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Genomic Profiles and Clinical Outcomes of Penile Squamous Cell Carcinoma With Elevated Tumor Mutational Burden

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Key Points

Question

Is there a role for comprehensive genomic profiling and immunotherapy in patients with advanced metastatic penile squamous cell carcinoma (PSCC)?

Findings

Among 397 patients with PSCC included in this cohort study, 15% had tumor mutational burden (TMB) of 10 mutations per megabase (mut/Mb) or higher, and 4% had TMB 20 mut/Mb or higher. Tumors with TMB 10 mut/Mb or higher were characterized by a distinct profile of co-occurring mutations with significantly more frequent *PIK3CA* and *KMT2D* genomic alterations and human papillomavirus infection.

Meaning

This cohort study suggests that patients with advanced metastatic PSCC characterized by high TMB values associated with particular genomic alterations may be candidates for immune checkpoint inhibitor therapy.

Abstract

Importance

Tumor mutational burden (TMB) is a putative biomarker of efficacy for immune checkpoint inhibitor (ICI) therapies of solid tumors, but not specifically for penile squamous cell carcinoma (PSCC).

Objective

To characterize biomarker features and ICI therapy outcomes associated with high TMB in PSCC in the routine clinical practice setting.

Design, Setting, and Participants

In this cohort study, 397 PSCC cases were analyzed to identify genomic alterations in more than 300 cancer-associated genes and genomic signatures, including TMB, using a hybrid capture–based comprehensive genomic profiling assay. Tumor mutational burden was categorized as low (<10 mutations per megabase [mut/Mb]), high (10-19 mut/Mb), or very high (≥20 mut/Mb). Germline status of genetic alterations was predicted using a validated somatic-germline computational method. Clinical outcomes of patients with metastatic PSCC receiving first-line ICI were abstracted using the deidentified nationwide Clinico-Genomic Database (CGDB) from January 1, 2011, through December 31, 2022.

Exposure

Comprehensive genomic profiling was performed using FoundationOne and FoundationOne CDx assays from Foundation Medicine Inc.

Main outcomes and measures

The spectrum of genetic alterations by TMB level in PSCC, the percentage of germline genetic alterations, and the outcome (overall survival with routine clinical treatment) by TMB of chemotherapy-naive patients with PSCC who received ICI treatment up front were assessed in this descriptive study.

Results

Among 397 patients (median [IQR] age, 65 [54-73] years; 266 [67.0%] of European, 83 [20.9%] of admixed American, and 34 [8.5%] of African or other genomic ancestry), the median (IQR) age (eg, 65 [53-73] years for low TMB vs 68 [61-78] years for TMB ≥10 mut/Mb) and genomic ancestry distribution (eg, European 228 of 339 [67.3%] for low TMB vs 38 of 58 [65.5%] for TMB ≥10 mut/Mb) were similar between TMB subgroups. There were 339 PSCC cases (85.4%) with low TMB, 40 cases (10.1%) with high TMB, and 18 cases (4.5%) with very high TMB. Comparisons of TMB of 10 mut/Mb or higher vs low TMB showed an enrichment of genetic alterations in *PIK3CA* (48.3% vs 18.3%; $P < .001$) and *KMT2D* (29.3% vs 7.7%; $P < .001$) and less frequent genetic alterations in *CDKN2A* (25.9% vs 45.7%; $P = .05$). Most genetic alterations did not co-occur. Human papillomavirus identification was more frequent as TMB increased: 28.3% for low TMB, 50.0% for high, and 72.2% for very high. In total, 95 of 1377 genetic alterations (6.9%) were germline. Of 10 patients identified from the CGDB receiving frontline ICIs, median (IQR) follow-up was 9.9 months. Four patients had overall survival with clinical treatment of more than 12 months, including 2 of 3 patients with TMB of 10 mut/Mb or higher.

Conclusions and Relevance

In this cohort study of advanced metastatic PSCC based on TMB levels, significant differences were observed for biomarkers in nearly 15% of patients with a TMB of 10 mut/Mb or higher. Germline testing and ICI-based therapy should be integrated into the management of selected PSCC cases.

This cohort study uses data from a nationwide database to characterize biomarker features and immune checkpoint inhibitor therapy outcomes associated with tumor mutational burden among patients with advanced metastatic penile squamous cell carcinoma.

Introduction

Locally advanced metastatic penile squamous cell carcinoma (PSCC) is a rare and deadly disease for which the prognosis closely depends on the primary tumor stage and the extent of involvement of regional lymph nodes.[1] The mainstay of treatment continues to rely on radical inguinal lymphadenectomy, with limited contribution to survival by adding perioperative systemic therapies or radiotherapy.[2,3] In the neoadjuvant setting for clinically lymph node–involved PSCC, the combination of paclitaxel, ifosfamide, and cisplatin was tested in a phase 2 trial conducted in the US and provided an objective response rate (ORR) of 50%.[4] The initial findings from that trial were further corroborated by additional retrospective studies, and clinical guidelines currently recommend an informed decision by the patient regarding the possibility of receiving neoadjuvant chemotherapy prior to extirpative surgery.[2,5] A previous meta-analysis on the outcomes of perioperative chemotherapy reported a pooled ORR of 53% (95% CI, 42-64), a pooled pathological complete response rate in patients who underwent radical inguinal lymphadenectomy of 16%, and an overall mortality rate of 55%.[6] The conclusion from those studies is that most patients with PSCC diagnosed with regional lymph node involvement need newer and more effective systemic therapies to improve outcomes.

Previous genomic studies originating from the Foundation Medicine Inc (FMI) database have shown that PSCC has distinctive genomic features when compared with metastatic cutaneous SCC of nonpenile UV light–exposed skin. Those studies have also identified opportunities for targeted therapies, including the mTOR pathway, DNA damage response pathway, and tyrosine kinase gene alterations (*FGFR3, EGFR,* and *ERBB2*).[7] Furthermore, human papillomavirus (HPV) infection characterizes a consistent subset of PSCC that appears to have a diverse tumor microenvironment and clinical course. In particular, HPV-positive PSCC is characterized by more pronounced T-cell infiltration, lower tumor programmed cell death ligand 1 (PD-L1) expression, and higher tumor mutational burden (TMB).[8,9] Tumor mutational burden has emerged to be a major surrogate biomarker of the efficacy of immune checkpoint inhibitor (ICI)–based therapy for a wide variety of malignant neoplasms but not specifically for PSCC.[10] The US Food and Drug Administration (FDA) has granted accelerated approval of pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors characterized by TMB of at least 10 mutations per megabase (mut/Mb) that have progressed or following standard treatment with no alternative therapeutic options.[11] In previous studies by members of our team evaluating various squamous cell carcinoma (SCC) lesions originating from the pelvic region, the percentages of cases with TMB of at least 10 mut/Mb were 15% for advanced PSCC, 24% for male anal SCC, 27% for cervical SCC, 22% for female anal SCC, and 28% for vaginal SCC.[12] In the present study, we investigated genomic biomarkers that characterized selected cases of PSCC with elevated TMB to identify optimal candidates for ICI or personalized medicine strategies.

Methods

This cohort study used 2 separate data sources: the FMI database and the Flatiron Health (FH)–FMI Clinico-Genomic Database (CGDB). Approval of the study protocol by the Western Copernicus Group Institutional Review Board was obtained prior to study conduct and included a waiver for the requirement to obtain informed consent via a Health Insurance Portability and Accountability Act waiver of authorization. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

FMI Database Analysis

In the FMI database, comprehensive genomic profiling (CGP) of clinically advanced cases of PSCC (defined as surgically incurable disease, including deeply invasive primary tumors, locally advanced primary tumors, or metastatic disease to lymph nodes or visceral organs, as diagnosed by the treating physician and confirmed on hematoxylin-eosin–stained slides) was performed using the FoundationOne and FoundationOne CDx assays (FMI) to identify genomic alterations in more than 300 cancer-associated genes and genomic signatures, as described previously, in a Clinical Laboratory Improvement Amendments–certified and College of American Pathologists–accredited laboratory.[13] Central pathology review was conducted using 1 tissue block per patient. All samples submitted for sequencing featured a minimum of 20% tumor cell nuclear area and yielded a minimum of 50 ng of extracted DNA. Comprehensive genomic profiling was performed on hybrid-capture, adapter ligation–based libraries to identify genomic alterations (base substitutions, small insertions and deletions, copy number alterations, and rearrangements) in coding exons (FoundationOne CDx: N = 309; FoundationOne: N = 395), additional selected introns of cancer-associated genes (FoundationOne CDx: N = 36; FoundationOne: N = 31), and TMB (mean coverage depth >600×).[14] We calculated TMB as the number of nondriver somatic coding mutations per megabase of the sequenced genome. In this study, very high TMB was defined as 20 mut/Mb or higher, high TMB as 10 to 19 mut/Mb, and low TMB as lower than 10 mut/Mb.

Microsatellite instability (MSI) was determined on at least 1500 loci.[14,15] Homologous recombination deficiency–specific genome-wide loss of heterozygosity was determined using validated algorithms that excluded whole-arm and whole-chromosome events.[16] Tumor cell PD-L1 expression was determined by immunohistochemistry (anti–PD-L1 antibody 22C3; Dako) and defined as tumor proportion score positive if 1% or higher and highly positive if 50% or higher. All genomic alterations studied included only those described as functional or pathogenic in the literature or those with a likely functional status (frameshift or truncation events in tumor suppressor genes).[17] Variants of unknown significance were not studied. For each profiling platform (FoundationOne and FoundationOne CDx), more than 40 000 common heterozygous single-nucleotide variant sites sequenced by CGP were identified. As self-reported race and ethnicity was not available, genomic ancestry was determined for each patient sample by using a single-nucleotide variant–based classifier to identify ancestral population groups (African, Admixed American [a mixture of parts of the ancestry DNA signatures of those with European, sub-Saharan African, and/or Indigenous American ancestry], East Asian, European, and South Asian), as previously reported, because FMI does not collect patient-reported ancestry.[18] Germline status was assessed using a validated somatic-germline computational method (somatic-germline zygosity) that was designed only for substitutions and indel variant types.[19] In addition, the genomic signature assignments used the Catalogue of Somatic Mutations in Cancer trinucleotide signatures and were attributed according to established computational methods.[20] The presence of HPV was determined by next-generation sequencing.[21]

CGDB Database Analysis

We also studied samples from patients with confirmed diagnosis of penile cancer who received first-line therapy for confirmed metastatic disease assessed using a rule-based heuristic, included in the US nationwide FH-FMI deidentified CGDB from January 1, 2011, through December 31, 2022. The deidentified data originated from approximately 280 US cancer clinics (approximately 800 sites of care). Retrospective longitudinal clinical data were derived from electronic health record data, comprising patient-level structured and unstructured data, curated via technology-enabled abstraction, and were linked to genomic data derived from FMI CGP tests in the FH-FMI CGDB by deidentified, deterministic matching.[22] Patient smoking status was extracted by natural language processing of electronic health record documents.[23] In this cohort, overall survival (OS) with routine clinical treatment was calculated from start of treatment in the metastatic setting to death from any cause, and patients without a record of mortality were right censored at the date of their last clinic visit or structured activity. Because patients could not enter the database until a CGP report was delivered, OS risk intervals were left truncated to the date of report to account for immortal time.

Statistical Analysis

All statistical analyses were performed using R software, version 4.2.2 (R Project for Statistical Computing). Proportions of categorical variables were compared using the Fisher exact test. Wilcoxon rank sum tests were used to test for differences between continuous variables. All *P* values were 2-sided, with values <.05 considered statistically significant, and multiple hypothesis testing correction was performed using the Benjamini-Hochberg procedure to calculate the false discovery rate.

Results

CGP Results From the FMI Database

In the total cohort of 397 patients with PSCC (median [IQR] age, 65 [54-73] years; 266 [67.0%] of European, 83 [20.9%] of admixed American, and 34 [8.5%] of African or other genomic ancestry), the median (IQR) age (65 [53-73] years for low TMB vs 68 [61-78] years for TMB ≥10 mut/Mb) and distribution of genomic ancestry (eg, European 228 of 339 [67.3%] for low TMB vs 38 of 58 [65.5%] for TMB ≥10 mut/Mb) were similar between TMB category subgroups, with a prevalence of European ancestry (Table). The distribution of PSCC TMB categories was 339 patients (85.4%) with low TMB, 40 patients (10.1%) with high TMB, and 18 patients (4.5%) with very high TMB (Figure 1). Due to the small number of patients in the very high TMB category, comparisons were dichotomized between the categories of low TMB and TMB of 10 mut/Mb or higher. The Table and eTables 1, 2, and 3 in Supplement 2 present the distributions of patient and disease characteristics and genomic alterations between the TMB categories. The median (IQR) age of patients with TMB of 10 mut/Mb or higher was 68 (61-78) years vs 65 (53-73) years in the low TMB cohort ($P = .09$). No significant differences between the TMB categories were found by genomic ancestry (eg, European ancestry, 67.3% vs 65.5%) or tumor PD-L1 expression (eg, for PD-L1 tumor proportion score 1%-49%, 46 of 111 [41.4%] vs 8 of 25 [32.0%]). Apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide–like (APOBEC) genomic mutational signature was more frequent in cases with TMB of 10 mut/Mb or higher (73.6%) vs low TMB (44.1%; $P = .05$). The identification of HPV was more frequent as TMB increased: 28.3% for low TMB, 50.0% for high TMB, and 72.2% for very high TMB groups. eFigure 1A in Supplement 1 displays a tile plot of the most frequent genomic alterations found in the entire cohort: the top-altered genes (≥10.0%) were *TP53* (54.4%), *TERT* (promoter, 44.1%), *CDKN2A* (42.8%), *PIK3CA* (22.7%), and *NOTCH1* (17.4%). Another potentially "actionable" genomic alteration was in the *EGFR* gene, observed in 10.8% of the cases. Comparisons of TMB of 10 mut/Mb or higher vs low TMB showed an enrichment of genetic alterations in *PIK3CA* (48.3% vs 18.3%; $P < .001$) and *KMT2D* (29.3% vs 7.7%; $P < .001$) and less frequent genetic alterations in *CDKN2A* (25.9% vs 45.7%, $P = .05$). eFigure 1B in Supplement 1 shows a tile plot of genomic alterations found in the population of 18 PSCC tumors with very high TMB: here, the enrichment in HPV-positive PSCC was evident, along with higher frequencies of *PIK3CA* (66.7%) and *KMT2D* (38.9%) genomic alterations. Those alterations were represented by short variants in all cases except for 1 case of *PIK3CA* amplification. An analysis of pairwise co-occurring short variants within the 2 categories of PSCC with low TMB and TMB of 10 mut/Mb or higher revealed quite a few recurrent pairs, including *PIK3CA* and *KMT2D* (eFigure 2A and B in Supplement 1). In addition, 2 cases with high MSI were reported. We also analyzed the principal Kyoto Encyclopedia of Genes and Genomes pathway distribution according to TMB category (eTables 1, 2, and 3 in Supplement 2). Several pathways were more frequently altered in PSCC with TMB of 10 mut/Mb or higher vs low TMB, including the cell cycle (58.6% vs 37.4%, $P = .04$), fatty acid metabolism (58.6% vs 32.7%, $P = .005$), mTOR (55.1% vs 33.3%, $P = .02$), and tryptophan metabolism (53.4% vs 21.8%, $P < .001$) pathways.

Landscape of Estimated Somatic vs Germline Genomic Alterations in PSCC

In total, 1377 of 1461 pathogenic short variant genomic alterations found in the entire cohort were assessable by the somatic-germline computational method: 95 (6.9%) were determined to be of likely germline origin, which requires confirmation by validated germline testing. Figure 2 displays the spec-

trum of the most frequent germline genomic alterations in our study, including *BRCA2* (4 of 7 or 42.9% somatic), *CHEK2* (3 of 5, or 40.0% somatic), *PMS2* (3 of 5, or 40.0% somatic), *ATM* (5 of 8, or 37.5% somatic), and *PTEN* (9 of 13, or 30.8% somatic).

OS With Frontline ICI Therapy in Routine Clinical Practice From the CGDB

We identified 30 patients with a median (IQR) age of 62 (52-71) years, 20 (66.7%) of whom presented with an Eastern Cooperative Oncology Group Performance Status scores of 0 or 1. Full baseline clinical and tumor characteristics of this cohort are provided in eTable in Supplement 1. Thirty patients had information on the type of first-line therapy that they received for metastatic disease between December 14, 2015, and November 10, 2022. Median (IQR) follow-up was 10 months. We included 10 patients who received ICI monotherapy, 2 patients who received cetuximab monotherapy, and 18 patients who received chemotherapy. Sixteen patients (53.3%) had received prior chemotherapy in the nonmetastatic setting, including 6 (60.0%) in the ICI-treated cohort. Information on TMB was missing in 3 cases. The OS outcomes for treatment in routine clinical practice according to the type of received therapy are displayed in the swimmer plot of Figure 3. An OS of 64 months (and continuing) with ICI was observed for a patient with high MSI penile cancer and a TMB of 12 mut/Mb. Four patients (40.0%) who initially received ICI demonstrated OS longer than 12 months, with an additional 3 patients who were still receiving ICI at the time of the last update. Of note, 2 of 3 patients with TMB 10 mut/Mb or higher demonstrated OS longer than 12 months with ICI therapy (while the third was censored at 10 months) vs 2 patients with TMB of 13 and 30 mut/Mb who displayed much shorter OS with chemotherapy given in routine clinical practice.

Description of Representative Clinical Cases of PSCC From the FMI Database

Case 1 A man 82 years of age with a partial penectomy presented with pT3 PSCC with basaloid features. Venous and lymphatic invasions were identified (eFigure 3A and B in Supplement 1). The patient rapidly developed metastasis. The tumor was negative for PD-L1 expression as assessed by immunohistochemistry. The CGP indicated that the tumor was MSI stable with a TMB of 30 mut/Mb. Multiple potential targets for therapies were also identified, including an *ERBB2* extracellular domain missense E265K-activating mutation* (eFigure 3C in Supplement 1) and mTOR pathway–activating alterations in *PIK3CA* E545K and *TSC1* Q527*. We also identified HPV-16 in this sample (11 933 reads per million). The *ERBB2* extracellular domain genomic alterations accounted for a frequency of 0.5% in the entire database, suggesting the consideration of this patient for potential inclusion in basket trials investigating novel ERBB2 inhibitors. Other therapeutic implications are represented by pembrolizumab as a US FDA-approved agent for trials investigating novel mTOR pathway inhibitors, or HPV-directed cell therapies or vaccines.

Case 2 A needle biopsy of an inguinal lymph node metastasis was obtained from a man 80 years of age with PSCC and a history of a radically resected pT4 colorectal carcinoma (eFigure 4A and B in Supplement 1). The assessed CGP indicated that the tumor had high MSI with a TMB of 33 mut/Mb. The potentially actionable genomic alterations included *BRAF* V600E, *BRAF* N581D, *BRCA2* I605fs*9, *NOTCH1* splice site 5018 + 2T>C, *NOTCH1* G1917fs*23, and *NOTCH1* R2327W (eFigure 4C in Supplement 1). The *NOTCH1* mutations accounted for 18.6% of the total mutations in PSCC with low TMB vs 10.3% in PSCC with a TMB of 10 mut/Mb or higher in the present study. Those alterations have been previously reported in PSCC by other studies.[24] The therapeutic options could include basket trials of *NOTCH1* in-

hibitors, including γ-secretase inhibitors, *BRAF* inhibitors, and poly(adenosine diphosphate ribose) polymerase inhibitors, as well as pembrolizumab as an FDA-approved agent. Germline testing would be recommended due to high MSI and a *BRCA2* mutation identified through CGP.

Discussion

To our knowledge, this cohort study is the largest to date to describe the landscape of clinically advanced PSCC genomic alterations in detail and correlated the findings with various TMB values. The study presents data from the most updated genomic database of FMI related to PSCC, expanding on evidence from previous studies reported from the initial database source.[7,25]

The results confirm that there is an opportunity to consider a genomically informed selection of patients with PSCC whose tumors can be characterized by biomarkers that have been associated with ICI or potential targeted therapy benefit. For example, the 14.6% of PSCC tumors with a TMB of 10 mut/Mb or higher—a bit lower compared with the percentage initially reported by members of our team from the same database[7]—is noteworthy. Authors have recently sought to evaluate the performance of the FDA-approved TMB algorithm to identify patients with favorable OS for single-agent ICI in a large cohort in a routine clinical practice setting. With few exceptions, higher TMB has been associated with more favorable OS in clinical practice among patients receiving ICI monotherapy across tumor types (not including PSCC), regardless of MSI status.[26] Despite widely varying distributions of TMB per tumor type, those data on routine clinical practice OS associations have been consistent with FDA approval of TMB 10 mut/Mb or higher using the FoundationOne CDx assay for guiding ICI monotherapy in advanced stage cancers across multiple tumor types. In the present study, we were able, for the first time, to expand the aforementioned observations to the field of rare urologic cancers, such as PSCC.

Within our study population we further recognized a cohort of tumors with TMB of 10 mut/Mb or higher that were characterized by a distinct molecular signature, with an enrichment of HPV-related tumors and increased frequencies of short variant alterations of the *PIK3CA* and *KMT2D* genes. Conversely, we found that *CDKN2A* short variants or copy number alterations (homozygous deletions) were enriched in the population of patients with low TMB tumors. Those findings may substantially influence the consideration of clinical trials evaluating putative therapeutic targets with novel therapies, including tyrosine kinase inhibitors and cyclin-dependent kinase 4 and 6 inhibitors, or via the pharmacological targeting of KMT2D-deficient tumors, as has been suggested by previous authors.[27] In tumors with very high TMB, those agents could be partnered in combinatorial therapies with ICI or with novel immunotherapeutic agents, cell therapies, or therapeutic vaccines targeting the HPV pathway, within clinical trials. Gene pathways analyses revealed further possibilities of ICI and targeted therapy in the broader population of PSCC with TMB of 10 mut/Mb or higher. In particular, fatty acid metabolism alterations may also contribute to ICI response as previously reported,[28] and tryptophan metabolism pathway genomic alterations would suggest an opportunity for indoleamine 2,3-dioxygenase 1 inhibitors. We also more frequently detected an APOBEC mutational signature in PSCC with TMB of 10 mut/Mb, as previously reported by other authors.[24]

These results could be important for improving the inclusion criteria for future clinical trials in PSCC. In fact, the available results reported in phase 2 trials or basket studies testing ICIs in unselected patients are inconclusive.[29,30] In a basket trial investigating the combination of nivolumab and cabozantinib, with or without ipilimumab, 3 patients with PSCC were included (all of whom received the triple combination): 1 partial response and 2 stable disease occurred.[29] Conversely, no partial response was reported

in 5 patients included in another study of nivolumab plus ipilimumab (2 stable disease and 3 progressive disease).[30] Atezolizumab was investigated as monotherapy or in combination with locoregional radiotherapy in a phase 2 trial including stage IV PSCC: the ORR was 44% with combination therapy and 17% with monotherapy.[31] Finally, various ICI regimens tested in a heterogeneous population of chemotherapy-naive and chemotherapy-treated PSCC were included in a retrospective study sponsored by the Global Society of Rare Genitourinary Cancers: the pooled ORR was 13%, with a median progression-free survival of 3.2 months.[32] There are also several trials in progress with ICIs, the most interesting being represented by the HERCULES study (first-line pembrolizumab and platinum-based chemotherapy, NCT04224740)[33] and the EPIC Trial sponsored by Cancer Research UK (cemiplimab, with or without chemotherapy).[34]

When analyzing OS data from patients with metastatic penile cancer receiving frontline ICI treatment in routine clinical practice, representing a unique cohort in the literature, we realized that sustained OS could be achieved with up-front ICI, therefore representing a therapeutic possibility instead of standard chemotherapy in selected patients. In particular, we observed that TMB (and the well-known high MSI status) appeared to be an important biomarker for the selection of first-line therapy, especially when focusing on patients exhibiting long-term survival. However, as the present study was only a descriptive analysis, those associations will need further validation in a larger cohort, primarily because we also observed patients with PSCC and low TMB having 16 and 29 months' OS in routine clinical practice.

Less frequent genomic alterations that emerged in our study may be also useful to provide rationale for inclusion of PSCC in basket trials testing ICIs in combination with targeted therapies. Published results to date point to the role of epidermal growth factor receptor (EGFR) targeting in PSCC. After the initial case report published by members of our team with panitumumab[35] and the following phase 2 trial of dacomitinib,[36] initial results with anti-EGFR tyrosine kinase inhibitors in combination with ICI, or in combination with ICI and chemotherapy, suggested the possibility to also improve outcomes in the perioperative setting.[36,37] In a small phase 2 trial conducted with 21 patients, the combination of toripalimab (anti–PD-1), chemotherapy, and nimotuzumab (anti-EGFR) resulted in a 61.1% pathological complete response rate.[37] Interpreting those results in the absence of biomarker data is difficult, and efforts in the next studies should prioritize the advances in our understanding of the biology underlying response to those agents. Furthermore, gene pathway analyses revealed an opportunity for mTOR inhibitor treatment among patients with PSCC and high TMB. Finally, we identified 6.9% of advanced PSCC cases that were predicted to have a germline mutation, with a prevalence of homologous recombination repair genes and genes involved in Lynch syndrome. That finding could be important to orient the next strategies of targeted therapies, a rationale for use of CGP in routine practice, and the possibility to extend genetic counseling and dedicated germline testing indications to selected patients with PSCC and their broader families (eg, cascade testing).

Limitations

This study has limitations. First, although there is currently a lack of more robust published clinical outcomes data, we need more data to corroborate the associations between OS and genomic biomarkers. Further important limitations include the retrospective and descriptive nature of the study; lack of randomized control groups; and the variability in therapies, surveillance, and follow-up protocols for patient treatments. Other limitations include a central pathology review of samples limited to 1 tissue block per patient, large time frame for sample collection, potential bias toward European ancestry, and lack of

association with other important analyses; for example, gene signature expression or single-gene expression findings, particularly those related to preexisting antitumor immunity or tumor T-cell infiltration, which may be additional biomarkers associated with response to ICI in PSCC.

Conclusions

The hypothesis-generating results of this cohort study support further study of TMB as a biomarker of ICI-based response in advanced PSCC, including for patients with TMB of 10 mut/Mb or higher who had tumor progression during conventional therapeutic options. The use of CGP for PSCC tumors may also help identify patients who may benefit from frontline ICI therapy based on the available OS data from routine clinical practice, with further potential opportunities resulting from targeted therapies in the future. The use of CGP may also inform eligibility for clinical trials and help identify candidates for genetic counseling and dedicated germline testing as part of routine disease management.

Notes

Supplement 1.

eTable. Clinical characteristics of the real-world clinical outcomes cohort

eFigure 1. Tile plot showing the distribution, type and frequency of single gene alterations* occurring in the entire population (A) or in the population of patients with TMB-very high PSCC (B)

eFigure 2. Tile plot displaying the frequency of pairwise co-occurring short variant alterations in the cohort of TMB-low (A) and TMB-high + very high (B) PSCC

eFigure 3. Low magnification (A) and high magnification (B) images of the primary PSCC which was used for sequencing are shown

eFigure 4. Low magnification (A) and high magnification (B) images of the primary PSCC which was used for sequencing are shown

Supplement 2.

eTable 1. FMI1: Absolute numbers indicating the distribution of cases across various TMB group comparisons

eTable 2. FMI2: Proportions and statistical comparisons between groups, without false discovery-rate correction (significant *P* values are highlighted in light green)

eTable 3. FMI3: Proportions and statistical comparisons between groups, after false discovery-rate correction (significant *P* values are highlighted in light green, *P* values that lost significance after false discovery-rate correction are yellow-highlighted)

Supplement 3.

Data Sharing Statement

References

1. Thomas A, Necchi A, Muneer A, et al.. Penile cancer. *Nat Rev Dis Primers*. 2021;7(1):11. doi: 10.1038/s41572-021-00246-5 [PubMed: 33574340] [CrossRef: 10.1038/s41572-021-00246-5]

2. Brouwer OR, Albersen M, Parnham A, et al.. European Association of Urology–American Society of Clinical Oncology Collaborative Guideline on Penile Cancer: 2023 Update. *Eur Urol*. 2023;83(6)548-560. doi: 10.1016/j.eururo.2023.02.027 [PubMed: 36906413] [CrossRef: 10.1016/j.eururo.2023.02.027]

3. Joshi SS, Handorf E, Strauss D, et al.. Treatment trends and outcomes for patients with lymph node–positive cancer of the penis. *JAMA Oncol*. 2018;4(5):643-649. doi: 10.1001/jamaoncol.2017.5608 [PMCID: PMC5885184] [PubMed: 29494739] [CrossRef: 10.1001/jamaoncol.2017.5608]

4. Pagliaro LC, Williams DL, Daliani D, et al.. Neoadjuvant paclitaxel, ifosfamide, and cisplatin chemotherapy for metastatic penile cancer: a phase II study. *J Clin Oncol*. 2010;28(24):3851-3857. doi: 10.1200/JCO.2010.29.5477 [PMCID: PMC2940402] [PubMed: 20625118] [CrossRef: 10.1200/JCO.2010.29.5477]

5. Clark PE, Spiess PE, Agarwal N, et al.; National Comprehensive Cancer Network . Penile cancer: clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. 2013;11(5):594-615. doi: 10.6004/jnccn.2013.0075 [PMCID: PMC4042432] [PubMed: 23667209] [CrossRef: 10.6004/jnccn.2013.0075]

6. Azizi M, Aydin AM, Hajiran A, et al.. Systematic review and meta-analysis: is there a benefit in using neoadjuvant systemic chemotherapy for locally advanced penile squamous cell carcinoma? *J Urol*. 2020;203(6):1147-1155. doi: 10.1097/JU.0000000000000746 [PubMed: 31928407] [CrossRef: 10.1097/JU.0000000000000746]

7. Jacob JM, Ferry EK, Gay LM, et al.. Comparative genomic profiling of refractory and metastatic penile and nonpenile cutaneous squamous cell carcinoma: implications for selection of systemic therapy. *J Urol*. 2019;201(3):541-548. doi: 10.1016/j.juro.2018.09.056 [PubMed: 30291913] [CrossRef: 10.1016/j.juro.2018.09.056]

8. Joshi VB, Spiess PE, Necchi A, et al.. Immune-based therapies in penile cancer. *Nat Rev Urol*. 2022;19(8):457-474. doi: 10.1038/s41585-022-00617-x [PubMed: 35851333] [CrossRef: 10.1038/s41585-022-00617-x]

9. Aydin AM, Chahoud J, Adashek JJ, et al.. Understanding genomics and the immune environment of penile cancer to improve therapy. *Nat Rev Urol*. 2020;17(10):555-570. doi: 10.1038/s41585-020-0359-z [PubMed: 32812000] [CrossRef: 10.1038/s41585-020-0359-z]

10. Klempner SJ, Fabrizio D, Bane S, et al.. Tumor mutational burden as a predictive biomarker for response to immune checkpoint inhibitors: a review of current evidence. *Oncologist*. 2020;25(1):e147-e159. doi: 10.1634/theoncologist.2019-0244 [PMCID: PMC6964127] [PubMed: 31578273] [CrossRef: 10.1634/theoncologist.2019-0244]

11. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med*. 2017;377(25):2500-2501. doi: 10.1056/NEJMc1713444 [PMCID: PMC6549688] [PubMed: 29262275] [CrossRef: 10.1056/NEJMc1713444]

12. Necchi A, Spiess PE, Bandini M, et al.. Advanced squamous cell carcinomas of the pelvic and perineal region: a comprehensive genomic profiling study. *Oncologist*. 2022;27(12):1016-1024. doi: 10.1093/oncolo/oyac144 [PMCID: PMC10259761] [PubMed: 35881043] [CrossRef: 10.1093/oncolo/oyac144]

13. Frampton GM, Fichtenholtz A, Otto GA, et al.. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023-1031. doi: 10.1038/nbt.2696 [PMCID: PMC5710001] [PubMed: 24142049] [CrossRef: 10.1038/nbt.2696]

14. Chalmers ZR, Connelly CF, Fabrizio D, et al.. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9(1):34. doi: 10.1186/s13073-017-0424-2 [PMCID: PMC5395719] [PubMed: 28420421] [CrossRef: 10.1186/s13073-017-0424-2]

15. Trabucco SE, Gowen K, Maund SL, et al.. A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1000 microsatellite instability-high cases in 67,000 patient samples. *J Mol Diagn*. 2019;21(6):1053-1066. doi: 10.1016/j.jmoldx.2019.06.011 [PMCID: PMC7807551] [PubMed: 31445211] [CrossRef: 10.1016/j.jmoldx.2019.06.011]

16. Coleman RL, Oza AM, Lorusso D, et al.; ARIEL3 investigators . Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017;390(10106):1949-1961. doi: 10.1016/S0140-6736(17)32440-6 [PMCID: PMC5901715] [PubMed: 28916367] [CrossRef: 10.1016/S0140-6736(17)32440-6]

17. Tate JG, Bamford S, Jubb HC, et al.. COSMIC: the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res*. 2019;47(D1):D941-D947. doi: 10.1093/nar/gky1015 [PMCID: PMC6323903] [PubMed: 30371878] [CrossRef: 10.1093/nar/gky1015]

18. Newburg J, Connolly C, Frampton G. Determining patient ancestry based on targeted tumor comprehensive genomic profiling. *Cancer Res*. 2019;79(13 suppl):1599. doi: 10.1158/1538-7445.AM2019-1599 [CrossRef: 10.1158/1538-7445.AM2019-1599]

19. Sun JX, He Y, Sanford E, et al.. A computational approach to distinguish somatic vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. *PLoS Comput Biol*. 2018;14(2):e1005965. doi: 10.1371/journal.pcbi.1005965 [PMCID: PMC5832436] [PubMed: 29415044] [CrossRef: 10.1371/journal.pcbi.1005965]

20. Petljak M, Alexandrov LB, Brammeld JS, et al.. characterizing mutational signatures in human cancer cell lines reveals episodic APOBEC mutagenesis. *Cell*. 2019;176(6):1282-1294.e20. doi: 10.1016/j.cell.2019.02.012 [PMCID: PMC6424819] [PubMed: 30849372] [CrossRef: 10.1016/j.cell.2019.02.012]

21. Knepper TC, Montesion M, Russell JS, et al.. The genomic landscape of merkel cell carcinoma and clinicogenomic biomarkers of response to immune checkpoint inhibitor therapy. *Clin Cancer Res*. 2019;25(19):5961-5971. doi: 10.1158/1078-0432.CCR-18-4159 [PMCID: PMC6774882] [PubMed: 31399473] [CrossRef: 10.1158/1078-0432.CCR-18-4159]

22. Singal G, Miller PG, Agarwala V, et al.. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non–small cell lung cancer using a clinicogenomic database. *JAMA*. 2019;321(14):1391-1399. doi: 10.1001/jama.2019.3241 [PMCID: PMC6459115] [PubMed: 30964529] [CrossRef: 10.1001/jama.2019.3241]

23. Adamson B, Waskom M, Blarre A, et al.. Approach to machine learning for extraction of real-world data variables from electronic health records. *Front Pharmacol*. 2023;14. doi: 10.3389/fphar.2023.1180962 [PMCID: PMC10541019] [PubMed: 37781703] [CrossRef: 10.3389/fphar.2023.1180962]

24. Chahoud J, Gleber-Netto FO, McCormick BZ, et al.. Whole-exome sequencing in penile squamous cell carcinoma uncovers novel prognostic categorization and drug targets similar to head and neck squamous cell carcinoma. *Clin Cancer Res*. 2021;27(9):2560-2570. doi: 10.1158/1078-0432.CCR-20-4004 [PubMed: 33441293] [CrossRef: 10.1158/1078-0432.CCR-20-4004]

25. Ali SM, Pal SK, Wang K, et al.. Comprehensive genomic profiling of advanced penile carcinoma suggests a high frequency of clinically relevant genomic alterations. *Oncologist*. 2016;21(1):33-39. doi: 10.1634/theoncologist.2015-0241 [PMCID: PMC4709208] [PubMed: 26670666] [CrossRef: 10.1634/theoncologist.2015-0241]

26. Gandara DR, Agarwal N, Gupta S, et al.. Tumor mutational burden (TMB) measurement from an FDA-approved assay and real-world overall survival (rwOS) on single-agent immune checkpoint inhibitors (ICI) in over 8,000 patients across 24 cancer types. *J Clin Oncol*. 2023;41(16 suppl):2503. doi: 10.1200/JCO.2023.41.16_suppl.2503 [CrossRef: 10.1200/JCO.2023.41.16_suppl.2503]

27. Dhar SS, Lee MG. Cancer-epigenetic function of the histone methyltransferase KMT2D and therapeutic opportunities for the treatment of KMT2D-deficient tumors. *Oncotarget*. 2021;12(13):1296-1308. doi: 10.18632/oncotarget.27988 [PMCID: PMC8238240] [PubMed: 34194626] [CrossRef: 10.18632/oncotarget.27988]

28. Jiang F, Luo F, Zeng N, et al.. Characterization of fatty acid metabolism-related genes landscape for predicting prognosis and aiding immunotherapy in glioma patients. *Front Immunol*. 2022;13:902143. [PMCID: PMC9315048] [PubMed: 35903107]

29. Apolo AB, Nadal R, Girardi DM, et al.. Phase I study of cabozantinib and nivolumab alone or with ipilimumab for advanced or metastatic urothelial carcinoma and other genitourinary tumors. *J Clin Oncol*. 2020;38(31):3672-3684. doi: 10.1200/JCO.20.01652 [PMCID: PMC7605393] [PubMed: 32915679] [CrossRef: 10.1200/JCO.20.01652]

30. McGregor BA, Campbell MT, Xie W, et al.. Results of a multicenter, phase 2 study of nivolumab and ipilimumab for patients with advanced rare genitourinary malignancies. *Cancer*. 2021;127(6):840-849. doi: 10.1002/cncr.33328 [PubMed: 33216356] [CrossRef: 10.1002/cncr.33328]

31. de Vries HM, Rafael TS, Gil-Jimenez A, et al.. Atezolizumab with or without radiotherapy for advanced squamous cell carcinoma of the penis (the PERICLES study): a phase II trial. *J Clin Oncol*. 2023;41(31):4872-4880. doi: 10.1200/JCO.22.02894 [PubMed: 37487169] [CrossRef: 10.1200/JCO.22.02894]

32. El Zarif T, Nassar AH, Pond GR, et al.. Safety and efficacy of immune checkpoint inhibitors in advanced penile cancer: report from the Global Society of Rare Genitourinary Tumors. *J Natl Cancer Inst*. 2023:djad155. doi: 10.1093/jnci/djad155 [PMCID: PMC11032703] [PubMed: 37563779] [CrossRef: 10.1093/jnci/djad155]

33. Cotait Maluf F, Trindade K, D'Almeida Preto D, et al.. A phase II trial of pembrolizumab combined with platinum-based chemotherapy as first-line systemic therapy in advanced penile cancer: HERCULES (LACOG 0218) trial. *J Clin Oncol*. 2023;41(suppl 6):TPS14. doi: 10.1200/JCO.2023.41.6_suppl.TPS14 [CrossRef: 10.1200/JCO.2023.41.6_suppl.TPS14]

34. Renninson E, Challapalli A, Foulstone E, et al.. A phase II trial of cemiplimab alone or in combination with standard of care chemotherapy in locally advanced or metastatic penile carcinoma (EPIC Trial). *J Clin Oncol*. 2022;40(suppl 16):TPS5094.doi: 10.1200/JCO.2022.40.16_suppl.TPS5094 [CrossRef: 10.1200/JCO.2022.40.16_suppl.TPS5094]

35. Necchi A, Nicolai N, Colecchia M, et al.. Proof of activity of anti–epidermal growth factor receptor-targeted therapy for relapsed squamous cell carcinoma of the penis. *J Clin Oncol*. 2011;29(22):e650-e652. doi: 10.1200/JCO.2011.34.8367 [PubMed: 21632506] [CrossRef: 10.1200/JCO.2011.34.8367]

36. Necchi A, Lo Vullo S, Perrone F, et al.. First-line therapy with dacomitinib, an orally available pan-HER tyrosine kinase inhibitor, for locally advanced or metastatic penile squamous cell carcinoma: results of an open-label, single-arm, single-centre, phase 2 study. *BJU Int*. 2018;121(3):348-356. doi: 10.1111/bju.14013 [PubMed: 28921872] [CrossRef: 10.1111/bju.14013]

37. An X, Yan R, Guo S, et al.. Anti-EGFR antibody plus anti-PD-1 antibody and chemotherapy as a neoadjuvant regimen for patients with locally-advanced penile squamous cell carcinoma: a prospective, single-arm, single-center, phase II clinical trial. *J Clin Oncol*. 2022;40(suppl 16):5037. doi: 10.1200/JCO.2022.40.16_suppl.5037 [CrossRef: 10.1200/JCO.2022.40.16_suppl.5037]

Figures and Tables

Table.

Genomic Alterations by TMB Level

Characteristic	Participants, No. (%)		Missing	*P* value[a]
	TMB low	TMB ≥10 mut/Mb		
No.	339	58	0	
Median (IQR) age, y	65 (53-73)	68 (61-78)		.09
Genomic alteration or tumor	5.5	6.2		.45
Genomic ancestry				
No.	339	58	0	
Admixed American[b]	72 (21.2)	11 (19.9)		>.99
African	25 (7.4)	9 (15.5)		.26
East Asian	9 (2.6)	0		>.99
European	228 (67.3)	38 (65.5)		>.99
South Asian	5 (1.4)	0		>.99
Microsatellite instability				
No.	328	57	12	
High	0	5 (8.8)		<.001
gLOH				
No.	210	46	141	
gLOH-high	13 (6.2)	3 (6.5)		>.99
COSMIC trinucleotide signature				
No.	34	53	310	
APOBEC	15 (44.1)	39 (73.6)		.05
MMR deficiency	3 (8.8)	7 (13.2)		>.99
Tobacco signature	1 (2.9)	0		.74
UV signature	1 (2.9)	3 (5.7)		>.99
PD-L1 IHC				
No.	111	25	261	
PD-L1 TPS <1%	24 (21.6)	10 (40.0)		.26
PD-L1 TPS 1%-49%	46 (41.4)	8 (32.0)		.78
PD-L1 TPS ≥50%	41 (36.9)	7 (28.0)		.78
Pathogenic genomic alteration				
No.	339	58	0	

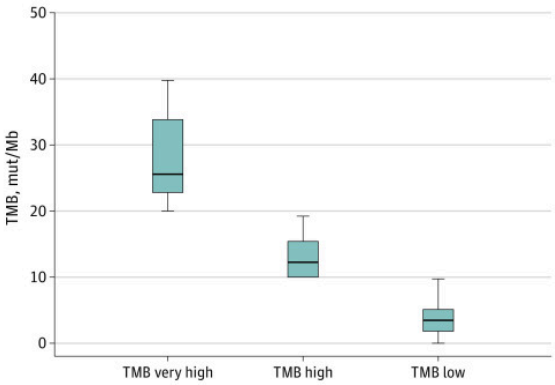
Abbreviations: APOBEC, apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide–like; COSMIC, Catalogue of Somatic Mutations in Cancer; gLOH, genome-wide loss of heterozygosity; IHC, immunohistochemistry; MMR, mismatch repair; mut/Mb, mutations per megabase; PD-L1, programmed cell death ligand 1; TMB, tumor mutational burden; TPS, tumor proportion score.

[a] False discovery rate corrected using Benjamini/Hochberg adjustment.

[b] Defined as a mixture of parts of the ancestry DNA signatures of those with European, sub-Saharan African, and/or Indigenous American ancestry.

[c] Homozygous deletion.

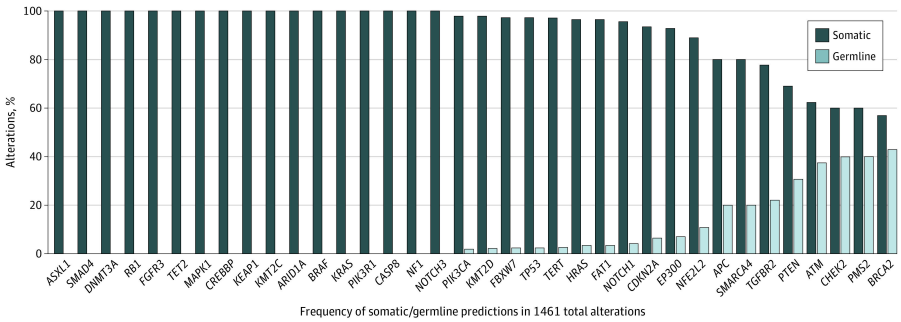
Figure 1.



Distribution of Tumor Mutational Burden (TMB) Values in All Patients With Advanced PSCC in the Foundation Medicine Database

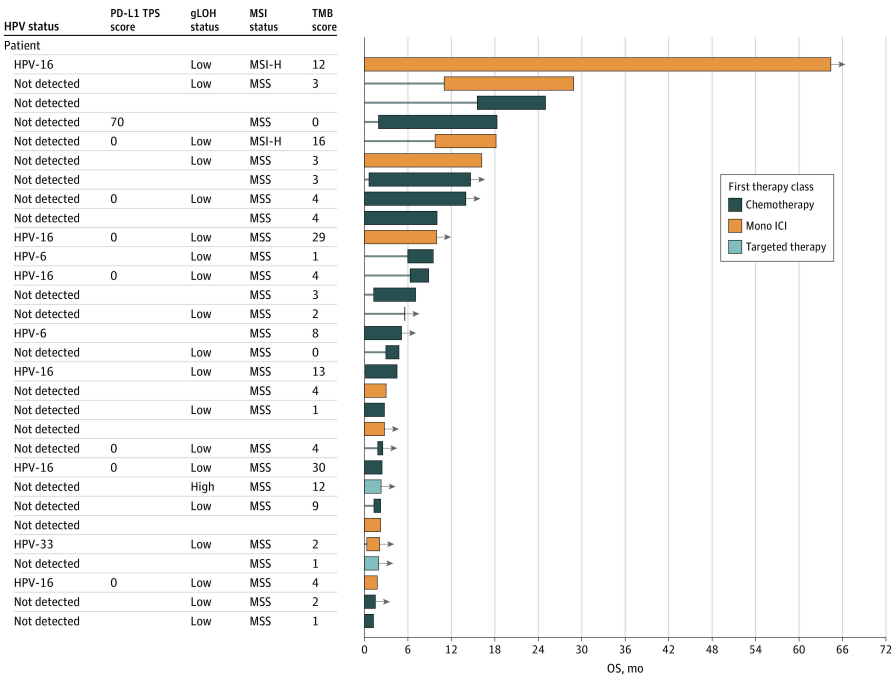
In box plots, the thick horizontal line indicates the median value; box ranges, 95% CIs; and whiskers, the range.

Figure 2.



Column Plot Illustrating Percentages of Somatic/Germline Predictions in 1461 Total Alterations

Figure 3.



HPV status	PD-L1 TPS score	gLOH status	MSI status	TMB score
Patient				
HPV-16		Low	MSI-H	12
Not detected		Low	MSS	3
Not detected				
Not detected	70		MSS	0
Not detected	0	Low	MSI-H	16
Not detected		Low	MSS	3
Not detected			MSS	3
Not detected	0	Low	MSS	4
Not detected			MSS	4
HPV-16	0	Low	MSS	29
HPV-6		Low	MSS	1
HPV-16	0	Low	MSS	4
Not detected			MSS	3
Not detected		Low	MSS	2
HPV-6			MSS	8
Not detected		Low	MSS	0
HPV-16		Low	MSS	13
Not detected			MSS	4
Not detected		Low	MSS	1
Not detected				
Not detected	0	Low	MSS	4
HPV-16	0	Low	MSS	30
Not detected		High	MSS	12
Not detected		Low	MSS	9
Not detected				
HPV-33		Low	MSS	2
Not detected			MSS	1
HPV-16	0	Low	MSS	4
Not detected		Low	MSS	2
Not detected		Low	MSS	1

Swimmer Plot of Overall Survival in Routine Clinical Practice From Start of First Systemic Antineoplastic Therapy in the Metastatic Setting

Bar color represents first-line therapy received (not all patients received therapy); gray segment, immortal time due to left truncation; arrow, patient who was right censored. gLOH indicates genome-wide loss of heterozygosity (score indicates status using a 16% cutoff[16]); HPV, human papillomavirus; ICI, immune checkpoint inhibitor; MSI, microsatellite instability; MSI-H, high microsatellite instability; MSS, microsatellite stable; PD-L1, programmed cell death ligand 1; TMB, tumor mutational burden; and TPS, tumor proportion score.