Genomic Profiles and Clinical Outcomes of Penile Squamous Cell Carcinoma With Elevated Tumor Mutational Burden
Andrea Necchi, MD; Philippe E. Spiess, MD, MS; Tiago Costa de Padua, MD; Roger Li, MD; Petros Grivas, MD, PhD; Richard S. P. Huang, MD; Douglas I. Lin, MD; Natalie Danziger, BS; Jeffrey S. Ross, MD; Joseph M. Jacob, MD, MCR; Rebecca A. Sager, MD; Alina Basnet, MD; Gerald Li, PhD; Ryon P. Graf, PhD; Dean C. Pavlick, PhD; Gennady Bratslavsky, MD

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 (continued)
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.


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. The mainstay of treatment continues to rely on radical inguinal lymphadenectomy, with limited contribution to survival by adding perioperative systemic therapies or radiotherapy. 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%. 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. 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%. 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). 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). 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. 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. 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. 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 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.\(^2\) Patient smoking status was extracted by natural language processing of electronic health record documents.\(^3\) 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 \(\geq 10\) 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 \(\geq 10\) 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 (\(\geq 10.0\%\)) 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Participants, No. (%)</th>
<th>TMB low</th>
<th>TMB ≥10 mut/Mb</th>
<th>Missing</th>
<th>P value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>339</td>
<td>58</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR) age, y</td>
<td>65 (53-73)</td>
<td>68 (61-78)</td>
<td></td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Genomic alteration or tumor</td>
<td>5.5</td>
<td>6.2</td>
<td></td>
<td>.45</td>
<td></td>
</tr>
<tr>
<td>Genomic ancestry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>339</td>
<td>58</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admixed American b</td>
<td>72 (21.2)</td>
<td>11 (19.9)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>25 (7.4)</td>
<td>9 (15.5)</td>
<td></td>
<td>.26</td>
<td></td>
</tr>
<tr>
<td>East Asian</td>
<td>9 (2.6)</td>
<td>0</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>228 (67.3)</td>
<td>38 (65.5)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>South Asian</td>
<td>5 (1.4)</td>
<td>0</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>328</td>
<td>57</td>
<td>12</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>5 (8.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gLOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>210</td>
<td>46</td>
<td>141</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gLOH-high</td>
<td>13 (6.2)</td>
<td>3 (6.5)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>COSMIC trinucleotide signature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>34</td>
<td>53</td>
<td>310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOBEC</td>
<td>15 (44.1)</td>
<td>39 (73.6)</td>
<td></td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>MMR deficiency</td>
<td>3 (8.8)</td>
<td>7 (13.2)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>Tobacco signature</td>
<td>1 (2.9)</td>
<td>0</td>
<td></td>
<td>.74</td>
<td></td>
</tr>
<tr>
<td>UV signature</td>
<td>1 (2.9)</td>
<td>3 (5.7)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>PD-L1 IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>111</td>
<td>25</td>
<td>261</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1 TPS &lt;1%</td>
<td>24 (21.6)</td>
<td>10 (40.0)</td>
<td></td>
<td>.26</td>
<td></td>
</tr>
<tr>
<td>PD-L1 TPS 1%-49%</td>
<td>46 (41.4)</td>
<td>8 (32.0)</td>
<td></td>
<td>.78</td>
<td></td>
</tr>
<tr>
<td>PD-L1 TPS ≥50%</td>
<td>41 (36.9)</td>
<td>7 (28.0)</td>
<td></td>
<td>.78</td>
<td></td>
</tr>
<tr>
<td>Pathogenic genomic alteration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>339</td>
<td>58</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD274 amplification</td>
<td>15 (4.6)</td>
<td>1 (1.8)</td>
<td></td>
<td>.78</td>
<td></td>
</tr>
<tr>
<td>CDKN2A</td>
<td>155 (45.7)</td>
<td>15 (25.9)</td>
<td></td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>EGFR</td>
<td>39 (11.5)</td>
<td>4 (6.9)</td>
<td></td>
<td>.74</td>
<td></td>
</tr>
<tr>
<td>FBXW7</td>
<td>30 (8.8)</td>
<td>4 (6.9)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>KMT2D</td>
<td>26 (7.7)</td>
<td>17 (29.3)</td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td>8 (2.4)</td>
<td>2 (3.4)</td>
<td></td>
<td>.95</td>
<td></td>
</tr>
<tr>
<td>MTAP lossb</td>
<td>11 (4.8)</td>
<td>3 (7.3)</td>
<td></td>
<td>.78</td>
<td></td>
</tr>
<tr>
<td>NOTCH1</td>
<td>63 (18.6)</td>
<td>6 (10.3)</td>
<td></td>
<td>.38</td>
<td></td>
</tr>
<tr>
<td>NOTCH2</td>
<td>5 (1.5)</td>
<td>1 (1.7)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td>62 (18.3)</td>
<td>28 (48.3)</td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>19 (5.6)</td>
<td>2 (3.4)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>RB1</td>
<td>11 (3.2)</td>
<td>5 (8.6)</td>
<td></td>
<td>.26</td>
<td></td>
</tr>
<tr>
<td>STK11</td>
<td>19 (5.6)</td>
<td>4 (6.9)</td>
<td></td>
<td>&gt;.99</td>
<td></td>
</tr>
<tr>
<td>TERT</td>
<td>157 (47.9)</td>
<td>18 (31.6)</td>
<td></td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>TPS3</td>
<td>193 (56.9)</td>
<td>23 (39.7)</td>
<td></td>
<td>.09</td>
<td></td>
</tr>
</tbody>
</table>

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.
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 spectrum 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

Figure 3. 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); 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.
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. The therapeutic options could include basket trials of NOTCH1 inhibitors, 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—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, 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.

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. 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. Conversely, no partial response was reported in 5 patients included in another study of nivolumab plus ipilimumab (2 stable disease and 3 progressive disease). 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. Finally, various ICI regimens tested in a heterogeneous population of chemotherapy-naïve 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. 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) and the EPIC Trial sponsored by Cancer Research UK (cemiplimab, with or without chemotherapy).

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 and the following phase 2 trial of dacomitinib, 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. 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. 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 (e.g., 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, long 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.
Incyte, Janssen, Merck, Rainer Therapeutics, and Roche outside the submitted work; and having a spouse with employment and stock in Bayer. Dr Spiess reported being the vice chair of the National Comprehensive Cancer Center bladder and penile cancer panel, president of Global Society of Rare Genitourinary Tumors, and a member of the American Society of Clinical Oncology/European Association of Urology penile cancer panel. Dr R. Li reported receiving grants from Predicine, Valar Labs, and Veracyte; receiving personal fees from Arquer Diagnostics, Bristol Meyers Squibb, CG Oncology, FerGene, Lucence, Merck, and UroGen Pharma; and receiving nonfinancial support from Janssen outside the submitted work. Dr Grivas reported receiving research funding from Bristol-Myers Squibb, G1 Therapeutics, Gilead Sciences, Merck KGaA, Mirati Therapeutics, MSD, Pfizer, and QED Therapeutics; receiving grants from Acirvon Therapeutics, ALX Oncology, Bavarian Nordic, Debiopharm Group, and GlaxoSmithKline; and receiving personal fees from 4D Pharma, Aadi Bioscience, Asieris Pharmaceuticals, Astellas, AstraZeneeca, BostonGene, Bristol Myers Squibb, CG Oncology, Dyania Health, Exelixis, Fresenius Kabi, G1 Therapeutics, Gilead Sciences, Guardant Health, ImmunityBio, Infinity Pharmaceuticals, Janssen, Lucence, Merck KGaA, Mirati Therapeutics, MSD, Pfizer, PureTech, QED Therapeutics, Regeneron, Roche, Seattle Genetics, Silverback Therapeutics, Strata Oncology, and UroGen Pharma outside the submitted work. Dr Huang reported receiving personal fees from Foundation Medicine Inc during the conduct of the study and outside the submitted work. Drs Lin, Danziger, G. Li, and Pavlick reported being employed by Foundation Medicine Inc and holding stock in F. Hoffmann-La Roche Ltd during the conduct of the study. Drs Ross and Graf reported being employed by Foundation Medicine Inc during the conduct of the study. No other disclosures were reported.

**Meeting Presentation:** Portions of this work were presented in an oral session at the Genitourinary Cancers Symposium, February 17, 2023, San Francisco, California.

**Data Sharing Statement:** See Supplement 3.

**REFERENCES**


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