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Association Between Human Papillomavirus Infection and Outcome of Perioperative Nodal Radiotherapy for Penile Carcinoma

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Abstract

Background: Data on the impact of human papillomavirus (HPV) infection status and outcomes for perioperative treatments for patients with lymph node–involved penile squamous-cell carcinoma (PSCC) are lacking.
Objective: To analyze the benefit from perioperative radiotherapy (RT) for PSCC according to HPV infection status.
Design, setting, and participants: In an international multicenter database of 1254 patients with PSCC who received inguinal lymph node dissection (ILND), 507 had suitable clinical information.
Intervention: ILND, with or without chemotherapy or RT for involved lymph nodes.
Outcome measurements and statistical analysis: Kaplan-Meier and restricted mean survival time (RMST) analyses for overall survival (OS) were performed for all patients and after propensity score-matching (PSM; n = 136), for which patient age, histology, type of penile surgical procedure, pathological tumor and nodal stage, ILND laterality, pelvic LND, and perioperative treatment were taken into account when assessing differences between HPV$^+$ and HPV– patients. Finally, we looked at genomic alterations in PSCC using data from the Foundation Medicine database (n = 199) to characterize HPV$^+$ PSCC.

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Results and limitations: Patients with HPV$^+$ PSCC (n = 86; 17%) had lower clinical N stage ($p < 0.001$) and inguinal lymph node metastasis density ($p < 0.001$). Perioperative RT was delivered in 49 patients (9.7%), with the vast majority receiving adjuvant RT (n = 40). HPV$^+$ patients had similar median OS (p = 0.1) but longer RMST than HPV– patients at different time points. Nevertheless, HPV$^+$ patients treated with perioperative RT exhibited longer median OS (p = 0.015) and longer RMST compared to HPV– patients. In the PSM cohorts, HPV$^+$ status remained significantly associated with longer OS after RT. The HPV– PSCC group had a higher frequency of *TP53* mutations compared to HPV$^+$ PSCC (75% vs 15%; $p < 0.001$). The results are limited by the retrospective nature of the data.

Conclusions: Perioperative RT was more effective in the HPV$^+$ PSCC subgroup. Reasons for the enhanced radiosensitivity may be related to the lack of *TP53* mutations.

Patient summary: We analyzed data from a large multicenter database for patients with penile cancer who had received inguinal lymph node dissection, with or without chemotherapy or radiotherapy. We found that for tumors positive for human papillomavirus (HPV), use of radiotherapy resulted in prolonged survival compared to HPV-negative tumors. On the basis of these results we are inspired to design studies on the use of radiotherapy in HPV-selected patients.
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Introduction

Penile squamous-cell carcinoma (PSCC) is a rare tumor in Western countries, accounting for less than 1% of all malignant tumors in these regions [1,2]. Peculiar features of PSCC include the heterogeneity of the geographical distribution [3] and an association with an underlying infection with oncogenic human papillomavirus (HPV), a driver of tumor development, in approximately 30–50% of patients, with more than 80% of such cases being attributable to infection with the high-risk HPV-16 genotype [4,5]. The latter feature naturally implies a possible contribution of preventive or therapeutic HPV vaccination in the treatment armamentarium for PSCC. Most noteworthy, multidisciplinary management of lymph node spread of PSCC provides a unique model for the management of genitourinary tumors, primarily in experienced centers, involving the use of regional lymphadenectomy as a backbone, with the addition of systemic chemotherapy or radiotherapy (RT) in selected cases [6,7].

In 2018 we started an international retrospective study of clinical cases registered in an international database. The aim of this study was to gather information on the therapeutic management of patients with PSCC who had received any type of inguinal lymph node dissection (ILND), with or without pelvic LND, in their clinical course [8,9]. Initial analyses from this database focused on prediction of ILN spread from the primary tumor [10]. In the present study, we focused on the association of HPV status with a survival benefit from RT. Although we know that HPV-positive (HPV$^+$) PSCC usually portends a more favorable prognosis and is characterized by a more indolent clinical course [11,12], we do not know if HPV$^+$ status confers different sensitivity to RT, as has been reported for other solid tumors [13,14]. Therefore, we tested HPV status as a possible predictive biomarker of the potential benefit of RT. Gathering this information could be very important for optimizing the use of adjuvant therapeutic solutions that are known to be moderately active among unselected patients.

Patients and methods

Patient selection

This retrospective study included individual patient-level data for men with PSCC treated from January 1980 to October 2019 at 11 tertiary care centers located in the USA, Europe, UK, Brazil, and China. The study was approved by the ethics committees at each participating institution. At the end of January 2020, data were extracted to select patients with suitable information for the present analysis. Details on the surgical templates and indications for pelvic LND across the centers are provided in the Supplementary material. All intraoperative and permanent sections were evaluated by dedicated genitourinary pathologists at each institution. Clinical and radiological examinations were performed by all centers according to routine practice. Staging was assigned according to the seventh edition of the American Joint Committee on Cancer staging manual [15]. Cases diagnosed before 2010 were reclassified according to this same edition. A flowchart of the patient selection process with reasons for patient exclusion is shown in Supplementary Figure 1. Perioperative RT was delivered based on lymph node status before or after ILND. Information on RT doses, fractions, and volumes, or on the use and type of chemosensitizing agents, was not available.

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Study objectives and statistical analyses

Descriptive analyses were based on standard methods. The study endpoint was overall survival (OS), calculated from the date of PSCC diagnosis to death from any cause.

The statistical significance of differences in medians and proportions was tested using Kruskal-Wallis and χ^2 tests, respectively.

The analyses were developed through several consecutive and logical steps. First, we explored differences in baseline characteristics and outcomes in the total population (n = 507). OS rates were assessed according to the perioperative treatment received (perioperative RT and chemotherapy) and according to geographical region.

Second, we assessed HPV status (HPV$^+$ vs HPV– patients) and predictors of HPV infection among geographical regions (Europe vs USA vs UK vs China), age at PSCC diagnosis, smoking status, circumcision, and histology, for which we used univariable and multivariable logistic regression analyses.

Third, we tested for OS differences between the HPV$^+$ and HPV– groups. Kaplan-Meier and restricted mean survival time (RMST) analyses were used to examine OS rates according to HPV status at different time points (3, 5, 7, and 10 yr of follow-up). In longitudinal clinical studies, RMST is useful for quantify underlying differences between groups with respect to a time-to-event endpoint, rather than hypothesis-testing procedures such as the log-rank test [16–19]. Results from the RMST were confutated by examining the estimate of the ratio of hazard functions (HPV$^+$ and HPV–) over time. The proportionality assumption was tested using the Schoenfeld residuals against transformed time. The presence of very small p values indicates that there are time-dependent coefficients (ie, the true hazard ratio [HR] is changing over time). Conversely, higher (close to 0.05) or nonsignificant p values indicate that the coefficient is not time-dependent (ie, the true HR is not changing over time). Multivariable Cox regression analyses tested the effect of HPV status on OS by accounting for predefined covariates.

Fourth, to determine whether HPV status has an influence on chemotherapy or RT efficacy, we tested OS rates according to perioperative chemotherapy or RT use across patients by HPV status.

Finally, for sensitivity analyses, we repeated analytical steps three and four after a 1:1 nearest-neighbor propensity–score matching (PSM; caliper = 0.05, n = 136) [20], where no difference between HPV$^+$ and HPV– status emerged in terms of the matching variables: patient age, histology, type of penile surgical procedure, pathological tumor and nodal stage, ILND laterality, use of pelvic LND, and use of perioperative chemotherapy and RT. All statistical tests were two-sided with the level of significance set at $p < 0.05$. Analyses were performed using R v.3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Genomic alterations in HPV$^+$ PSCC in the Foundation Medicine database

The Foundation Medicine database was queried for all cases with clinically advanced PSCC, and genomic alterations were analyzed according to HPV status as determined by a comprehensive genomic profiling (CGP) assay. In total, 199 PSCC samples from patients with advanced/metastatic PSCC were subjected to a hybrid capture–based CGP assay (FoundationONE) in a single certified laboratory with Clinical Laboratory Improvement Amendments certification (Foundation Medicine, Inc., Cambridge, MA, USA). Approval for this portion of the study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional review board (Protocol No. 20152817). Genomic alteration frequencies were compared using Fisher's exact test. Details of the genomic analyses for these tumor samples are reported in the Supplementary material.

Results

Patient and treatment characteristics and associated survival

A total of 507 patients were included in the database. Of these, 226 patients received perioperative chemotherapy as neoadjuvant (n = 110; 21.7%) or adjuvant therapy (n = 116; 22.9%). A total of 49 patients received perioperative RT as neoadjuvant (n = 9; 1.8%) or adjuvant (n = 40; 7.9%) therapy. OS for these patients is shown in Supplementary Figure 2A. OS data for subgroups according to geographical region, use of perioperative chemotherapy, and use of perioperative RT are shown in Supplementary Figure 2B–D. In general, when considering the entire population, the efficacy of additional therapies was seemingly poor or minimal, as we have previously reported [8].

HPV rates and predictors

In total, there were 86 (17%) HPV$^+$ and 421 (83%) HPV– PSCC cases. The baseline characteristics are shown in Table 1. HPV infection was detected by immunohistochemistry (IHC; as p16 staining) in 263 cases (51.9%), by in situ hybridization in 241 cases (47.5%), and by polymerase chain reaction in three patients (0.6%). The distribution of HPV positivity according to geographical area is shown in Supplementary Figure 3. In the study cohort, patients with HPV$^+$ PSCC had a lower burden of inguinal lymph node metastases (ILNM). This was identified in terms of both pathologic N stage ($p < 0.001$) and ILNM density ($p < 0.001$). Results for the logistic regression analyses are shown in Supplementary Table 1.

Ancestry analysis of the sequencing data revealed similar frequencies for East Asian, European, and South Asian patients. The HPV$^+$ cases featured a higher number of African ancestry cases (11% vs 5%), which was not significant, and a lower frequency of admixed American cases (8% vs 23%), which was significant (p = 0.01).

Survival analyses across HPV$^+$ and HPV– patients regardless of treatment received

The median follow-up was 70 mo (interquartile range [IQR] 35–122). In the total patient population, median OS was similar between HPV$^+$ and HPV– patients (p = 0.1; Fig. 1A).

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Table 1 – Baseline characteristics of the study population

Variable	Overall (n = 507)	HPV– (n = 421)	HPV$^+$ (n = 86)	p value
Median age at diagnosis, yr (IQR)	59 (50–68)	58 (49–68)	60 (51–69)	0.4
Median inguinal LNM density, % (IQR)	14.3 (5–25)	14.3 (6.7–25)	6.9 (0–22.2)	<0.001
Geographic distribution, n (%)				<0.001
Europe	388 (76.5)	358 (85)	30 (34.9)	
China	61 (12)	29 (6.9)	32 (37.2)	
UK	15 (3)	11 (2.6)	4 (4.6)	
USA	43 (8.5)	23 (5.5)	20 (23.3)	
HPV assessment technique, n (%)				<0.001
In situ hybridization	241 (47.5)	163 (38.8)	78 (90.7)	
p16 (immunohistochemistry)	263 (51.9)	257 (61.0)	6 (7.0)	
Polymerase chain reaction	3 (0.6)	1 (0.2)	2 (2.3)	
Smoking status, n (%)				0.1
Current/former	195 (38.4)	163 (38.7)	32 (37.2)	
Never	119 (23.5)	92 (21.9)	27 (31.4)	
NA	193 (38.1)	166 (39.4)	27 (31.4)	
Histology, n (%)				0.2
SCC	473 (93.3)	396 (94.1)	77 (89.5)	
Non-SCC	34 (6.7)	25 (5.9)	9 (10.5)	
Pathologic T stage, n (%)				0.047
<T2	193 (38.1)	170 (40.4)	23 (26.8)	
T2	205 (40.4)	166 (39.4)	39 (45.3)	
T3–4	109 (21.5)	85 (20.2)	24 (27.9)	
Pathologic N stage, n (%)				<0.01
N0	114 (22.5)	83 (19.7)	31 (36)	
N1–2	168 (33.1)	139 (33.0)	29 (33.8)	
N3	225 (44.4)	199 (47.3)	26 (30.2)	
Inguinal LND, n (%)				<0.001
Monolateral	143 (28.2)	137 (32.5)	6 (7.0)	
Bilateral	364 (71.8)	284 (67.5)	80 (93)	
LNM laterality, n (%)				<0.001
Monolateral	125 (24.7)	107 (25.4)	18 (20.9)	
Bilateral	146 (28.8)	117 (27.8)	29 (33.8)	
No LNM	99 (19.5)	68 (16.2)	31 (36.0)	
NA	137 (27.0)	129 (30.6)	8 (9.3)	
Surgical procedure, n (%)				<0.01
Local/no procedures	62 (12.2)	56 (13.3)	6 (7.0)	
Partial penectomy	296 (58.4)	230 (54.6)	66 (76.7)	
Total penectomy	144 (28.4)	130 (30.9)	14 (16.3)	
NA	5 (1.0)	5 (1.2)	0 (0)	
Perioperative CTx, n (%)				<0.001
No CTx	212 (41.8)	181 (43.0)	31 (36.0)	
Perioperative CTx	226 (44.6)	206 (48.9)	20 (23.3)	
NA	69 (13.6)	34 (8.1)	35 (40.7)	
Perioperative RT, n (%)				<0.001
No RT	388 (76.5)	354 (84.1)	34 (39.5)	
Perioperative RT	49 (9.7)	32 (7.6)	17 (19.8)	
NA	70 (13.8)	35 (8.3)	35 (40.7)	
Pelvic LND, n (%)				<0.01
Yes	245 (48.3)	216 (51.3)	29 (33.7)	
No	262 (51.7)	205 (48.7)	57 (66.3)	

CTx = chemotherapy; HPV = human papillomavirus; IQR = interquartile range; LND = lymph node dissection; LNM = lymph node metastases; NA = not available; RT = radiotherapy; SCC = squamous-cell carcinoma.

However, RMST analyses showed longer OS for HPV$^+$ than for HPV– patients at 3 yr (3.5 mo, 95% confidence interval [CI] 1.6–5.3; $p < 0.001$), 5 yr (7.2 mo, 95% CI 3.0–11.4; $p = 0.001$), and 7 yr (9.7 mo, 95% CI 2.1–16.6; $p = 0.006$) of follow-up, but not at 10 yr (8.3 mo, 95% CI –3.7 to 20.2; $p = 0.2$). The overall HR estimate for HPV$^+$ status of 0.7 (95% CI 0.4–1.1; $p = 0.1$) changed over time ($p = 0.008$; Fig. 1B). In multivariable Cox regression analyses (Supplementary Table 2), HPV$^+$ status was not associated with OS (HR 0.7, 95% CI 0.4–1.1; $p = 0.1$).

Survival analyses across HPV$^+$ and HPV– patients according to perioperative RT and chemotherapy

When considering only patients treated with perioperative RT, HPV$^+$ status was associated with longer median OS ($p = 0.015$; Fig. 2A) and longer RMST at 3 yr (9.5 mo, 95% CI 5.7–13.2; $p < 0.001$), 5 yr (23.3 mo, 95% CI 15.5–31.1; $p < 0.001$), 7 yr (32.3 mo, 95% CI 17.8–46.9; $p < 0.001$), and 10 yr (39.1 mo, 95% CI 11.8–66.4; $p = 0.005$) of follow-up when compared with HPV– patients. In this case, the overall HR

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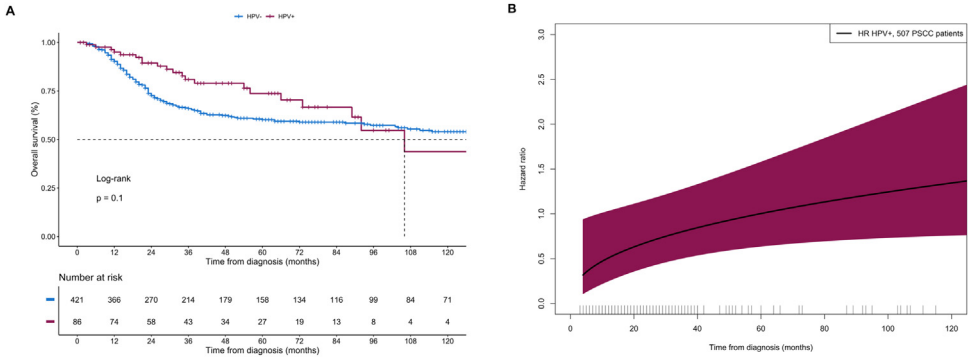


Fig. 1 – (A) Kaplan-Meier curves of overall survival for the total study population according to human papillomavirus (HPV) status. (B) Plot showing the hazard ratio (HR) trend for overall survival for HPV$^+$ infection status, indicated from the time of penile squamous-cell carcinoma (PSCC) diagnosis.

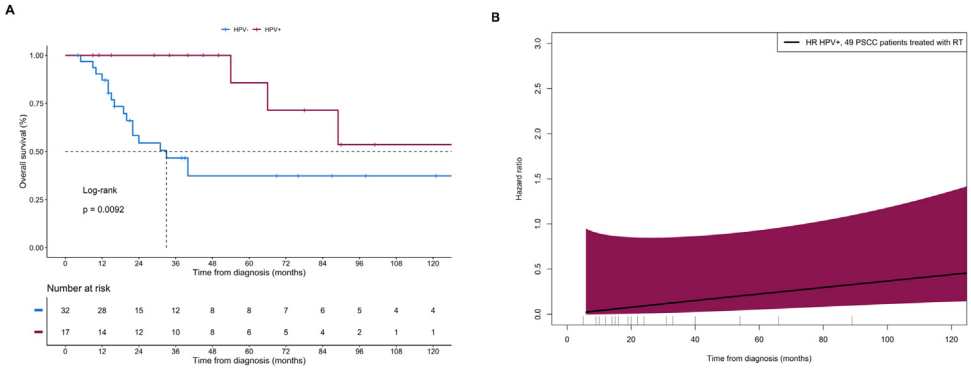


Fig. 2 – (A) Kaplan-Meier curves of overall survival in the population of radiotherapy (RT)-treated patients according to human papillomavirus (HPV) infection status. (B) Plot showing the hazard ratio (HR) for overall survival for HPV$^+$ infection status, indicated from the time of penile squamous-cell carcinoma (PSCC) diagnosis.

estimate of 0.2 (95% CI 0.1–0.8; $p = 0.02$) did not change over time ($p = 0.06$; Fig. 2B).

These findings were not confirmed in the subgroup of patients treated with perioperative chemotherapy ($p = 0.45$). In multivariable Cox regression analyses (Supplementary Table 2), patients with HPV$^+$ PSCC exhibited significantly better OS than patients with HPV– PSCC (HR 0.12, 95% CI 0.03–0.48; $p = 0.003$).

Sensitivity analyses after PSM

After PSM, we identified 68 HPV$^+$ and 68 HPV– patients with nonsignificant differences in clinical and pathological characteristics (Supplementary Table 3).

In the PSM cohort, OS differences between the HPV– and HPV$^+$ groups disappeared in terms of both median OS ($p = 0.62$; Fig. 3A) and RMST differences. Accordingly, the HR estimate of 0.8 (95% CI 0.4–1.6; $p = 0.6$) was more stable over time ($p = 0.04$; Fig. 3B).

Conversely, in the subgroup of PSM patients treated with perioperative RT, HPV$^+$ status remained associated with longer median OS ($p = 0.006$; Fig. 4A) and longer RMST differences at 3 yr (11.1 mo, 95% CI 4.5–17.6; $p = 0.001$), 5 yr (27.6 mo, 95% CI 15.3–39.9; $p < 0.001$), 7 yr (39.5 mo, 95% CI 19–60; $p < 0.001$), and 10 yr (50.5 mo, 95% CI 15.3–85.7; $p = 0.005$) of follow-up. Accordingly, the HR estimate of 0.2 (95% CI 0.1–0.7; $p = 0.009$) did not change over time ($p = 0.06$; Fig. 4B). Again, these findings were not observed for perioperative chemotherapy ($p = 0.2$).

CGP of HPV$^+$ versus HPV– tumors from the Foundation Medicine database

In total, 62 HPV$^+$ and 137 HPV– cases with CGP data were identified. The median age of HPV$^+$ patients was 64.5 yr (IQR 58–73.7) versus 63 yr (IQR 54–71) for HPV– patients.

The genomic alterations in these subgroups are shown in Figure 5 (raw data available in the Supplementary material). The most frequently altered genes (>20%) among HPV$^+$ cases were *PI3KCA* (38.7% in HPV$^+$ vs 17% in HPV–; $p < 0.0001$) and *KMT2D* (25.8% in HPV$^+$ vs 3% in HPV–; $p < 0.0001$). Among HPV– cases the most frequently altered genes were *TP53* (75.2% in HPV$^+$ vs 15% in HPV–; $p < 0.0001$), *CDKN2A* (65% in HPV$^+$ vs 1% in HPV–; $p < 0.0001$), and *TERT* (promoter region; 60.2% in HPV$^+$ vs 9% in HPV–; $p < 0.0001$). Microsatellite instability-high status was observed for one (1.6%) HPV$^+$ and one (0.7%) HPV– case. The median TMB among HPV$^+$ cases was 5.2 mutations/Mb (IQR 2.7–10.3);

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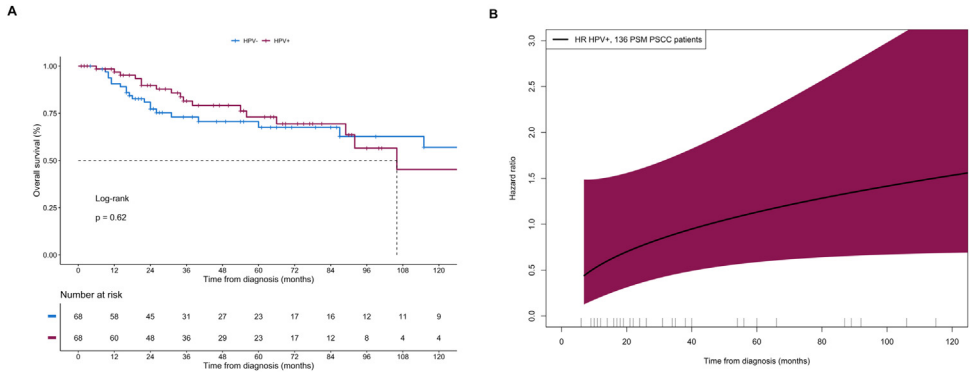


Fig. 3 – (A) Kaplan-Meier curves of overall survival in the propensity score–matched (PSM) population according to human papillomavirus (HPV) status. (B) Plot showing the hazard ratio (HR) trend for overall survival for HPV⁺ infection status, indicated from the time of penile squamous-cell carcinoma (PSCC) diagnosis.

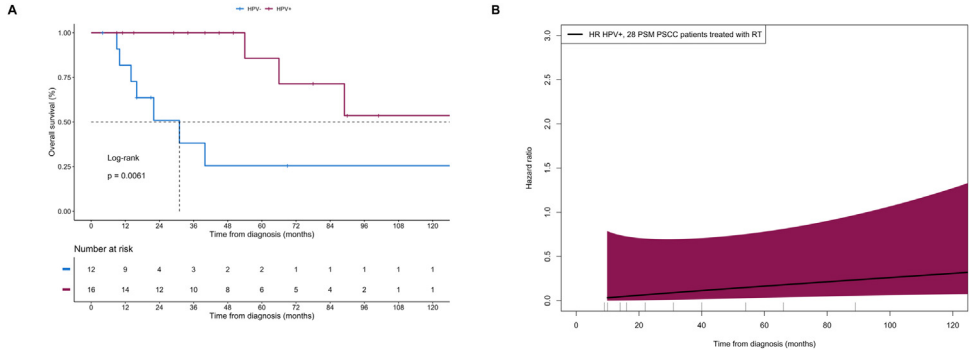


Fig. 4 – (A) Kaplan-Meier curves of overall survival in the propensity score–matched (PSM) population of radiotherapy (RT)-treated patients according to human papillomavirus (HPV) status. (B) Plot showing the hazard ratio (HR) trend for overall survival for HPV⁺ infection status, indicated from the time of penile squamous-cell carcinoma (PSCC) diagnosis.

27% of cases had a TMB of ≥10 mutations/Mb and 13% had ≥20 mutations/Mb. For the HPV– cases, the median TMB was 3.2 mutations/Mb (IQR: 1.7-5.2); 10% had a TMB of ≥10 mutations/Mb and 2% had ≥20 mutations/Mb.

Discussion

In PSCC, identification of novel clinical or tumor-related factors for selection of the most suitable candidates for multidisciplinary treatment represents one of the main goals of clinical research.

This study, which used one of the largest cohorts of PSCC patients who underwent ILND, provides new insights into the role of HPV infection as a biomarker of clinical outcomes for patients treated using a combined modality approach. There are heterogeneous data in the literature regarding the role of HPV in PSCC. The main limitation is that these studies included patients with mixed clinical stages who were treated with different surgical approaches and additional therapies [5,11,12].

We know that metastatic lymph node spread in PSCC marks a profound difference between curable disease (absence of lymph node involvement) and high-risk disease (ILN spread), which can easily become incurable disease, depending on the burden of metastatic lymph node spread. Therefore, by focusing on PSCC patients who have homogeneously undergone ILND in their clinical course, we set optimal conditions for analyzing the effect of additional therapies and tumor biomarkers.

The study revealed interesting findings that could be important to test in prospective randomized clinical trials. The most important is certainly the effectiveness of perioperative RT in the subgroup of patients with HPV⁺ tumors. This effect was maintained over time, as demonstrated by the relative "stability" of the HR for HPV against the OS endpoint. Because of the lack of data showing a benefit of RT, in particular postoperative RT for lymph node–involved PSCC, use of RT has not been widely reported for this tumor type [21].

The current version of the European Association of Urology (EAU) guidelines for PSCC supports RT in select patients with extranodal extension, as a benefit emerged from previous analyses we performed for the present database [22]. In addition, an exquisite effect of adjuvant inguinopelvic chemoRT in the select HPV⁺ PSCC population was originally reported for a limited number of patients by authors from the Moffitt Cancer Center [23]. Better disease-specific survival and a lower risk of regional recurrence were observed with this approach.

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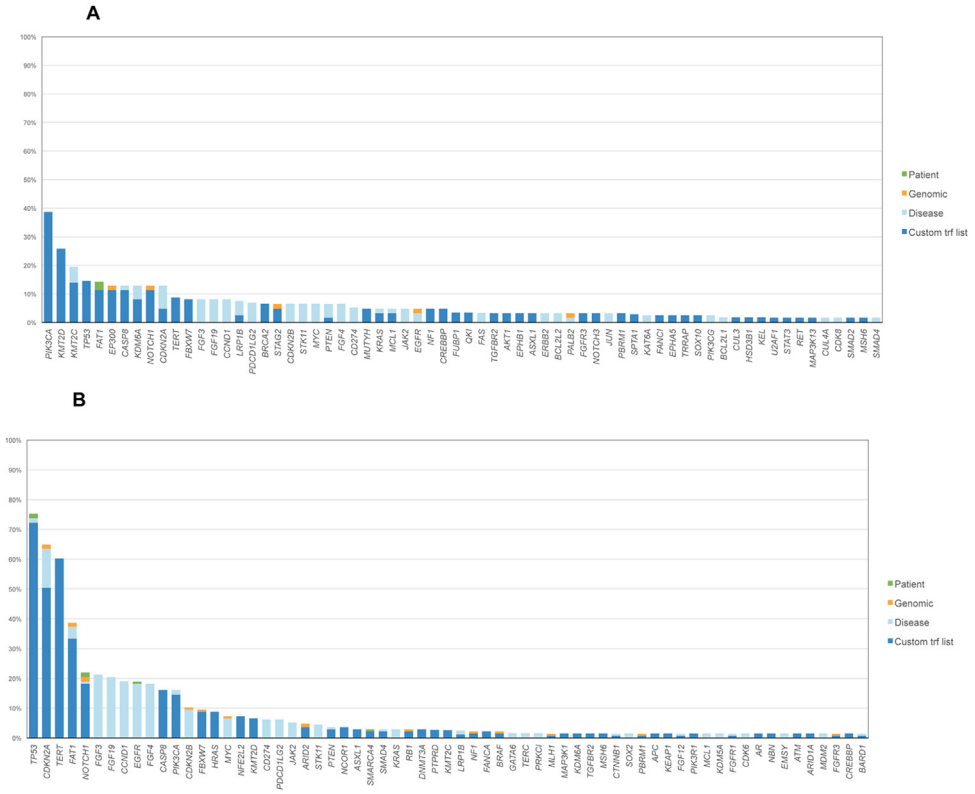


Fig. 5 – Plots showing the most frequent genomic alterations observed in the penile squamous-cell carcinoma population from the Foundation Medicine database for (A) HPV$^+$ and (B) HPV– samples. HPV = human papillomavirus.

For most of the cases reported here (approx. 75%), RT was delivered postoperatively on the basis of lymph node status in the draining bed. Unfortunately, as a major limitation of our findings, we were unable to separately analyze the impact of preoperative and postoperative treatments because of the limited number of cases and events, but for RT in particular we could assume that the major impact of our findings is related to adjuvant RT. Further limitations include a lack of information regarding the use and type of chemosensitizing agents and the fields for RT in the database. In addition, owing to the database characteristics, we were unable to adjust the multivariable analyses according to year of treatment, which is a potentially important bias because it is expected that multidisciplinary treatments may vary over time, although their global impact on survival remains limited.

Despite these limitations, the results provide a rationale for prospectively comparing the efficacy of RT (as chemoRT) as an adjuvant therapy after ILND or as a definitive approach, compared with ILND alone, in patients with ILN spread from HPV$^+$ PSCC.

Interestingly, the results confirm the lack of a well-suited role for perioperative chemotherapy in locally advanced PSCC, despite the current guideline indications. There are conflicting data on the impact of chemotherapy on OS, although neoadjuvant chemotherapy is still suggested by the EAU guidelines for unresectable locally advanced PSCC, based on limited prospective data [24].

The availability of a predictive biomarker of the effect of RT could be exceptionally useful for these rare tumors, for which an effect of small magnitude of perioperative treatments is typically diluted in unselected populations, and is usually not clinically meaningful. This is the typical case for PSCC, for which neither perioperative chemotherapy nor perioperative RT provided a survival improvement, in agreement with data from the US National Cancer Data Base [25]. To the best of our knowledge, although there is a significant amount of literature on this subject, there is currently no specific genomic alteration "signature" that is accepted as a validated predictor of radiosensitivity or radioresistance. Although only hypothesis-generating, a recent study demonstrated heterogeneity in intrinsic PSCC radiosensitivity using a genome-adjusted radiation dose [26,27], which was derived in part by assessing the relationship between *TP53* mutation status and in vitro cell survival following 2 Gy of RT [28]. According to this study, approximately 50% of the patients in our cohort who received uniform RT dosing (50–54 Gy) would have achieved durable locoregional control, suggesting that personalized RT dosing might further optimize these outcomes. Previous studies of DNA damage repair (DDR) genes have generally shown that inactivating mutations in such genes (eg, *BRCA1*, *BRCA2*, *ATM*, *ATX*, *RAD21*, *RAD51*, *XPD*, and others) are linked to responsiveness to platinum-based and other DNA-damaging chemotherapies. This could be also true, to some extent, for predicting the benefit from RT

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[29,30]. In our study we observed a higher number of *BRCA2* mutations in the HPV$^+$ group, but we did not observe any differences for the other DDR genes, all of which are rarely altered in PSCC. Conversely, the median TMB was higher in HPV$^+$ than in HPV– tumors. HPV– PSCC also had significant alterations in other genomic-fidelity regulators such as *CDKN2A* and *TERT* (both >40%).

The major genomic difference, however, is the significantly lower number of *TP53* mutations among HPV$^+$ patients. This is well described in the literature on head and neck and cervical SCC and there is a long-held theory that HPV– tumors rely on mutations in *TP53* to deregulate the cell cycle, whereas the viral "genome" itself is sufficient in HPV$^+$ tumors [31]. Of course the other theory is that HPV– SCC was once HPV$^+$, but when a *TP53* mutation occurs the "requirement" for HPV-mediated cell-cycle dysregulation is no longer necessary as the tumor progresses. Either way, the hypothesis here could be that the lower *TP53* mutation frequency for HPV$^+$ tumors impacts their sensitivity to RT. Interestingly, we previously reported that p53 IHC expression was also associated with inferior outcomes among patients treated with adjuvant cisplatin-based chemotherapy [32]. Therefore the lack of *TP53* mutation among HPV$^+$ cases could be a further reason to use cisplatin as a chemosensitizing agent, and this is definitely well suited as a stratification factor for randomized studies. The major limitation here is that we analyzed unpaired patient populations from clinical and genomic data sets. Thus, genomic analyses may not reflect the clinical findings from the multi-institutional data set. Lastly, our study was certainly affected by the heterogeneous assessment of HPV across institutions and by the lack of a standardized procedure for testing HPV positivity, as already reported by previous authors. In particular, future studies should try to minimize the bias related to differences between the various methods for HPV assessment. As an example, in a recent systematic review of the literature, the percentage p16^{INK4a} positivity was 79.6% among HPV DNA-positive cases of PSCC versus 18.5% for HPV DNA-negative patients [4].

Conclusions

In conclusion, using two large sets of clinical and genomic data for patients with PSCC, we provide results suggesting a potential role of (perioperative) RT for HPV$^+$ PSCC with lymph node involvement, mainly in the adjuvant setting. In addition, HPV$^+$ PSCC tumors are characterized by higher mutation burden and lower *TP53* mutation frequency compared to HPV– tumors. The latter feature could be used as a stratifying biomarker in prospective randomized clinical trials.

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Study concept and design: Necchi.
Acquisition of data: All authors.
Analysis and interpretation of data: Necchi, Bandini, Ross.
Drafting of the manuscript: Necchi.
Critical revision of the manuscript for important intellectual content: All authors.
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euo.2020.10.011.

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