cohort of patients from two tertiary cancer centers. Second, we used targeted-sequencing panels that did not assess all 29 members of the mSWI/SNF complex. However, we did assess the most frequently mutated genes within this complex (20, 21). Third, we only evaluated two genotypes (LOF and WT), while excluding missense mutations and heterozygous deletions from our analysis. Nonetheless, we deemed that this type of analysis was necessary to answer our hypothesis because we could not assess with confidence the true functional impact of these GAs. Fourth, this study did not investigate the possibility that driver mutations in other genes may influence clinical benefit to ICIs. For example, alterations in *TP53, MYC, BRAF, EGFR*, and *HER2* have previously been shown to impact clinical outcomes in patients treated with ICIs (62–66). This could be addressed in future studies by investigating how these genes interact with the mSWI/SNF complex in neural networks and impact outcomes to ICIs (67). Finally, we were unable to determine with certainty whether variants were somatic versus germline in the DFCI cohort, but we attempted to correct for that by excluding variants that were observed at a frequency >0.1% in the Exome Aggregation Consortium (ExAC) database.

In conclusion, this work provides a step forward in understanding complex and multivariable mechanisms driving tumor response to therapy, while validating and challenging simultaneously previously reported correlations from smaller studies on the association of mSWI/SNF GAs and clinical benefit from ICIs. It also highlights the intricacy of the mSWI/SNF complex and its disease-specific function, advocating for further efforts to discern the biology of mSWI/SNF complex and its interaction with immune checkpoint blockade.

Disclosure of Potential Conflicts of Interest

Z. Bakouny reports receiving a commercial research grant from Genentech/imCORE and other commercial research support from Bristol-Myers Squibb. D.A. Braun is a consultant/advisory board member for Schlesinger Advisor, Octane Global, Defined Health, Dedham Group, Adept Field Solutions, Slingshot Insights, Blueprint Partnerships, Charles River Associates, Trinity Group, Insight Strategy, and Bristol-Myers Squibb and reports receiving other remuneration from LM Education/Exchange Services. S.A. Shukla is a consultant/advisory board member for Neon Therapeutics and Bristol-Myers Squibb, and has ownership interest (including patents) in Agenus Inc., Agios Pharmaceuticals, BreakBio Corp., Bristol-Myers Squibb, and NewLink Genetics. A.D. Cherniack has an immediate family member who is an employee of LabCorp, reports receiving a commercial research grant from Bayer, and has ownership interest (including patents) in Merck. G. Sonpavde is a consultant for EMD Serono, Merck, Bristol-Myers Squibb, AstraZeneca, and Genentech; reports receiving a commercial research grant from AstraZeneca; and reports receiving other remuneration from Bristol-Myers Squibb and AstraZeneca. R.I. Haddad is a consultant for Bristol-Myers Squibb, Pfizer, Merck, Genentech, AstraZeneca, Eisai, and GlaxoSmithKline. M.M. Awad is a consultant for Genentech, Bristol-Myers Squibb, Merck, AstraZeneca, Maverick, Blueprint, Syndax, Ariad, Nektar, and Gritstone and reports receiving commercial research grants from Genentech, Lilly, AstraZeneca, and Bristol-Myers Squibb. M. Giannakis reports receiving a commercial research grant from Bristol-Myers Squibb, other commercial research support from Merck, and speakers bureau honoraria from AstraZeneca. F.S. Hodi is a consultant/advisory board member for Bristol-Myers Squibb, Merck, Verastem, Torque, Bicara, Amgen, EMD Serono, Novartis, Sanofi, Takeda, Genentech, Surface, Compass Therapeutics, and Aduro; reports receiving commercial research grants from Bristol-Myers Squibb and Novartis (to his institution); and has ownership interest (including patents) in MICA-related disorders (patent royalties to his institution per institutional policies). X.S. Liu is founder of and a consultant for GV20 Oncotherapy, is a scientific advisory board member for 3DMedCare, is a consultant for Genentech, and reports receiving commercial research grants from Sanofi and Takeda. S. Signoretti is a consultant for AstraZeneca and Merck; is a senior editor for AACR; is co-chair of the Renal Task Force for NCI; reports receiving commercial research grants from Bristol-Myers Squibb, Exelixis, AstraZeneca, and Novartis; and reports receiving other remuneration from Biogenex. C. Kadoch is scientific founder of; fiduciary board of directors member, scientific advisory board member, and consultant for; and has ownership interest (including patents) in Foghorn Therapeutics, Inc. E.M. Van Allen is a consultant for Tango Therapeutics, Genome Medical, Invitae, Janssen, and Ervaxx; reports receiving commercial research grants from Novartis and Bristol-Myers Squibb; and holds ownership interest (including patents) in Ervaxx, Syapse, Tango Therapeutics, Genome Medical, and SWI/SNF genes and response to immunotherapy. T.K. Choueiri is a consultant for Merck, Bristol-Myers Squibb, Foundation Medicine, Pfizer, and Exelixis and reports receiving commercial research grants from Pfizer, Bristol-Myers Squibb, Exelixis, and Novartis. No potential conflicts of interest were disclosed by the other authors.

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