original reports

Novel Genetic Subtypes of Urothelial Carcinoma With Differential Outcomes on Immune Checkpoint Blockade

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abstract

PURPOSE Immune checkpoint blockade (ICB) therapy has significantly improved clinical outcomes in bladder cancer. Identification of correlates of benefit is critical to select appropriate therapy for individual patients.

METHODS To reveal genetic variables associated with benefit from ICB, we performed whole-exome sequencing on tumor specimens from 88 patients with advanced bladder cancer treated with ICB.

RESULTS We identified several genetic factors that correlated with progression-free and overall survival after ICB therapy including *ARID1A* mutation, tumor mutational burden, intratumoral heterogeneity, the ratio of nonsynonymous to synonymous mutations in the immunopeptidome (immune dN/dS), and tumor cell purity. In addition, we noted that neutrophil-to-lymphocyte ratio and smoking history were negatively associated with overall survival. These genetic characteristics define four molecular subtypes demonstrating differential sensitivity to ICB. We validated the association of these four subtypes with clinical benefit from ICB in an independent cohort (IMvigor210). Finally, we showed that these molecular subtypes also correlate with outcome, although with distinct relationships, among patients not treated with ICB using The Cancer Genome Atlas (TCGA) bladder cancer cohort. Using parallel RNA sequencing data, the subtypes were also shown to correlate with immune infiltration and inflammation, respectively, in the IMvigor210 and TCGA cohorts.

CONCLUSION Together, our study defines molecular subgroups of bladder cancer that influence benefit from ICB.

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INTRODUCTION

The introduction of immune checkpoint blockade (ICB) therapy has revolutionized the treatment of advanced bladder cancer. Nevertheless, long-lasting clinical benefit from ICB is observed in only a subset of patients,[1-3] and the 5-year survival rate remains low (6%).[4] Therefore, identifying clinical and molecular features that can determine response to ICB is essential for appropriate selection of therapy for each patient. Furthermore, clinical trials with unselected populations have shown divergent outcomes and conflicting results despite clear activity in some patients.[5-10]

Previous studies have demonstrated that tumor-related factors such as tumor mutational burden (TMB)[11-14] and PD-L1 expression[14,15] can independently predict the efficacy of ICB. Gene expression signatures quantifying angiogenesis activity, TGFβ, tissue inflammation, and tumor microenvironment (TME) subtypes (immune-excluded, immune-desert, or inflamed[14]) can further improve predictions of patient response beyond what is achievable using only clinical factors such as tumor stage and grade.[2] Interestingly, Goswami et al[16] showed that *ARID1A* mutation plus CXCL13 expression levels can predict ICB response in metastatic urothelial carcinoma. Such genetic predictors are more clinically useful than gene expression signatures given the increasingly routine use of tumor genomic profiling. Toward more practical genetic predictors of ICB benefit, we examined the mutational landscape of tumors from patients with advanced urothelial carcinoma treated with ICB and identified genetic subgroups with differing survival outcomes after treatment.

METHODS

Patient Data

Patients with metastatic bladder cancer treated with ICB at our institution with available pretreatment tumor tissue and paired normal DNA samples were identified. Clinical characteristics and outcomes were collected. Progression-free survival (PFS) was defined as time from start of ICB treatment to disease progression or death. Overall survival (OS) was defined as time from start of ICB treatment to death. The study was approved by the Memorial Sloan Kettering institutional review board, and all patients provided written informed consent.

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Can novel genomic-based classifiers predict outcomes of immune checkpoint blockade (ICB) treatment in advanced bladder cancer?

Knowledge Generated

Whole-exome sequencing on tumor specimens from 88 patients with advanced bladder cancer treated with ICB was performed. Four molecular subtypes were identified on the basis of tumor mutational burden, tumor cell purity, and ARID1A mutation with distinct clinical outcomes and were validated in the IMvigor210 trial cohort.

Relevance *(M.A. Carducci)*

The validation of molecular subtypes in urothelial cancer and correlation with response to ICB using only DNA sequencing may eventually be clinically useful if can be conducted timely to aid treatment decision making. This data set also reinforces prior ARID1A findings establishing it as a consistent genomic alteration associated with better outcomes with ICB.*

*Relevance section written by *JCO* Associate Editor Michael A. Carducci, MD, FACP, FASCO.

Whole-Exome Sequencing

From 200 ng of DNA from each tumor and matched normal blood sample, libraries for whole-exome sequencing were generated using the KAPA Hyper Prep Kit (Roche, Indianapolis, IN). Each library contained either a 6- or 8-base pair (bp) single index and were quantified before enrichment using the Quant-iT dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA) and Agilent TapeStation. For each library, 500 ng was used in a single-plex, overnight hybridization reaction at 62°C with IDT xGen Exome Research Panel and IDT xGe Universal Blockers. Enriched libraries were normalized using bead-based normalization, and 10 samples were pooled for sequencing. Samples were sequenced by 151 bp paired-end reads on an Illumina NovaSeq 6000 S4 flow cell using the Xp workflow for individual lane loading (10-plex per lane). On average, each sample yielded 431 million unique reads, passing the filter and median target coverage depth of 728×. All samples were required to have a median target coverage of 150× to be included in our analysis.

Raw exome sequencing reads were aligned to the hg19 reference genome using Burrows-Wheeler Aligner (BWA-MEM). Somatic variants were called using Strelka-2.9.10 (Illumina, San Diego, CA) on paired tumor-normal BAM files after removing duplicate reads. Low-confidence single-nucleotide variants were removed if they did not meet the following criteria: tumor variant allele fraction (VAF) $\geq$0.05, read depth $\geq$50 in tumor samples and $\geq$20 in normal, $\geq$5 reads in the tumor, and VAF normal/VAF tumor $<$0.2. Only variants called on both strands were called at high confidence. TMB was defined as the total number of somatic mutations identified normalized to exonic coverage in megabases.

Validation Cohorts

Raw exome sequencing data on The Cancer Genome Atlas Bladder Urothelial Carcinoma (TCGA-BLCA) cohort were obtained from the GDC portal,[17] and those on the IMvigor210 cohort were obtained from the European Genome-phenome Archive under accession ID EGAD00001004218. Both data sets were analyzed using the pipeline described above. Processed RNA sequencing data on the IMvigor210 cohort were downloaded from IMvigor210CoreBiologies,[14] a fully documented software and data package for the R statistical computing environment,[18] and those on the TCGA bladder cancer cohort were obtained from the study by Thorsson et al.[19]

Statistical Analysis

R 4.0.3 was used for all statistical analyses. Continuous data are reported as medians and IQRs. *P* values for Kaplan-Meier analyses were derived using the log-rank test. Hazard ratios (HRs) for survival analysis were calculated using uni- or multivariable Cox proportional hazards models in R.

To dichotomize continuous variables into high versus low, we tested whether each feature was associated with PFS using a previously suggested threshold (median or top 20%[11]). Alternatively, to obtain the optimum threshold, we performed a grid search and corrected the maximal *P* value and corresponding CIs by generating a null reference.[20,21] The null reference was found by permuting the covariate of interest and calculating the maximal log-rank statistic for each permutation. The *P* value is the proportion of permutations for which the test statistic exceeds that for the original configuration. The 95 CI for the HR was obtained using the critical value from the permutation distribution corresponding to the confidence level instead of the standard normal value.

Features that were significantly associated with PFS ($P < .05$) on univariate analysis were included in the multivariable model. In this model, optimal thresholds were selected for each feature to identify the best correlates of benefit from ICB.

RESULTS

We identified 92 patients with advanced urothelial carcinoma treated with ICB with available pretreatment tumor and matched normal DNA samples, 88 of which had samples of sufficient quality for downstream analysis. Patients were mostly male (70%), with a history of smoking (65%). Disease originated from the bladder in 65%, from the upper tract (ureter or renal pelvis) in 30%, and the urethra in 5%. At the start of ICB treatment, the median age was 69 years (range, 43–93), 65% had visceral metastases, and 73% were treated with prior platinum-based chemotherapy. Patients were treated with either a PD-1 inhibitor (45%) or a PD-L1 inhibitor (55%) intravenously until disease progression, completion of two years of therapy for pembrolizumab-treated patients, or unacceptable toxicity. The median PFS was 3.4 months (range, 0.2-80 months). The median OS was 11.2 months (range, 0.2-80 months).

To interrogate potential genomic correlates of benefit from ICB, we performed whole-exome sequencing (WES). Somatic variant calling identified a variety of mutations across the cohort (Figs 1A and 1B). To determine whether the mutational landscape of our cohort is representative of patients with bladder cancer overall, we compared it with that in the TCGA after reprocessing of its raw data using an identical bioinformatics pipeline, which confirmed concordance (Fig 1B). The most commonly mutated genes included *TP53*, *ARID1A*, *KMT2D/C*, *KDM6A, FGFR3*, and *RB1*, which were also frequently mutated in the TCGA bladder cancer cohort.

Next, we evaluated whether any of the most frequently mutated genes were associated with PFS or OS after ICB therapy. We observed a strong association between *ARID1A* mutation and positive outcomes after ICB treatment (PFS, $P = .002$; OS, $P = .025$; Fig 2A). Interestingly, we observed a trend toward association between *KMT2D* mutation and longer PFS ($P = .06$).

Because TMB is a strong predictor of clinical benefit from ICB treatment,[11,22,23] motivating the US Food and Drug Administration approval of pembrolizumab for patients with solid tumors with high TMB, defined as >10 mut/Mbp,[13] we next evaluated its association with outcomes in our cohort. Although a trend toward association between TMB > 10 mut/Mbp and longer PFS was observed, this cutoff did not optimally enrich for patients who benefited from ICB ($P = .15$; HR, 1.5), in agreement with previous studies in various tumor types.[11,24-26] Therefore, we performed a grid search to identify the optimal threshold, yielding a cutoff of nonsynonymous TMB > approximately 3 mut/Mbp for both PFS ($P = .003$; HR, 0.44) and OS ($P = .08$; HR, 0.55; Fig 2A). These P values and the corresponding 95% CIs were corrected for multiple comparisons (see the Methods section). Although 24 insertion or deletion (INDEL) mutations best stratified PFS and OS, the association of this feature with outcomes did not reach statistical significance after multiple comparisons correction (PFS, $P = .308$, OS, $P = .153$; Fig 2A).

Other studies[27] have highlighted the importance of mutation clonality and intratumoral heterogeneity (ITH) in prediction of ICB treatment outcomes. Clonal mutational load correlated with PFS and OS; however, consistent with analysis of the IMvigor210 cohort,[28] this metric did not outperform standard TMB (Fig 2A). ITH, measured using the MATH score (on the basis of cancer cell fraction after purity and coverage normalization),[29,30] was also strongly associated with ICB outcome when the top 20% was used as threshold (PFS, $P = .011$; HR, 2.05; Fig 2A).

Motivated by this association of tumor clonality and low ITH with benefit from ICB treatment, we next sought to determine whether tumor immunoevolutionary metrics affect outcomes on ICB. We measured clonal evolution under selective pressure imposed by the immune system by determining the ratio of nonsynonymous to synonymous mutations (dN/dS) in the immunopeptidome, a method used to quantify selection pressure on mutations.[31,32] Interestingly, we found that patients with ICB-treated bladder cancer with high dN/dS ratios had superior PFS after ICB therapy ($P = .029$; HR, 0.46; Fig 2A).

Diversity of patient HLA genotype could enhance the breadth of antigen presentation. Previously, we have found that greater HLA diversity promotes antitumor immune responses and is associated with higher response rates to ICB in some cancer types like melanoma.[33-35] However, in this urothelial cancer cohort, we found no significant association between HLA zygosity and ICB response (Fig 2A).

Genomic instability, specifically the overall frequency of copy number alterations, has been reported to associate with poor prognosis and/or poor response to ICB treatment in a pan-cancer study.[36] In our cohort, we did not observe an association of ploidy, another measure of genomic instability, with either PFS or OS. A number of studies have reported the prognostic value of tumor purity in multiple cancer types.[37-39] Using the top 20%-25% as the tumor cell purity threshold,[11] we observed a strong association between high tumor cell purity and poor outcomes on ICB (PFS, $P = .022$; HR, 1.89; Fig 2A).

Several clinicopathologic factors have been shown to affect the efficacy of ICB. Although several studies have linked smoking with poorer ICB response, likely because of a compromised immune system,[40] no direct association

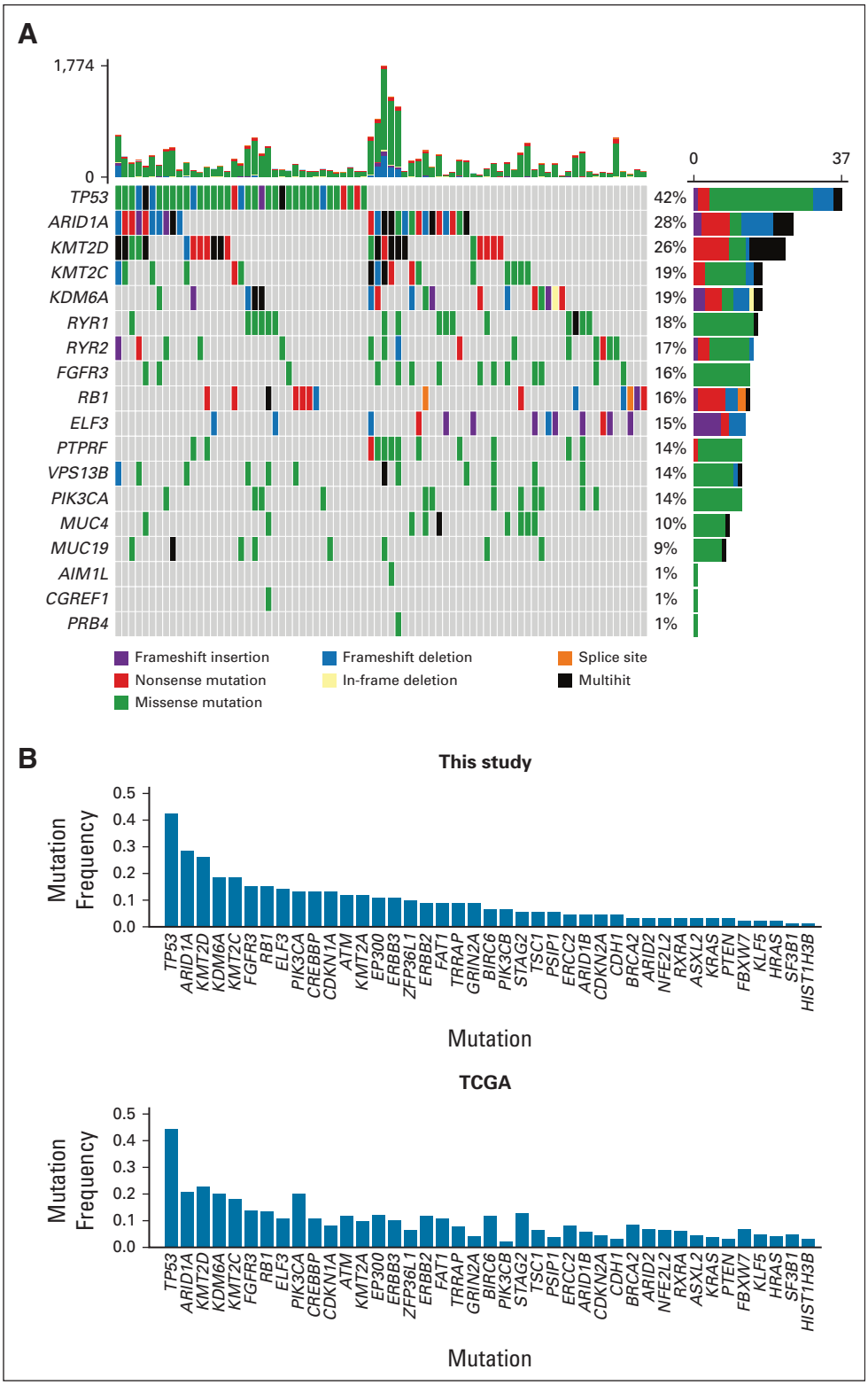

FIG 1. Genomic alterations in 88 patients with advanced bladder cancer treated with ICB. (A) Left, OncoPrint of the 18 most commonly altered genes, color coded by alteration type; right, frequency of each mutation. Top bar plot in OncoPrint shows the total number of mutations in each patient. (B) Frequency of common mutations in our cohort versus TCGA bladder cancer cohort. ICB, immune checkpoint blockade; TCGA, The Cancer Genome Atlas.

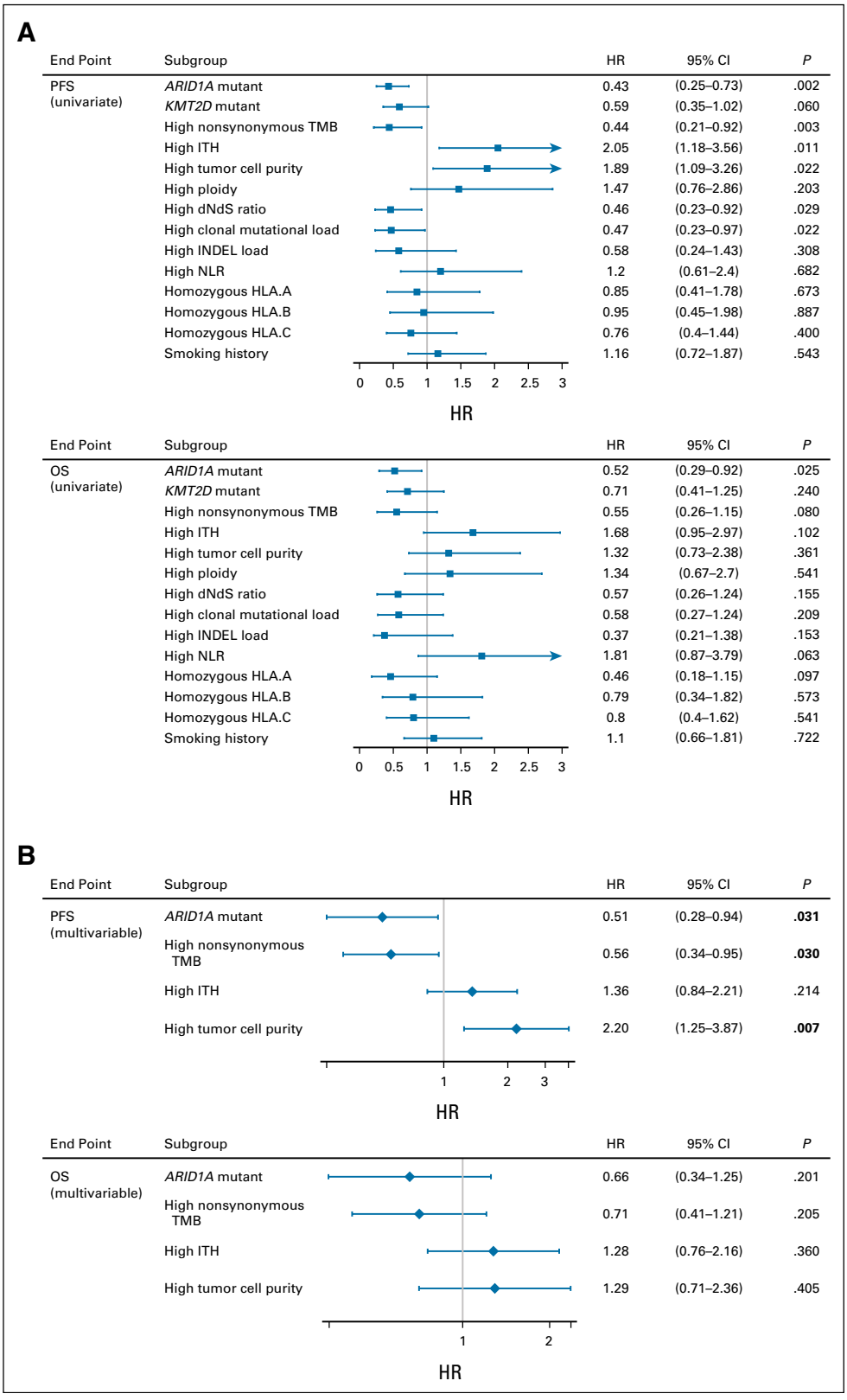

FIG 2. Correlates of survival and response to ICB. (A) Univariate and (B) multivariable analyses of significant predictors of ICB benefit. *P* values and CIs are corrected for multiple comparisons where grid searches were used to obtain optimal thresholds (TMB, ITH, and INDEL rate). *P* values derived by the log-rank test. dN/dS, ratio of nonsynonymous to synonymous mutations in the immunopeptidome; HR, hazard ratio; ICB, immune checkpoint blockade; INDEL, insertion or deletion; ITH, intratumoral heterogeneity; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PFS, progression-free survival; TMB, nonsynonymous tumor mutational burden.

between smoking status and ICB benefit was observed in our cohort (Fig 2A). Neutrophil-to-lymphocyte ratio (NLR), defined by the absolute counts of neutrophils and lymphocytes, has also been associated with worse OS and PFS after ICB therapy.[41] In our bladder cohort, NLR was strongly predictive of OS before multiple comparisons correction ($P = .018$, corrected $P = .063$; HR, 1.81) but not PFS ($P = .433$, $P = .682$; HR, 1.2). Similarly, low pretreatment eosinophil count has been correlated with worse outcomes in advanced urothelial cancer treated with ICB.[42] In our cohort, the neutrophil-to-eosinophil ratio (NER) is a negative predictive marker that outperforms NLR with a HR of 2.95 for OS ($P = .001$; Data Supplement [online only]). As information regarding NER is not provided in the IMvigor210 or TCGA publicly available data, we were not able to externally validate this finding.

Having illustrated the relevance of several potential biomarkers, we sought to investigate whether a combination of these factors can be used to define distinct bladder cancer subtypes with differential clinical outcomes on ICB. Although others have reported transcriptomic subtypes,[14,43] a more comprehensive model has not yet been introduced. Hence, we performed multivariable analysis and included variables that demonstrated a significant association with PFS on univariate analysis ($P < .05$), including nonsynonymous TMB, *ARID1A* mutation, tumor cell purity, and ITH (Fig 2B). Among factors associated with mutational load (ie, INDEL load, clonal TMB, and dN/dS), we included only nonsynonymous TMB because it showed the strongest association with survival. Using the best threshold from univariate analysis, the association between these factors remained strong in a multivariable survival model although ITH did not achieve statistical significance. Mutation in *ARID1A*, which encodes a basic subunit of Switch/Sucrose-Nonfermentable chromatin–remodeling complexes, was associated with a higher mutational load (Wilcoxon $P = .00002$). However, *ARID1A* mutation was a predictor of outcomes after ICB therapy even within TMB-high patients, suggesting a partially independent impact on tumor response to ICB therapy ($P = .031$). Interestingly, tumor cell purity outperformed other factors and was a negative predictor of ICB benefit ($P = .007$).

Next, we evaluated whether TMB, tumor cell purity, and *ARID1A* mutation can classify patients into molecular subtypes with distinct clinical outcomes. On the basis of our previous results, we identified four molecular subtypes using a rule-based classifier. We identified the following subgroups: tumors with low TMB (subtype 1); tumors with high TMB and high tumor cell purity (low immune infiltration, subtype 2); tumors with high TMB, low tumor cell purity, and *ARID1A* mutation (subtype 3); and tumors with high TMB, low tumor cell purity, and wild-type *ARID1A* (subtype 4; Fig 3A). As shown above, *ARID1A* mutation was a positive predictor of PFS and OS on ICB. Comparing subtype 3 (*ARID1A*-mutant) with subtype 4, we observed that subtype 3 included tumors with low ITH, high clonal TMB, and a high dN/dS (Fig 3B).

To establish the clinical relevance of our proposed molecular subtypes, we compared survival after treatment with ICB among subtypes (Fig 3C). As expected, subtype 1 demonstrated the worst clinical outcome with a median PFS of <6 months. Conversely, subtype 3, harboring the highest mutational load and *ARID1A* mutation, had the longest PFS. Despite high mutational load, patients whose tumors had higher tumor cell purity (subtype 2) had poor outcomes although small numbers preclude any definite conclusions for this subtype.

To test the generalizability of our findings, using our reanalyzed WES data from IMvigor210,[14] patients from that cohort were classified into subtypes 1-4. As in previously validated TMB harmonization methods,[11,44] high and low tumor cell purity and TMB were defined in IMvigor210 using quantile categories rather than the same numerical threshold to avoid cohort-specific and batch effect biases related to sequence depth or differences in tissue collection or dissection method. Strikingly, OS was strongly associated with tumor cell purity, TMB, and *ARID1A* mutation, as observed in our cohort (Fig 4A). Classification into subtypes 1-4 also distinguished OS in the IMvigor210 cohort, validating our findings (Fig 4B). Response data from IMvigor210 support these findings (Data Supplement) although numbers for subtype 2 are too small to draw reliable conclusions. Because the immunophenotypes of the IMvigor210 samples were previously determined by RNA sequencing,[14] we examined how these differed among molecular subtypes and correlated with specific molecular features. As expected, tumor cell purity was strongly associated with the immune-desert phenotype (Fig 4C). Similarly, subtypes 3 and 4 included more inflamed tumors, whereas subtype 2 was enriched for the immune-desert phenotype (Fig 4D). We also estimated the T-cell fraction by measuring T-cell receptor excision circle loss during V(D)J recombination of the T-cell receptor-α gene (*TCRA*, also known as *TRA*),[45] which was also associated with the inflamed phenotype (Fig 4E) and clinical benefit from ICB in IMvigor210 (Fig 4F). Therefore, we assessed whether patients may be classified into molecular subtypes using *TCRA* estimated T-cell fraction instead of tumor cell purity; this approach maintained distinction of OS on ICB (Fig 4G). However, estimating T-cell fraction by this approach was only possible for tumors in which the exome capture probe for WES covered the *TCRA* gene.

Finally, we evaluated whether our molecular classification could also provide prognostic information for patients with bladder cancer who were not treated with ICB. Classification of tumor samples from the TCGA bladder cancer cohort into subtypes 1-4 segregated patient groups with differing survival; however, the pattern of relative survival among subtypes differed from that in ICB-treated patients (Fig 5A). Notably, in the TCGA cohort, which represents primarily

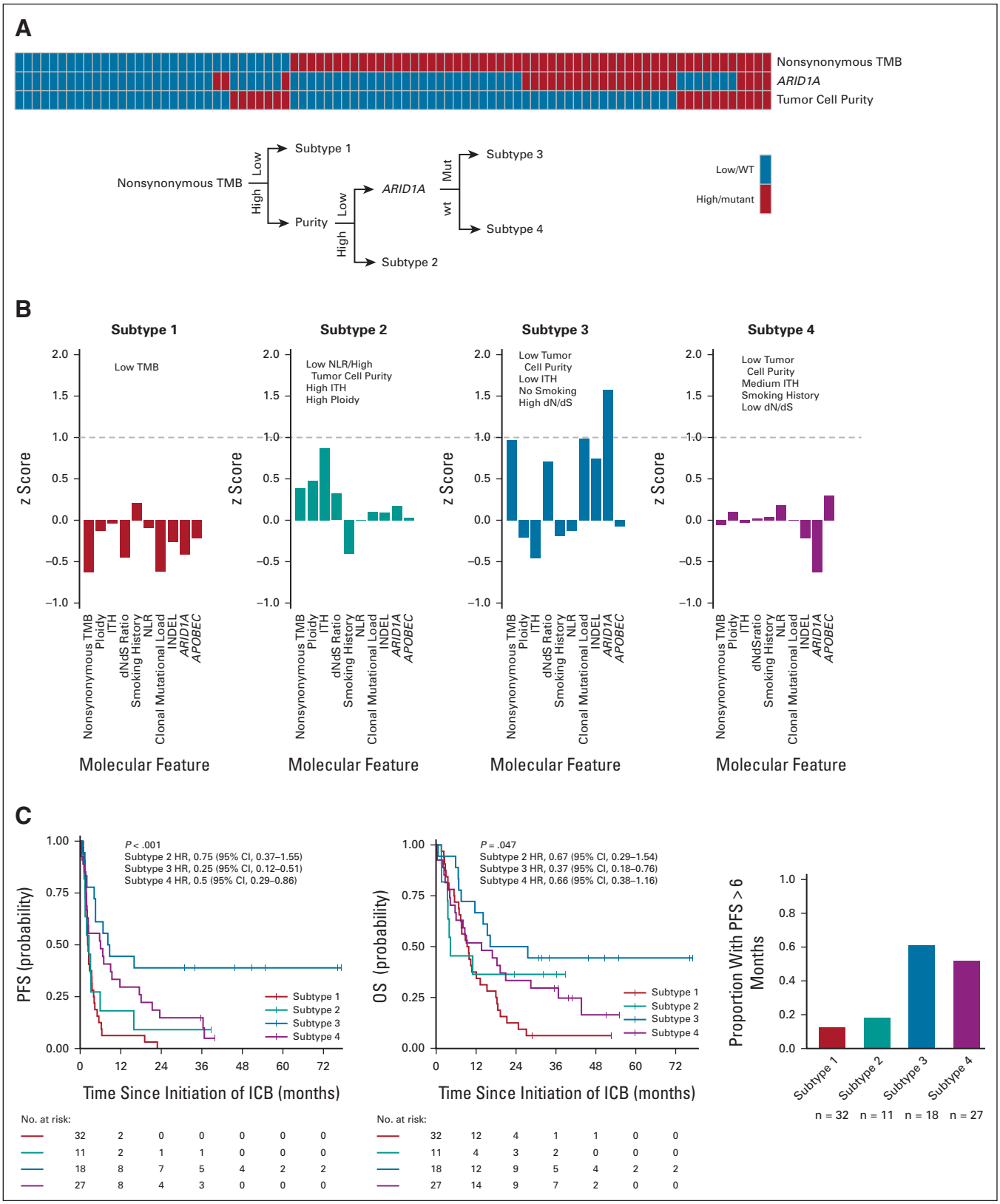

FIG 3. Differential outcomes of four molecular subtypes of bladder cancer after ICB therapy. (A) Proposed molecular subtypes of bladder cancer illustrated by OncoPrint and classification schema. (B) Genomic characteristics of bladder cancer subtypes. For each subtype, enrichment or depletion of each molecular feature is measured by z-score. (C) Association between survival and molecular subtypes. Kaplan-Meier curves showing PFS (left) and OS (middle) of each subtype. Right, proportion of patients with a PFS of > 6 months in each molecular subtype. *P* values calculated by the log-rank test. HR, hazard ratio; ICB, immune checkpoint blockade; Mut, mutated; OS, overall survival; PFS, progression-free survival; purity, tumor cell purity; TMB, tumor mutational burden; wt, wild-type.

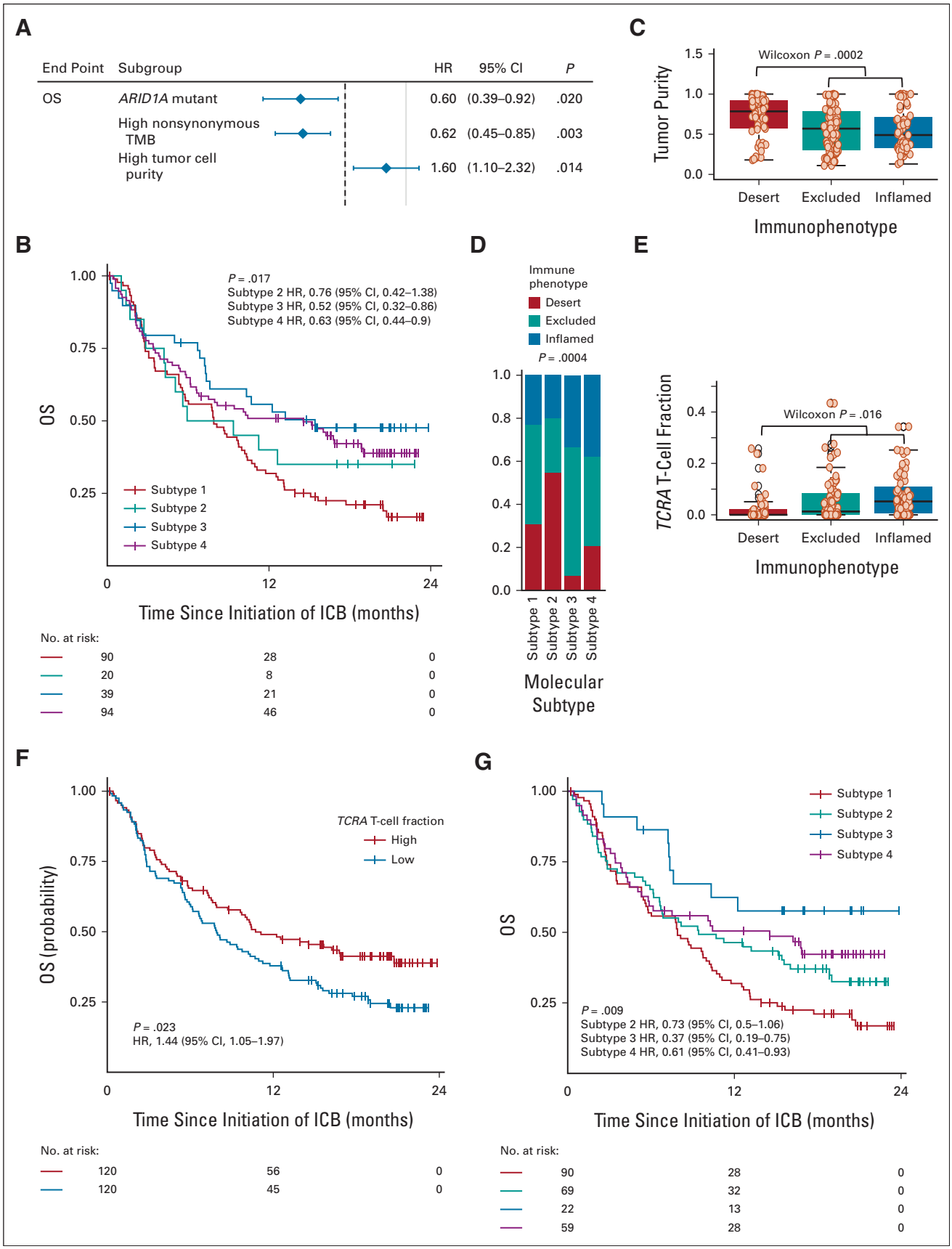

FIG 4. Validation of predictive power of molecular subtypes of bladder cancer in the IMmvigor210 cohort. (A) Multivariable analysis of the association of three biomarkers with OS after ICB therapy. (B) Kaplan-Meier curves showing OS according to the molecular subtype. (continued on following page)

FIG 4. (Continued). Association between (C) tumor cell purity, (D) molecular subtype, and (E) T-cell fraction as determined by quantitation of *TCRA* reads versus immunophenotype as determined by RNA sequencing. In (C) and (E), black lines are median values, box boundaries indicate IQRs, and whiskers represent 1.5 IQR. Kaplan-Meier plots of OS according to (F) *TCRA* T-cell fraction and (G) molecular subtype using T-cell fraction in place of tumor cell purity. *P* values calculated by the log-rank test. HR, hazard ratio; ICB, immune checkpoint blockade; Mut, mutated; OS, overall survival; PFS, progression-free survival; purity, tumor cell purity; TMB, tumor mutational burden; wt, wild-type.

localized disease, the most common subtype was subtype 2, which was associated with better survival, as opposed to the ICB-treated cohort, which represents advanced disease.

Conversely, the *ARID1A*-mutant subtype 3 patients did not fare significantly better than *ARID1A*-wt (subtype 4). Consistent with our observations in the IMvigor210 cohort (Figs 4C and 4E), we observed the lowest IFN scores in subtype 2 (high tumor cell purity) followed by subtype 1 (TMB-low; Fig 5B), suggesting that this molecular classification can capture features of both the tumor and the TME.

DISCUSSION

ICB can yield deep and durable responses, but predicting which patients will benefit remains challenging. Although previous studies have identified molecular markers associated with clinical benefit from ICB therapy in bladder cancer, including PD-(L)1 expression, immunophenotype, and minimal residual disease status,[14,15,43] such markers require specific assays. Toward more clinically applicable predictors, we focused on genetic and clinicopathologic features and identified several that correlate with benefit from ICB therapy. In general, these features reflect tumor immunogenicity and include TMB, tumor clonality or ITH, dN/dS, T-cell infiltration, and tumor cell purity.

We found that *ARID1A* mutations were associated with better ICB treatment outcomes, as previously reported in analyses of the IMvigor210[14,16] and CheckMate275 cohorts.[16] Similarly, *ARID1A* knockdown enhanced sensitivity to ICB in a murine model of bladder cancer.[16] Despite this agreement, further studies are required to evaluate whether this relationship is causative or purely correlative because of the higher mutational load observed in *ARID1A*-mutated tumors.

We found that high tumor cell purity correlated with poor outcomes on ICB. One interpretation of this finding is that high tumor cell purity reflects low immune cell infiltration, which can be associated with poor immune checkpoint therapy response.[14] However, our findings related to tumor cell purity estimation should be treated with caution. First, computational estimates of tumor cell purity cannot differentiate between immune, stromal, and normal adjacent cells. Second, as shown previously,[46] laser-capture microdissection can affect tumor cell purity measurements.

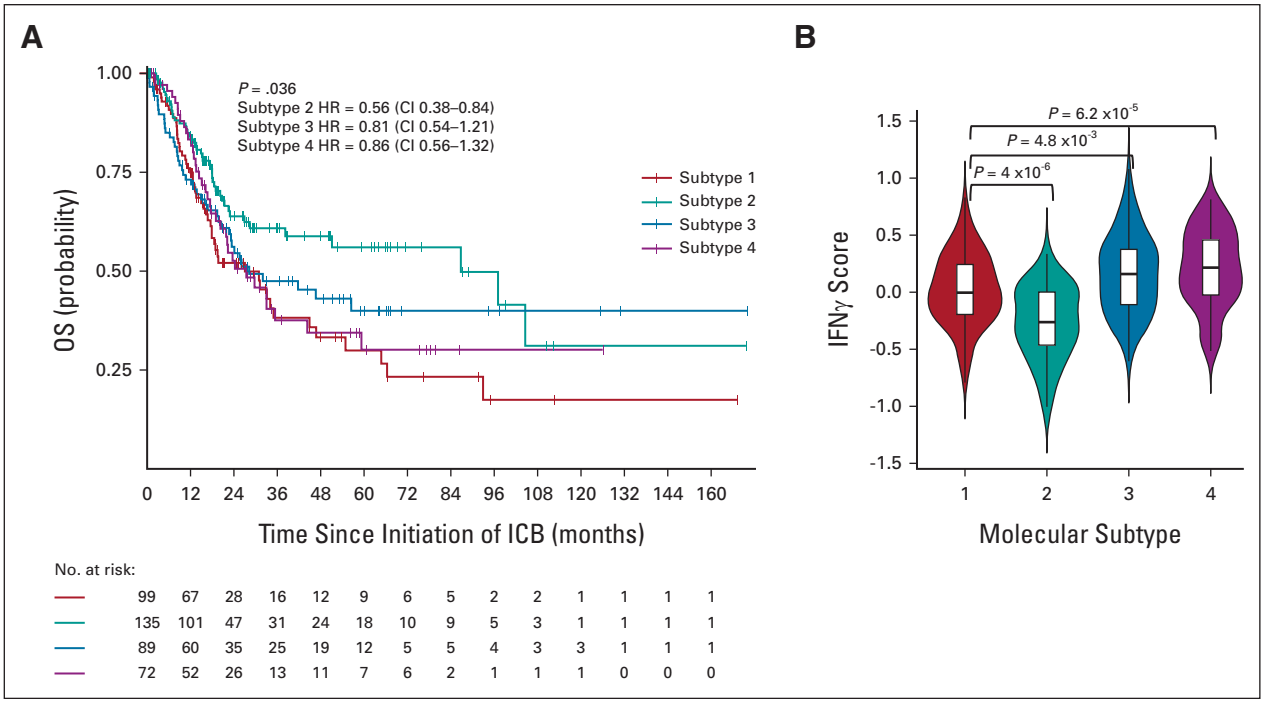

FIG 5. Molecular subtypes also distinguish outcomes and immunophenotypes in non–ICB-treated patients. (A) Kaplan-Meier plots of OS of the TCGA bladder cancer cohort according to the molecular subtype. (B) Association between IFN**?** score calculated from TCGA RNA sequencing data and molecular subtype. Black lines indicate medians, box boundaries the IQR, and whiskers 1.5 IQR. HR, hazard ratio; ICB, immune checkpoint blockade; OS, overall survival; TCGA, The Cancer Genome Atlas.

Therefore, consistent dissection across patients is required to avoid batch-to-batch variability. To address this issue, we used a threshold of the top 20%-25% to distinguish high versus low tumor cell purity, mitigating the effects of cohort-to-cohort variability.

Our finding that subtype 2 was more frequent and associated with better survival in the TCGA cohort may suggest that high TMB and high purity tumors are associated with a propensity to progress more slowly or metastasize less frequently. Along with these characteristics, these tumors have low NLR, which is associated with lower levels of myeloid-dependent immunosuppression in tumors. Therefore, another possibility is that they possess a baseline immune activity that is independent of response to treatment.

Importantly, our study proposes a novel molecular subtyping of bladder cancer on the basis of genetic features easily quantified using DNA. This approach can be adapted to targeted sequencing panels and clinical WES. The stability of DNA enhances the feasibility of our approach in clinical environments. Although RNA-based signatures may further enhance the predictive value of our model, clinical implementation of RNA-based assays has historically been challenging. Future studies will be needed to confirm the general clinical utility of our findings.

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EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.22.02144.

DATA SHARING STATEMENT

The data generated in this study are available from cBioPortal.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Novel Genetic Subtypes of Urothelial Carcinoma with Differential Outcomes on Immune Checkpoint Blockade

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Patents, Royalties, Other Intellectual Property: Neoantigens and uses thereof for treating cancer Systems and methods for determining the likely responsiveness of a human cancer subject to a checkpoint blockade immunotherapy regimen are provided. Sequencing reads are obtained from samples from the subject representative of the cancer. A human leukocyte antigen type and a plurality of clones are determined from the sequencing reads. For each clone, an initial frequency Xa in one or more samples is determined and a corresponding clone fitness score of the clone is computed, thereby computing clone fitness scores. Each such fitness score is computed by identifying neoantigens in the respective clone, computing a recognition potential for each neoantigen, and determining the corresponding clone fitness score of the respective clone as an aggregate of these recognition potentials. A total fitness, quantifying the likely responsiveness of the subject to the regimen, is computed by summing the clone fitness scores across the plurality of clones, RNA-containing compositions, and methods of their use. The present invention is related to a composition comprising an isolated, single-stranded RNA molecule having a nucleotide sequence comprising 20 or more bases, a pattern of CpG dinucleotides defined by a strength of statistical bias greater than or equal to zero, and a pharmaceutically acceptable carrier suitable for injection. The present invention is also related to a kit comprising a cancer vaccine and the composition of the present invention as an adjuvant to the cancer vaccine. The present invention is further related to a method of treating a subject for a tumor and a method of stimulating an immune response, Compositions and methods for inhibiting cancers and viruses. The present invention is related to compositions comprising isolated, single-stranded RNA molecules and pharmaceutically acceptable carriers suitable for injection. The present invention is related to methods for stimulating an immune response and treating tumors. The present invention is further related to kits comprising a cancer vaccine and compositions of the present invention for use as an adjuvant to cancer vaccines, Repeat RNA as biomarkers of tumor immune response Methods for predicting response to immunotherapy and selecting immunotherapy for treating cancer, for example, cancer of epithelial origin, in a subject, Models for predicting mutant p53 fitness and their implications in cancer therapy. The present technology is related to methods, computing devices, and systems for predicting the fitness of mutant p53 on the basis of the loss of transcription factor function and immunogenicity of a particular TP53 mutation. The fitness of mutant p53 may be used to determine whether a patient will benefit from a particular anticancer therapy such as immune checkpoint inhibitor therapy, adoptive T-cell therapy, or prophylactic cancer vaccine therapy
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