PENILE CANCER IN GENITOURINARY ONCOLOGY

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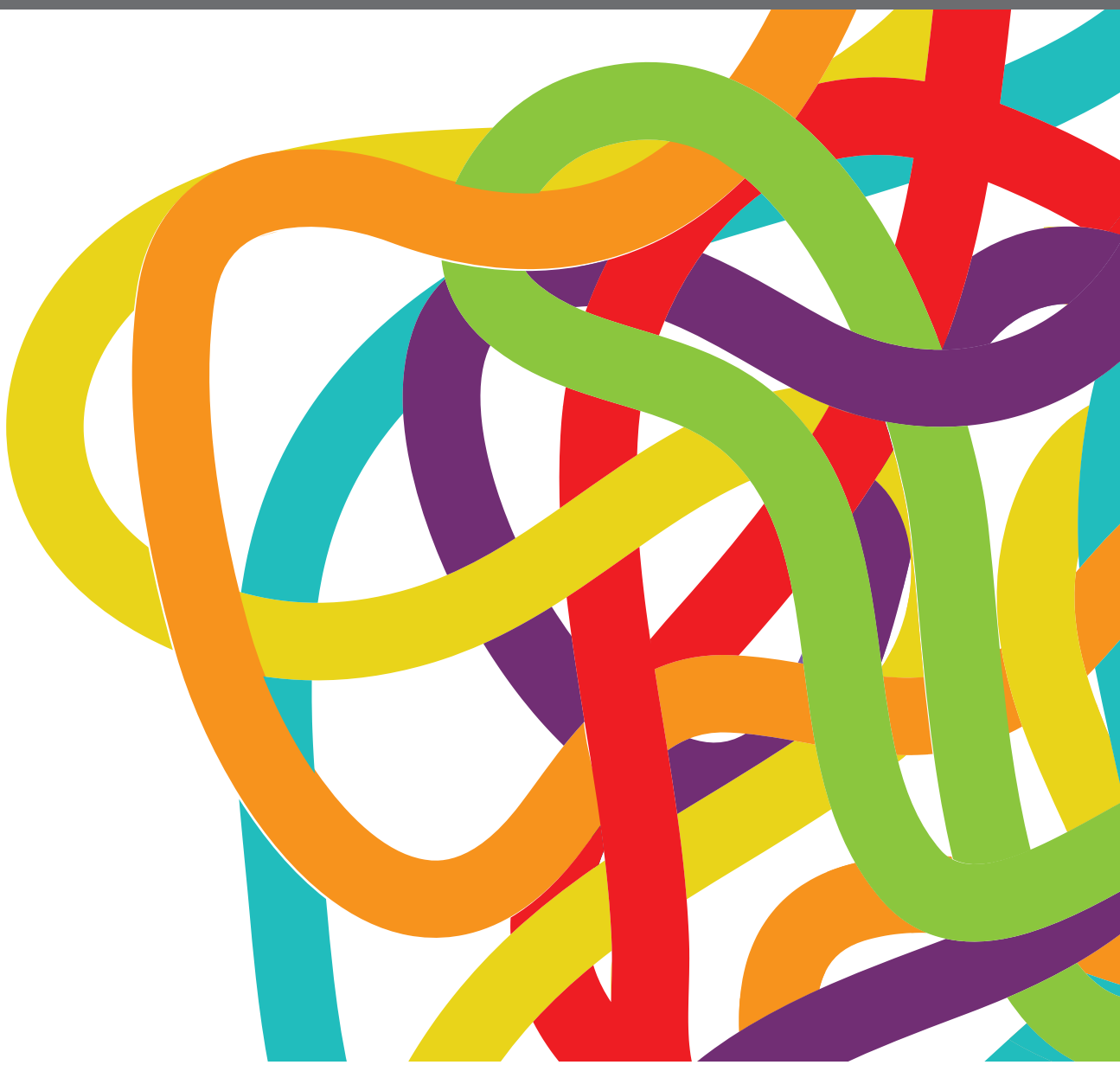

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PENILE CANCER IN GENITOURINARY ONCOLOGY

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Editorial: Penile cancer in genitourinary oncology

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Editorial on the Research Topic
Penile cancer in genitourinary oncology

The Research Topic *Penile Cancer in Genitourinary Oncology* as part of the Frontiers in Oncology has explored hot topics in this rare disease that needs further development. Authors from Asia, Europe, North and South America have explored important diagnostic, prognostic and therapeutic aspects.

Lack of significant progress in the treatment and management of penile cancer patients in the United States in recent decades is suggested by the increasing trend in incidence-based mortality (IBM) with no significant improvement in the 5-year relative survival rate observed using population-level data from the SEER database [Deng et al.].

A large multicenter Chinese study including 340 penile cancers has identified that phimosis has decreased its prevalence through the years studied while HPV infections rose, becoming a more important etiological factor. HPV types 16 and 18 comprised 88.6% of infections detected and when added type 33, 91% of infections studied are accumulated [Gu et al.].

Other Chinese group sequenced 35 penile cancer patients' complete exome in order to define a correlation between gene copy number alterations and the disease prognosis. The 5-year survival rate of patients with *MYCN* and *FAK* amplification was 69.2%, and 65.6%, compared to 94.4% and 94.7% in the non-amplification groups, respectively [Yu et al.].

Lymph node status is the most important prognostic factor of penile cancer, and examined lymph node (ELN) count and lymph node density (LND) were independent prognostic factor for overall survival when analyzing 528 patients in the Surveillance, Epidemiology, and End Results cohort from 2010 to 2015 and 156 patients in a Chinese cohort (2006-2016). Using the ROC curve, the recommended cutoff values of ELN and LND were 13 and 9.3%, respectively (P <0.001) [Gao et al.].

Based on the indication for complementary treatment and the already known poor prognosis of extra lymph node extension (ENE) in penile squamous cell carcinomas, a Chinese group studied 234 patients who underwent bilateral inguinal lymph node dissection surgery and has developed a nomogram based on pathological staging and

clinical-laboratory data including platelet-lymphocyte ratio and squamous cell carcinoma antigen, capable of predicting the risk of ENE presence [Wu et al.].

Also, deep inguinal lymph node metastasis (ILNM) was the most accurate factor for predicting pelvic lymph node metastasis (PLNM) in a different cohort of 189 Chinese patients. Once involvement of deep ILNs indicates poor prognosis, authors propose that patients with deep ILNM should be staged as pN3 and referred for pelvic lymph node dissection [Yang et al.].

Radioisotope-guided dynamic sentinel node biopsy (DSNB) simultaneously or secondarily after penile surgery has potential to limit morbidity. Among 41 Germany patients, the morbidity rate was 15.8% per inguinal region and intervention was only required in six groins (7.9%). While DSNB is limited by false-negative results, it might be reduced when performed simultaneously to primary tumor resection [Nemitz et al.].

Incipient data has showed therapeutic potential in the use of immune checkpoint inhibitors against penile cancer progressing after chemotherapy, which usually have 06 months median overall survival.

Two patients successfully treated with pembrolizumab are reported, one with high tumor mutational burden and complete response for 38 months and other with PDL-1 expression in penile tumor tissue with partial response for 18 months [Chahoud et al.]. Other two patients with advanced penile squamous cell carcinoma who were administered chemotherapy combined with sintilimab also showed sustained partial and complete response for one and two years, respectively [Mei et al.].

In a European survey, guideline adherence for most treatment recommendations increases with growing annual penile cancer caseload. The probability of a guideline-adherent recommendation increased with each patient treated per year. The type of hospital care (academic vs. non-academic) did not affect guideline adherence in any scenario [Lebentrau et al.].

Brazilian authors brought a compilation on non-coding RNA and its role in penile cancer, as well as the opportunity to use paraffin samples, allowing retrospective studies. Micro RNAs, pi-RNAs, and long non-coding RNAs (LncRNAs) are already known as corelated to disease and are possible tumor biomarkers with potential diagnostic and prognostic targeting in the near future [Pinho et al.]. This research topic selection highlights the current penile cancer landscape, from new technologies with prognostic ability, with the lymph node dissemination still as the Achilles' heel, to the great therapeutic potential of immunotherapies for the near future.

Author contributions

LR: Supervision; KB, DC, LD: Manuscript writing/editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Development and Validation of a Clinical Prognostic Model Based on Immune-Related Genes Expressed in Clear Cell Renal Cell Carcinoma

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Background: Clear cell renal cell carcinoma (ccRCC) is the most frequent and terminal subtype of RCC. Reliable markers associated with the immune response are not available to predict the prognosis of patients with ccRCC. We exploited the extensive number of ccRCC samples from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) repository to perform a comprehensive analysis of immune-related genes (IRGs).

Methods: Based on TCGA data, we incorporated IRGs and their expression profiles of 72 normal and 539 ccRCC samples. Univariate Cox analysis was used to evaluate the relationship between overall survival (OS) and IRGs expression. The Lasso Cox regression model identified prognostic genes used to establish a clinical immune prognostic model. The TF–IRG network was used to study the potential molecular mechanisms of action and properties of ccRCC-specific IRGs. Multivariate Cox analysis established a clinical prognostic model of IRGs.

Results: We found a significant correlation among 15 differentially expressed IRGs with the OS of patients with ccRCC. Gene function enrichment analysis showed that these IRGs are significantly associated with response to receptor ligand activity. Lasso Cox regression analysis identified 10 genes with the greatest prognostic value. A clinical prognostic model based on six IRGs, which performed well for predicting prognosis, revealed significant associations of patients' survival with age, sex, stage, tumor, node, and metastasis. Moreover, these findings reflect the infiltration of tumors by various immune cells.

Conclusion: We identified six clinically significant IRGs and incorporated them into a clinical prognostic model with great significance for monitoring and predicting prognosis of ccRCC.

Keywords: clear cell renal cell carcinoma, TCGA, GEO, immune-related genes, clinical prognostic model, tumor microenvironment

INTRODUCTION

Renal cell carcinoma (RCC) is a frequent cause of mortality of patients with urinary cancer, accounting for 2% of malignant tumors of adults (1). Annually, there are ∼350,000 new cases of RCC worldwide, leading to ≥140,000 annual fatalities (2). Clear cell renal cell carcinoma (ccRCC) is the most frequent and lethal subtype, accounting for 75% of RCCs (3). Although the treatment of ccRCC has significantly improved during the past 10 years, there are limitations to its diagnosis, treatment, and prognosis. Distant metastasis occurs in 30% of patients with ccRCC who undergo surgery during the early stages of disease (4). Further studies of the mechanisms of ccRCC occurrence and development are therefore required, as well as efforts to develop new diagnostic methods and to identify potential biomarkers.

The components of the tumor microenvironment, which contribute to the development of tumors, include immune cells, stromal cells, extracellular matrix molecules, cytokines, and chemokines (5). These components reflect the evolutionary nature of tumor progression, which promotes immune escape, tumor growth, and metastasis (6). Moreover, new therapeutic targets have been identified through studies of these components and their complex interactions (5). For example, Li et al. (7) investigated the prognostic value of immune-related genes (IRGs) to establish an individual's immune characteristics and to improve predictions of the prognosis of patients with non-small cell lung cancer (7). Thus, understanding the molecular and cellular composition and function of the ccRCC tumor microenvironment is required to improve prognosis and to identify new biomarkers (8, 9).

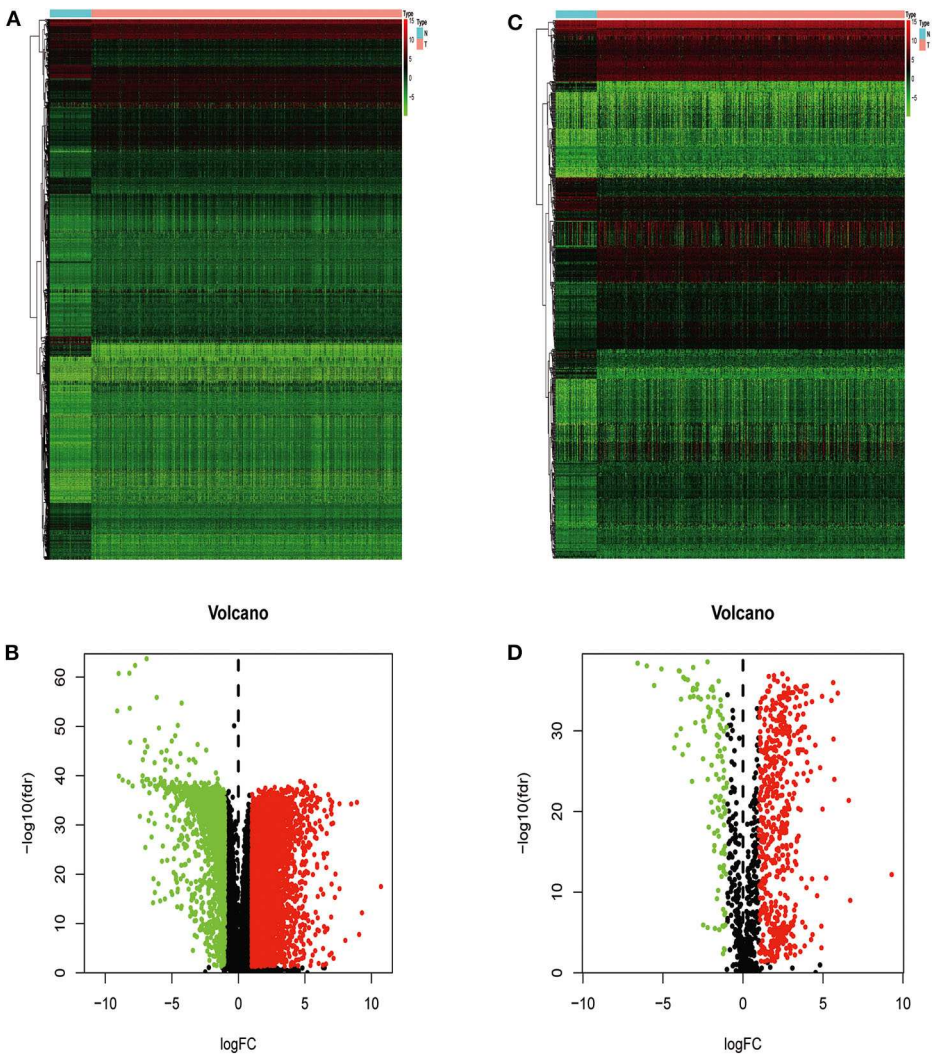

FIGURE 1 | Differentially expressed immune-related genes. Heatmap **(A)** and volcano plot **(B)** showing genes differentially expressed between clear cell renal cell carcinoma (ccRCC) and normal tissues. Green, red, and black dots represent genes expressed at relatively lower, higher, or equal levels. The differentially expressed immune-related genes (IRGs) are shown in a heatmap **(C)** and volcano plot **(D)**. Green, red, and black dots represent genes expressed at relatively lower, higher, or equal levels.

Publicly available gene expression datasets and the emergence of related platforms such as The Cancer Genome Atlas (TCGA) database provide readily accessible and convenient platforms for rapid and accurate identification of biomarkers for monitoring tumors (10, 11). For example, Yoshihara et al. (8) studied the tumor microenvironment by analyzing the expression of specific molecular biomarkers of immune and stromal cells using an estimation algorithm employing stromal and immune scores. Such estimation algorithms evaluate the prognosis of many tumors and identify biomarkers (8, 9, 12, 13). However, there is no definitive threshold to aid studies of the associations of clinical correlates and prognostic significance with the tumor microenvironment and ccRCC.

Here we aimed to comprehensively study the possible clinical efficacy of IRGs in the ccRCC tumor microenvironment to stratify prognosis, as well as their potential value as biomarkers for targeted therapy. For this purpose, we combined the expression profiles of IRGs with clinical information to evaluate overall survival (OS). We systematically analyzed the expression of ccRCC IRGs and their associations with prognosis to develop personalized prognostic markers. Furthermore, bioinformatics analysis was used to identify potential regulatory mechanisms. The results of this study will provide the basis for research related to immunization and provide a theoretical basis for the development of individualized therapy.

MATERIALS AND METHODS

Data Collection and Clinical Samples

We acquired ccRCC transcriptomic sequencing data from TCGA data (https://portal.gdc.cancer.gov/), including 539 ccRCC and 72 normal samples. Patients' clinical information was extracted as well. Gene expression matrix files and clinical information from the GSE29609 dataset were obtained from the Gene Expression Omnibus (GEO) repository. The list of IRGs was exported

TABLE 1 | Gene function enrichment of differentially expressed immune related genes.

Ontology	ID	Description	*p*.adjust	Count
BP	GO:0002460	Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	3.78E-106	138
BP	GO:0002449	Lymphocyte mediated immunity	8.76E-106	136
BP	GO:0002429	Immune response-activating cell surface receptor signaling pathway	1.69E-94	137
BP	GO:0002768	Immune response-regulating cell surface receptor signaling pathway	1.76E-93	140
BP	GO:0016064	Immunoglobulin mediated immune response	1.76E-93	105
BP	GO:0019724	B cell mediated immunity	2.81E-93	105
BP	GO:0006959	Humoral immune response	3.63E-92	126
BP	GO:0002455	Humoral immune response mediated by circulating immunoglobulin	3.63E-92	91
BP	GO:0006958	Complement activation, classical pathway	3.20E-90	87
BP	GO:0050900	Leukocyte migration	1.36E-85	137
CC	GO:0009897	External side of plasma membrane	1.98E-83	118
CC	GO:0042571	Immunoglobulin complex, circulating	2.96E-59	52
CC	GO:0019814	Immunoglobulin complex	2.99E-59	53
CC	GO:0042611	MHC protein complex	4.97E-26	21
CC	GO:0043235	Receptor complex	1.18E-24	64
CC	GO:0072562	Blood microparticle	7.47E-21	37
CC	GO:0071556	Integral component of lumenal side of endoplasmic reticulum membrane	7.61E-17	17
CC	GO:0098553	Lumenal side of endoplasmic reticulum membrane	7.61E-17	17
CC	GO:0042613	MHC class II protein complex	8.96E-16	13
CC	GO:0012507	ER to Golgi transport vesicle membrane	2.60E-11	18
MF	GO:0003823	Antigen binding	9.43E-163	140
MF	GO:0048018	Receptor ligand activity	7.44E-75	124
MF	GO:0034987	Immunoglobulin receptor binding	2.39E-57	52
MF	GO:0005125	Cytokine activity	6.65E-54	75
MF	GO:0005126	Cytokine receptor binding	3.96E-45	75
MF	GO:0004896	Cytokine receptor activity	6.41E-36	42
MF	GO:0004252	Serine-type endopeptidase activity	4.44E-35	62
MF	GO:0008236	Serine-type peptidase activity	9.84E-33	62
MF	GO:0017171	Serine hydrolase activity	2.15E-32	62
MF	GO:0008083	Growth factor activity	2.70E-29	47

from the immunology database and analysis portal (ImmPort) database that provides immunology data (14). Moreover, the database provides a list of IRGs associated with processes that mediate the immune response.

TABLE 2 | The top 10 most significant Kyoto Encyclopedia of Genes and Genomes pathways (KEGG).

ID	Description	P.adjust	Count
hsa04060	Cytokine-cytokine receptor interaction	1.88E-67	117
hsa04061	Viral protein interaction with cytokine and cytokine receptor	1.41E-35	52
hsa04650	Natural killer cell mediated cytotoxicity	2.82E-25	49
hsa04612	Antigen processing and presentation	4.09E-21	35
hsa04640	Hematopoietic cell lineage	4.17E-18	36
hsa04658	Th1 and Th2 cell differentiation	1.93E-16	33
hsa04062	Chemokine signaling pathway	1.97E-15	46
hsa04659	Th17 cell differentiation	3.35E-15	34
hsa04514	Cell adhesion molecules (CAMs)	5.70E-13	37
hsa04630	JAK-STAT signaling pathway	1.31E-11	37

TABLE 3 | First reported immune microenvironment- related genes in ccRCC.

Gene symbol	logFC	*p*-value	FDR
AEN	1.39002	<0.001	<0.001
ANGPTL7	−1.06387	<0.001	<0.001
APLN	2.486788	<0.001	<0.001
AZGP1	−1.75511	<0.001	<0.001
BLNK	−1.15577	<0.001	<0.001
BMP5	−1.27325	<0.001	<0.001
BMP8A	1.442843	<0.001	<0.001
C3AR1	1.909827	<0.001	<0.001
CARD11	2.086722	<0.001	<0.001
CKLF	1.085822	<0.001	<0.001
CSF3R	2.789211	<0.001	<0.001
EBI3	2.313785	<0.001	<0.001
FAM3B	−4.0026	<0.001	<0.001
FCGR2B	2.001925	<0.001	<0.001
FPR1	1.584903	<0.001	<0.001
HCST	1.980888	<0.001	<0.001
HSPA6	2.069337	<0.001	<0.001
IGHA2	2.137115	<0.001	<0.001
IGHJ2	2.46991	<0.001	<0.001
IL2RA	2.276302	<0.001	<0.001
INPP5D	1.711738	<0.001	<0.001
PPARA	−1.00738	<0.001	<0.001
RAET1E	−1.80059	<0.001	<0.001
TNFSF14	3.76326	<0.001	<0.001

logFC, log fold change (tumor tissues vs. normal tissues); FDR, false discovery rate.

Analysis of Differentially Expressed Genes

The edgeR package was used to screen IRGs differentially expressed between ccRCC and normal samples (15). Log_2 transformation was used to standardize the raw data. We applied differential gene expression (DGE) analysis using cut-off values of $|\log_2$ fold change$| > 1$ and FDR <0.05. Then, we extracted the differentially expressed IRGs from all DEGs. The molecular mechanisms potentially responsible for the differential expression of IRGs were investigated using functional enrichment analysis of the GO and KEGG pathways (16–18) using the clusterProfiler package (19).

Survival Analysis

Clinical information were acquired from TCGA data and the GEO database. To analyze OS, we used the R survival and survminer packages. We conducted single-variable Cox analysis using the R survival package to identify survival-related IRGs.

Molecular Characteristics of Prognosis-Related IRGs

Analyses of the differential expression of IRGs related to the prognosis of patients with ccRCC may have clinical value. To investigate functional interactions, we constructed a protein–protein interaction (PPI) network using the STRING database (http://string-db.org) (20). PPI networks show direct or indirect interactions of gene products. Cytoscape was used to visualize the results of the PPI network (21). Moreover, transcription factors (TFs) directly control gene expression. We focused on potential target transcription factors (TFs) of these prognosis-related IRGs. To identify the regulatory links between the TFs and the transcriptome, we employed the Cistrome Cancer database (http://cistrome.org/), which incorporates TCGA data with >2,300 ChIP-seq data and analyses of chromatin accessibility. We constructed a regulatory network of potential TFs and current IRGs by considering TFs of clinical significance.

Construction and Verification of a Prognostic Model

We used the Lasso method to select the main IRGs from the important cohort of the Cox univariate regression analysis, which identifies the subclass of IRGs associated with the prognosis with ccRCC. This was achieved by considering lowering the regression coefficient by suppressing the penalty score compared with its size. Finally, a few indicators with nonzero weights persisted, while those of most possible indicators approached zero. Therefore, the proportional hazards regression calculated using the Lasso method further reduced the representation of immune-related genes. We next generated a sample of an existing sample dataset using 1,000 iterations, selected IRGs repeated 900 times, and used the "glmnet" R package to complete the Lasso Cox analysis. Finally, we used β coefficients of multiple regression analysis to establish a prognostic immune correlation model. These coefficients were multiplied by the expression level of each immune-related gene.

Clinical and Immune Correlations of the Prognostic Model

The classification of patients into high- and low-risk groups was performed according to their risk scores, and prognosis was evaluated. The TIMER database (https://cistrome.shinyapps.io/timer/) analyzes and visualizes the abundance of tumor-infiltrating immune cells (22). Here we analyzed these data for patients with ccRCC and calculated their correlations with IRGs to establish a model of clinical prognosis and immune cell infiltration.

Statistical Analysis

We identified the functions of the prognostic features using the survivalROC R package to calculate survival according to the area under the curve (AUC) of the receiver operator characteristic (ROC) curve (23). Significant and acceptable predictive values were defined as AUC $\geq$ 0.75 and AUC $\geq$ 0.6, respectively.

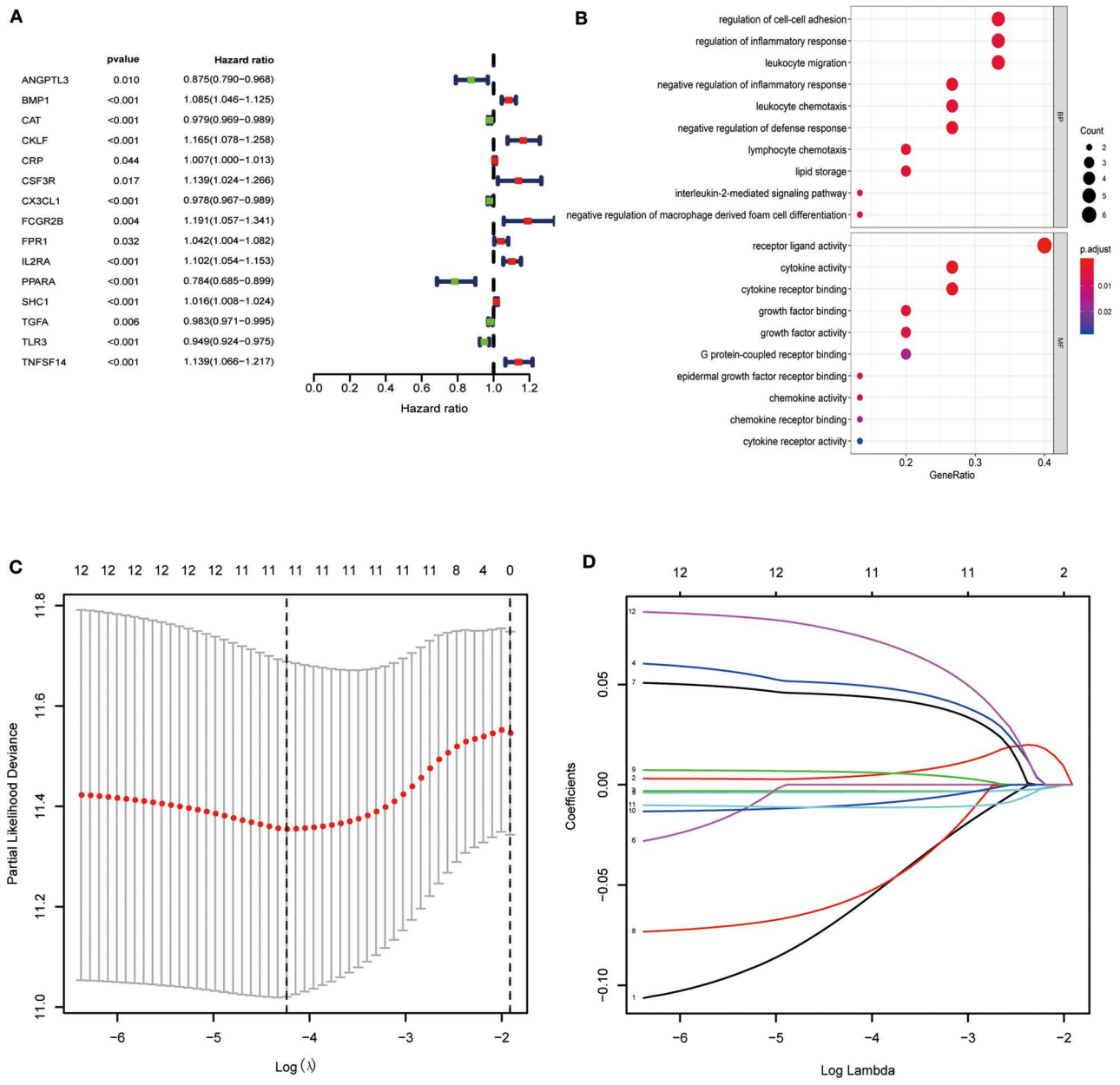

FIGURE 2 | Identification of immune-related genes with the most significant prognostic value. **(A)** Forest plot of hazard ratios showing the prognostic value of survival-related immune-related genes. **(B)** Gene function enrichment (GO) analysis of survival-related immune-related genes. **(C,D)** The Lasso regression method based on the glmnet package was used to identify the 21 most prognostic IRGs in TCGA training group.

Statistical analysis was performed using R software, and $P < 0.05$ indicates a significant difference.

RESULTS

Identification of Differentially Expressed IRGs

We extracted 7,369 genes and 611 samples from TCGA ccRCC data, including 1,902 upregulated genes and 5,467 downregulated genes (**Figures 1A,B**). We extracted 681 differentially expressed IRGs from this set of genes, which included 116 downregulated and 565 upregulated genes (**Figures 1C,D**). Gene function enrichment analysis showed that the immune response-regulating cell surface receptor signaling pathway, the external side of the plasma membrane, and antigen binding were the most common biological terms among biological processes, cell components, and molecular functions, respectively (**Table 1**). Furthermore, KEGG pathway analysis revealed that these IRGs (**Table 2**) are significantly involved in cytokine–cytokine receptor interactions, viral protein interactions with cytokines, and natural killer cell-mediated cytotoxicity. **Table 3** showed the first reported IRGs in ccRCC.

Identification of Prognosis-Related IRGs

We found a significant association of 15 IRGs with OS. A forest hazard map shows that most of these IRGs serve as risk factors for ccRCC (**Figure 2A**), and gene function enrichment analysis revealed that these IRGs are significantly associated with response to receptor ligand activity (**Figure 2B**). Furthermore, Lasso Cox regression analysis identified 10 genes with the highest prognostic values (**Figures 2C,D**).

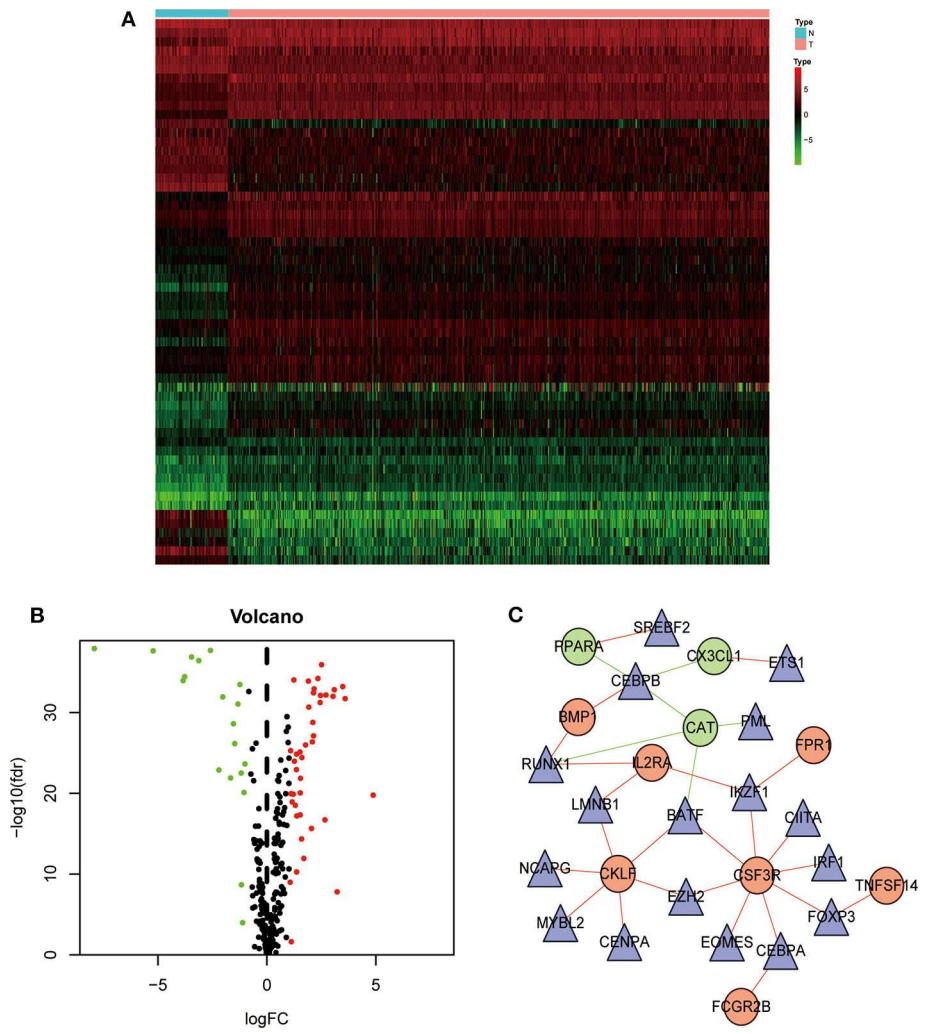

FIGURE 3 | Construction of a TF–immune-related gene regulatory network. Differentially expressed transcription factors (TFs) are shown in a heatmap **(A)** and a volcano plot **(B)**. Green, red, and black dots represent genes expressed at relatively lower, higher, or equal levels. **(C)** A regulatory network comprising TFs and IRGs. Triangles represent TFs, and red and green indicate risk and protective factors, respectively.

A Gene Regulatory Network Comprising TFs and IRGs

We next analyzed the regulatory mechanisms of TF genes and IRGs to identify the molecular mechanisms linked to their clinical significance. When we analyzed the expression profiles of 318 TFs, we identified 60 differentially expressed TFs (**Figures 3A,B**). A regulatory network constructed using these 60 TFs and 15 IRGs. The critical values were correlation coefficient = 0.4 and $P = 0.6$. The resulting TF-based regulatory networks clearly illustrated the regulatory relationships between these IRGs (**Figure 3C**).

TABLE 4 | Information on IRGs used to construct clinical prognostic models.

IRGs	Coef	HR	*p*-value
ANGPTL3	−0.1200	0.8870	0.0209
IL2RA	0.0577	1.0594	0.0401
PPARA	−0.1445	0.8655	0.0431
SHC1	0.0105	1.0106	0.0492
TGFA	−0.0159	0.9843	0.0152
TNFSF14	0.1075	1.1135	0.0046

IRGs, immune-related genes; Coef, Cox-PH coefficient; HR, Hazard Ratio.

Development of a Clinical Prognostic Model

Here we identified six IRGs according to the results of the Lasso Cox model analysis, which were used to develop a prognostic model of the IRGs, *ANGPTL3*, *IL2RA*, *PPARA*, *SHC1*, *TGFA*, and *TNFSF14* (**Table 4**). The risk score was calculated as follows: [expression level *ANGPTL3* * (−0.1200)] + [expression level *IL2RA* * (0.0577)] + [expression level *PPARA* * (−0.1445)] + [expression level *SHC1* * (0.0105)] + [expression level *TGFA* * (−0.0159)] + [expression level *TNFSF14* * (0.1075)].

TABLE 5 | Clinical characteristics of ccRCC patients included in this study.

Variables	Total TCGA-KIRC	Training group	Testing group	GEO cohort
	(*N* = 504)	(*N* = 252)	(*N* = 252)	(*N* = 39)
Age (Mean ± SD)	60.47 ± 12.16	61.71 ± 11.82	59.24 ± 12.39	61.38 ± 12.77
Survival time (y)	3.27 ± 2.18	3.13 ± 2.21	3.40 ± 2.15	2.99 ± 1.67
Status				
Alive	339 (67.26)	169 (67.06)	170 (67.46)	22 (56.41)
Dead	165 (32.74)	83 (32.93)	82 (32.54)	17 (43.59)
Gender				
Male	331 (65.67)	157 (62.30)	174 (69.05)	
Female	173 (34.33)	95 (37.70)	78 (31.95)	
Stage				
I	249 (49.70)	123 (49.00)	126 (50.40)	
II	53 (10.57)	30 (11.95)	23 (9.20)	
III	117 (23.35)	58 (23.11)	59 (23.60)	
IV	82 (16.37)	40 (15.94)	42 (16.80)	
Grade				
1	10 (2.01)	3 (1.21)	7 (2.80)	
2	215 (43.26)	110 (44.53)	105 (42.00)	
3	198 (39.84)	103 (41.70)	95 (38.00)	
4	74 (14.89)	31 (12.55)	43 (17.20)	
T				
1	255 (50.60)	127 (50.40)	128 (50.79)	11 (28.21)
2	65 (12.90)	36 (14.29)	29 (11.51)	5 (22.73)
3	173 (34.33)	82 (32.54)	91 (36.11)	22 (56.1)
4	11 (2.18)	7 (2.78)	4 (1.59)	1 (2.56)
M				
0	400 (83.68)	201 (84.45)	199 (82.92)	26 (66.67)
1	78 (16.32)	37 (15.55)	41 (17.08)	13 (33.33)
N				
0	224 (93.33)	112 (93.33)	112 (93.33)	32 (82.05)
1	16 (6.67)	8 (6.67)	8 (6.67)	7 (17.95)

Data are shown as n (%). T, tumor; M, metastasis; N, node.

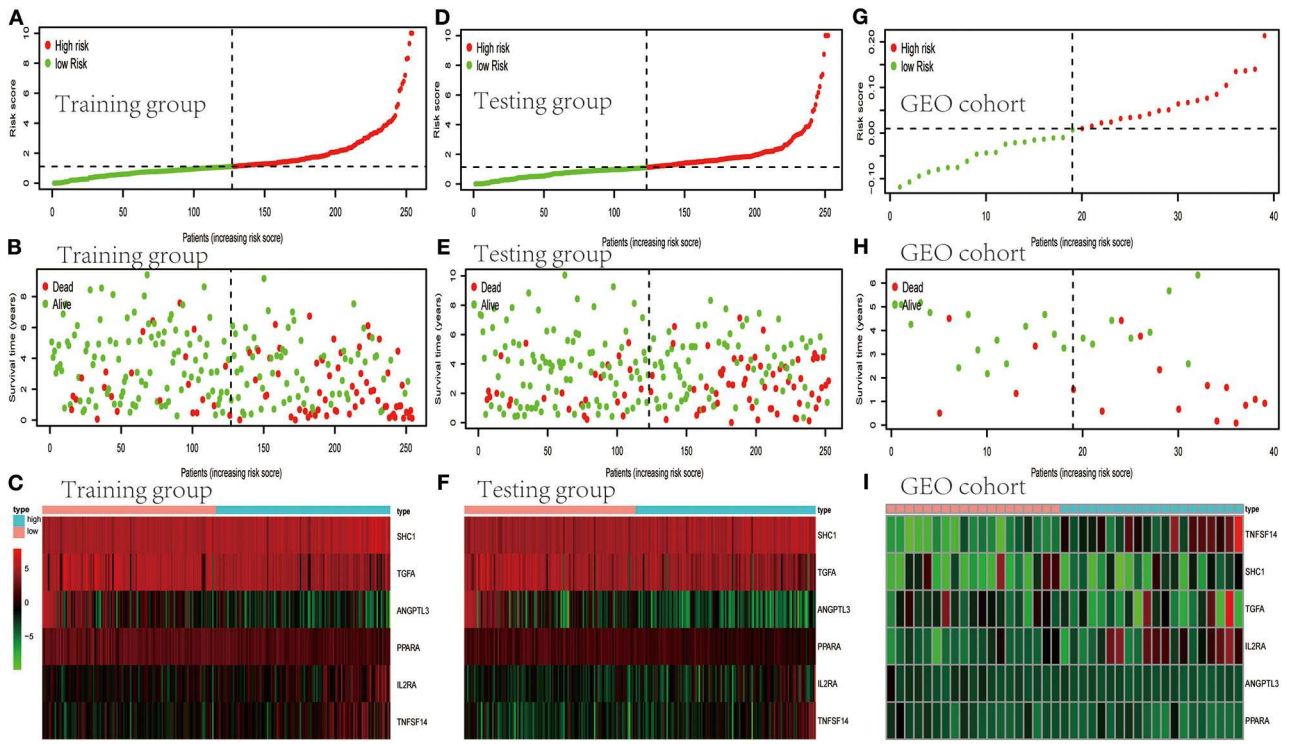

FIGURE 4 | Evaluation and validation of clinical prognostic models employing IRGs. **(A,D,G)** Distribution of patients according to risk index. **(B,E,H)** Survival. **(C,F,I)** Heatmaps of IRG expression profiles.

Evaluation of the Prognostic Performance of the Clinical Prognostic Model Based on IRGs

TCGA clinical data of 504 patients with ccRCC included age, sex, stage, tumor, node, metastasis stage, and survival. These patients were randomly divided into a training ($n = 252$) or test ($n = 252$) group. **Table 5** shows their clinical information. According to the risk scores of the prognostic model, patients with ccRCC were divided into a low- or high-risk group (**Figure 4A**). As the risk score increased, the longevity of patients decreased (**Figure 4B**). **Figure 4C** shows differential expression of the IRGs between the low- and high-risk groups. The clinical prognostic model yielded a risk score that predicted that the OS rates of the low- and high-risk groups were significantly different (**Figure 5A**). The AUC of the ROC curve was 0.772, indicating that the prognostic features based on IRGs were highly accurate for predicting survival (**Figure 5B**). Furthermore, univariate analysis revealed that the risk score significantly correlated with shorter OS (HR: 2.50; 95% CI: 1.64–3.83; $P < 0.001$). Other clinicopathologic variables associated with poor survival included stage, and grade as well as tumor, node, and metastasis stage. Multivariate analysis indicated that the risk score served as an independent prognostic factor (HR: 2.20; CI: 1.33–3.63, $P = 0.002$) (**Figures 6A,B**, **Table 6**).

Validation of the Clinical Prognostic Model

To determine whether the clinical prognostic model was reliable when applied to different populations, we used the same formula to evaluate the test group and the GEO cohort (GSE29609), which was consistent with the results of the training group. The GSE29609 data include 39 patients with ccRCC (**Table 5**). Patients were divided into high- or low-risk groups according to the risk value of the model (**Figures 4D,G**). Increased risk was associated with more deaths (**Figures 4E,H**). The results show further that the prediction potential of the clinical prognostic model was suitable for different populations. **Figures 4F,I** show the expression data of selected IRGs for different risk groups. Furthermore, the probability of survival of the high-risk group was lower than that of the low-risk group (**Figures 5C,E**). Next, we evaluated the accuracies of the clinical prognostic model applied to the test group and GEO cohort, for which the AUCs of the ROC curve were 0.678 and 0.781, respectively (**Figures 5D,F**). These results indicate that the clinical prognostic model accurately predicted the prognosis of patients with ccRCC.

Clinical and Immune Correlations of the Prognostic Model

The correlation between the IRGs analyzed using the clinical prognostic model with clinical and demographic characteristics was analyzed as a function of age, sex, stage, and TNM stage (**Figure 7**). Furthermore, to determine whether the immune prognostic model accurately reflected the state of the tumor immune microenvironment, we analyzed the relationship between risk scores and immune cell infiltration. The results show that the risk score was significantly related to $CD8^+$T

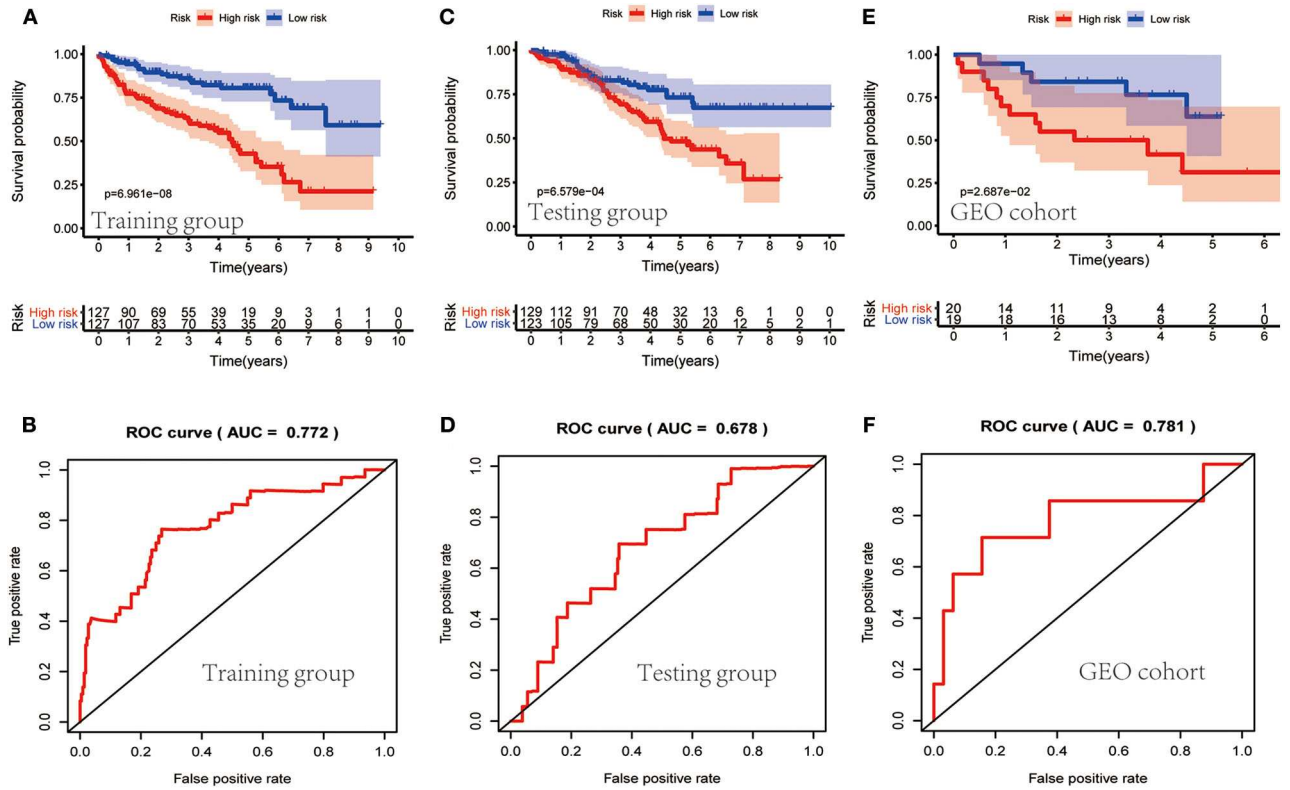

FIGURE 5 | Evaluation of the clinical prognostic model. **(A,C,E)** The risk scores of the clinical prognostic model predict survival. **(B,D,F)** The receiver operating characteristic (ROC) curve of survival-dependent receiver verifies the prognostic value of the model.

TABLE 6 | Univariate analysis and multivariate analysis of the correlation between the risk score calculated by the clinical prognosis model and OS.

	Univariate analysis		Multivariate analysis	
Clinicopathologic	**HR (95%CI)**	***p*-value**	**HR (95%CI)**	***p*-value**
Age	1.00 (0.97–1.02)	0.765		
Gender	0.85 (0.45–1.60)	0.616		
Grade	2.86 (1.72–4.75)	<0.001	1.49 (0.84–2.65)	0.174
Stage	1.90 (1.44–2.51)	<0.001	0.67 (0.20–2.25)	0.520
T	2.31 (1.56–3.41)	<0.001	2.01 (0.62–6.47)	0.242
M	3.79 (1.91–7.52)	<0.001	5.14 (0.872–30.35)	0.071
N	4.47 (1.31–15.19)	0.016	2.64 (0.66–10.66)	0.712
Risk score	2.50 (1.64–3.83)	<0.001	2.20 (1.33–3.63)	0.002

Bold values indicate P < 0.05. HR, hazard ratio; CI, confidence interval. T, Tumor; N, Node; M, Metastasis.

cells ($p < 0.001$), neutrophils ($p < 0.001$), and dendritic cells ($p < 0.001$) (**Figure 8**).

DISCUSSION

The role of IRGs in tumorigenesis and development is established. However, systematic, comprehensive data that identify their roles in patients with ccRCC are insufficient. To address this deficiency in our knowledge, here we analyzed the ccRCC dataset of TCGA to establish a clinical prognostic model employing differentially expressed IRGs that accurately predicted the clinical outcomes of patients according to their clinicopathological characteristics. Moreover, these IRGs are closely associated with the occurrence and development of ccRCC and therefore may serve as significant clinical biomarkers. These results show that our clinical prognostic model predicted patients' outcomes as well as identified potential targets of immunotherapy.

Specifically, we identified 15 IRGs closely related to the survival of patients, including six protective factors and nine risk factors. Functional enrichment analysis showed that these IRGs are significantly associated with response to receptor ligand

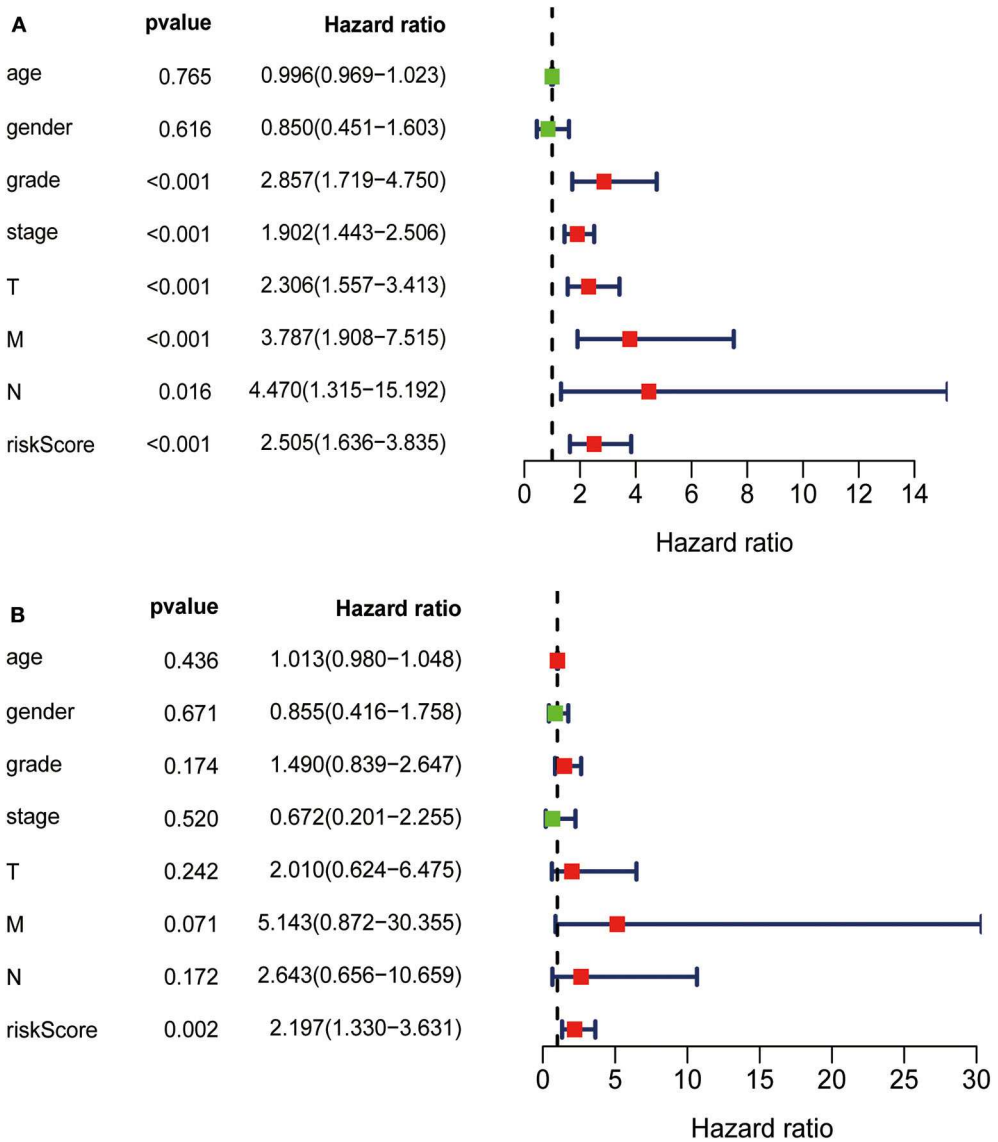

FIGURE 6 | (A) Univariate analysis revealed that the risk score correlated with shorter OS, stage, grade, and TNM. **(B)** Multivariate analysis revealed that the risk score served as an independent prognostic factor.

activity. To improve the accuracy of the clinical prognostic model, we used the Lasso Cox regression model to identify IRGs with the greatest prognostic value. Moreover, to study the molecular mechanisms that explain the possible clinical value of these IRGs, we established a TF-mediated network that considered significant differentially expressed TFs regulated by these IRGs. The regulatory network contained 17 TFs and 10 IRGs. Our TF–IRG regulatory network will provide guidance for future mechanistic analyses.

The present clinical prognostic model comprised six IRGs with prognostic significance. For example, angiopoietin-like proteins (ANGPTLs) (24) mediate lipid metabolism, inflammation, cancer cell infiltration, and hematopoietic stem cell expansion (24–28). Low levels of ANGPTL3 in RCC tissue are associated with poor prognosis (29), and ANGPTL3 inhibits metastasis of RCC by regulating the activities of MMPs and epithelial-mesenchymal transition (EMT)-related pathways (29). SHC1 is expressed at higher levels in RCC tissues compared with normal tissues, suggesting its requirement for the progression of ccRCC (30). SHC1 regulates PTRF through the AKT pathway to contribute to the occurrence and development of ccRCC (30).

Signaling through NF-κB-mediate pathways promotes tumor cell proliferation, inhibits apoptosis, induces angiogenesis and the EMT, and promotes distant metastasis. The activation of NF-κB may reshape local metabolism and energize the immune system, thereby promoting tumor growth (31, 32). TNFSF14 induces the noncanonical NF-κB pathway in certain types of cancer cells to promote tumor development (33). The nuclear transcription factor peroxisome proliferator-activated receptor-α (PPARA), a key mediator of lipid metabolism, serves as a

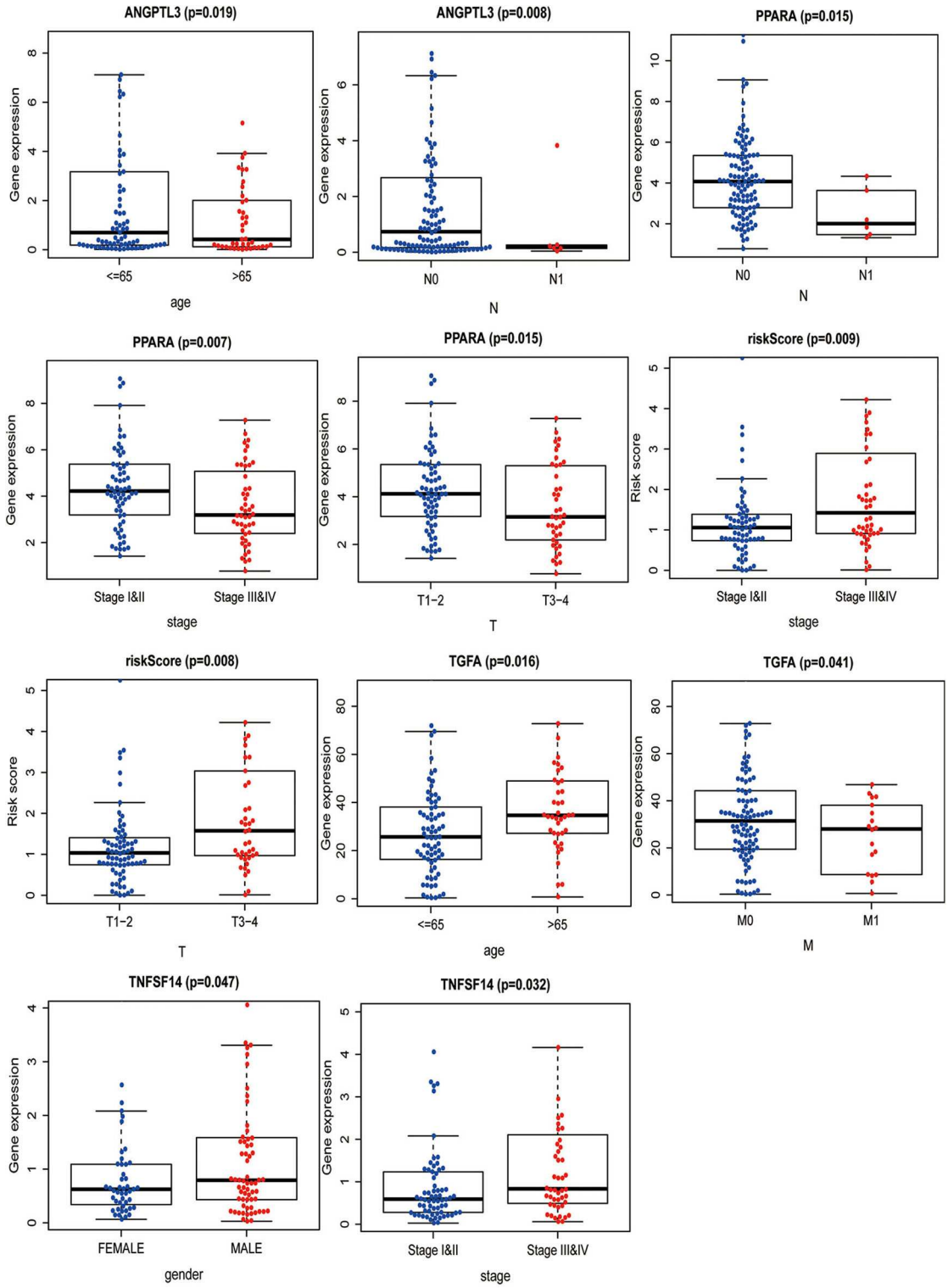

FIGURE 7 | Correlation between IRGs and patients' clinical and demographic characteristics.

biomarker for ccRCC (34). The high levels of IL2RA in activated circulating immune cells and Tregs is exploited for IL-2 immunotherapy of tumors and autoimmune diseases; and certain polymorphisms of *IL2RA* are related to the risk of kidney cancer (35, 36). Thus, these IRGs therefore provide a new direction for our research.

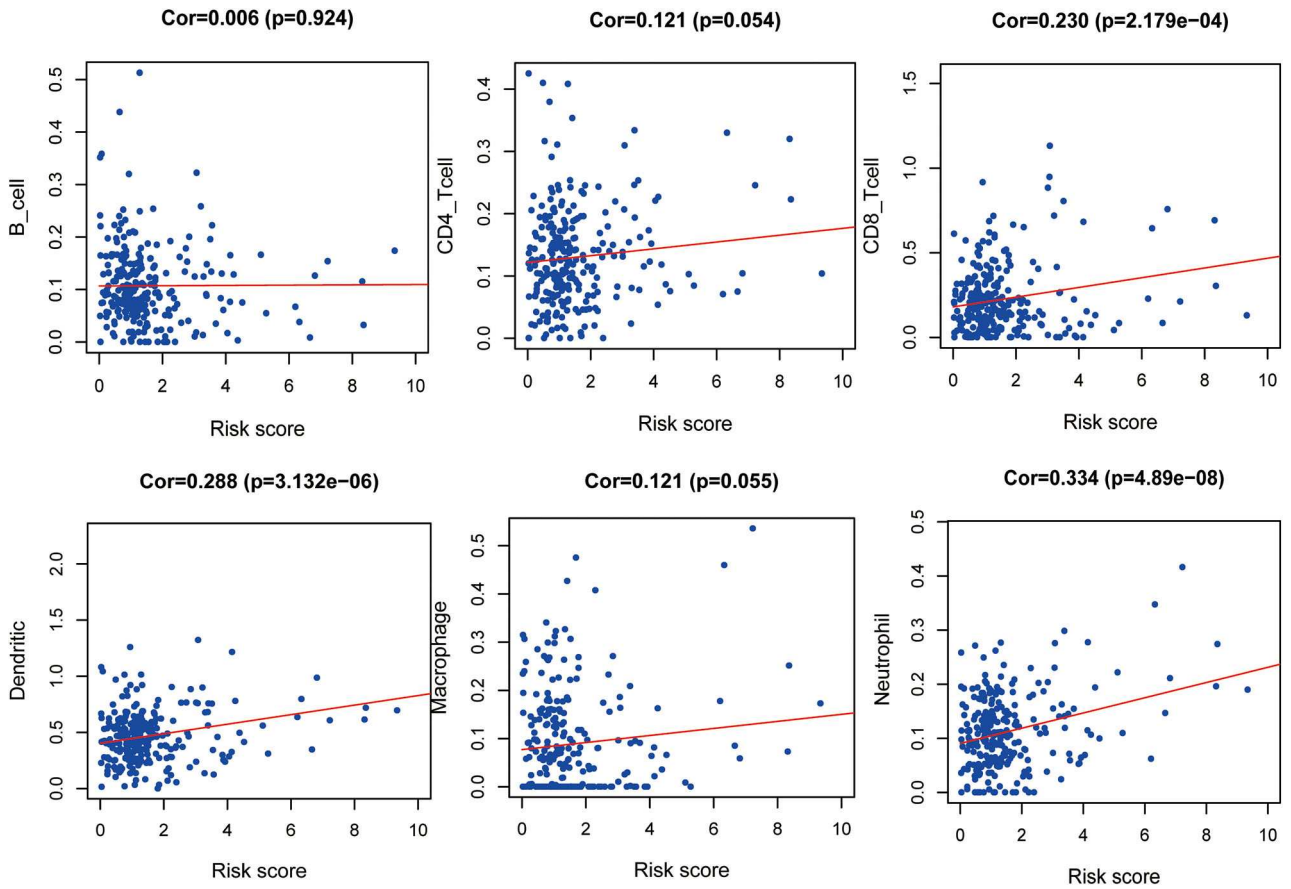

FIGURE 8 | Relationship between risk score and immune cell infiltration.

To evaluate the prognostic value of our clinical prognostic model, we determined the OS of patients with ccRCC in the training group. The prognostic model classified these patients into high- or low-risk groups for shorter survival according to risk scores. Moreover, when we generated risk curves by combining the changes in levels of six IRGs with clinical parameters, and by combining the risk scores of the prognostic model, we were able to monitor the progression of ccRCC. ROC curves indicated that high accuracy of the clinical prognostic model. All results were verified using a testing group and the GEO cohort. Multivariate analysis further confirmed that the risk score served as an independent predictor of OS of patients with ccRCC. Moreover, the prognostic model predicted the survival of patients as well as disease progression. Thus, this model will likely serve as a valuable tool to evaluate the prognosis of patients with ccRCC.

Moreover, our clinical prognostic model showed good clinical feasibility. For example, the six IRGs performed moderately for predicting prognosis and were associated with age, sex, grade, stage, and TNM stage. To analyze tumor–immune interactions, it is essential to characterize immune infiltration. Our analysis shows that the levels of the six IRGs positively correlated with the infiltration of neutrophils, dendritic, and CD8+ T cells. The role of neutrophils in cancer is multifactorial, and they participate in different stages of cancer development, including occurrence, growth, proliferation, and metastasis (37, 38). Furthermore, neutrophils promote tumor proliferation by weakening the immune system (39). Dendritic cells are required for the immune response through attracting antitumor T cells in the TME. However, during tumor development, dendritic cells may convert from immunostimulators to immunosuppressors (40). These results suggest that high-risk patients harbor relatively higher numbers of infiltrating dendritic cells, CD8+ T cells, and neutrophils. Moreover, our results suggest that these six IRGs may predict increased immune cell infiltration.

Previously, Ghatalia et al. and Wang et al. reported the ccRCC immune model, but the research of Ghatalia et al. was mainly based on ccRCC patients who received nephrectomy, and discussed the relationship between the characteristics of tumor infiltrating immune cells and the recurrence rate of local renal cancer (41). The difference is that our study is based on ccRCC patients and established a clinical immune gene model to predict the clinical prognosis of ccRCC patients. Another analysis of TGCA RCC data identified a prognostic 6-DEG classifier, including genes encoding IL21R, ATP6V1C2,

GBP1, P2RY10, GBP4, and TNNC2 (42). Further analysis using this model revealed significant associations between immune/stromal scores and clinicopathological staging. The expression patterns of these genes expressed in the tumor microenvironment provide a powerful indicator of prognosis of patients with RCC. The differences in predictive IRGs identified by Wang et al. (42) Our present study may be explained by the former's use of the ESTIMATE package of R to score the immune/stromal of TCGA samples and then to screen differentially expressed genes using the Lasso Cox regression model to build a prognostic six gene-based clinical model to predict the survival of patients with ccRCC. In contrast, here we screened for differentially expressed IRGs acquired from the ImmPort database, and we then identified IRGs related to survival among the differentially expressed genes and used the Lasso Cox regression model to select IRGs with the highest ability to predict prognosis to construct the prognostic model. Furthermore, our prognostic model was validated using TCGA and GEO data, which yielded consistent, stable, and universal results.

In conclusion, our study identified and validated a clinical prognostic model comprising six IRGs, which served as an independent prognostic factor for patients with ccRCC. Moreover, the prognostic significance of this model may contribute to monitoring ccRCC occurrence and to predict prognosis. Our results provide new insights into approaches to develop new immunotherapies for ccRCC.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found in The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) and Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/).

AUTHOR CONTRIBUTIONS

SR, WW, and HS wrote the manuscript. CZ conducted bioinformatics analyses. MS and YW collected and processed data. XZ and HH prepared figures and tables. BL prepared the literature search and the bibliography, and references. ZW designed the article. CC reviewed the final draft of the manuscript.

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REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Forman D. Global cancer statistics. *CA Cancer J Clin*. (2011) 62:60–9. doi: 10.3322/caac.20107
2. Capitanio U, Montorsi F. Renal cancer. *Lancet*. (2016) 387:894–906. doi: 10.1016/S0140-6736(15)00046-X
3. Yuan J, Dong R, Liu F, Zhan L, Liu Y, Wei J, et al. The miR-183/182/96 cluster functions as a potential carcinogenic factor and prognostic factor in kidney renal clear cell carcinoma. *Exp Ther Med*. (2019) 17:2457–64. doi: 10.3892/etm.2019.7221
4. Cohen HT, Mcgovern FJ. Renal-cell carcinoma. *N Engl J Med*. (2005) 353:2477. doi: 10.1056/NEJMra043172
5. Iyengar NM, Gucalp A, Dannenberg AJ, Hudis CA. Obesity and cancer mechanisms: tumor microenvironment and inflammation. *J Clin Oncol*. (2016) 34:4270–6. doi: 10.1200/JCO.2016.67.4283
6. Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. *Mol Cancer*. (2019) 18:10. doi: 10.1186/s12943-018-0928-4
7. Li B, Cui Y, Diehn M, Li R. Development and validation of an individualized immune prognostic signature in early-stage nonsquamous non–small cell lung cancer. *JAMA Oncol*. (2017) 3:1529. doi: 10.1001/jamaoncol.2017.1609
8. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun*. (2013) 4:2612. doi: 10.1038/ncomms3612
9. Jia D, Li S, Li D, Xue H, Yang D, Liu Y. Mining TCGA database for genes of prognostic value in glioblastoma microenvironment. *Aging*. (2018) 10:592–605. doi: 10.18632/aging.101415
10. Wu J, Xu WH, Wei Y, Qu YY, Zhang HL, Ye DW. An integrated score and nomogram combining clinical and immunohistochemistry factors to predict high ISUP grade clear cell renal cell carcinoma. *Front Oncol*. (2018) 8:634. doi: 10.3389/fonc.2018.00634
11. Xu WH, Qu YY, Wang J, Wang HK, Wan FN, Zhao JY, et al. Elevated CD36 expression correlates with increased visceral adipose tissue and predicts poor prognosis in ccRCC patients. *J Cancer*. (2019) 10:4522–31. doi: 10.7150/jca.30989
12. Alonso MH, Aussó S, Lopez-Doriga A, Cordero D, Guinó E, Solé X, et al. Comprehensive analysis of copy number aberrations in microsatellite stable colon cancer in view of stromal component. *Br J Cancer*. (2017) 117:421–3. doi: 10.1038/bjc.2017.208
13. Shah N, Wang P, Wongvipat J, Karthaus WR, Abida W, Armenia J, et al. Regulation of the glucocorticoid receptor via a BET-dependent enhancer drives antiandrogen resistance in prostate cancer. *Elife Sci*. (2017) 6:e27861. doi: 10.7554/eLife.27861
14. Bhattacharya S, Andorf S, Gomes L, Dunn P, Schaefer H, Pontius J, et al. ImmPort: disseminating data to the public for the future of immunology. *Immunol Res*. (2014) 58:234–9. doi: 10.1007/s12026-014-8516-1
15. Robinson MD, Mccarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. (2010) 26:139–40. doi: 10.1093/bioinformatics/btp616
16. Liang HW, Ye Z-H, Yin S, Mo W-J, Wang H, Zhao J-C, et al. A comprehensive insight into the clinicopathologic significance of miR-144-3p in hepatocellular carcinoma. *Oncotargets Ther*. (2017) 10:3405–19. doi: 10.2147/OTT.S138143
17. Zhang Y, Huang JC, Cai KT, Yu XB, Chen YR, Pan WY, et al. Long noncoding RNA HOTTIP promotes hepatocellular carcinoma tumorigenesis and development: a comprehensive investigation based on bioinformatics, qRTPCR and metaanalysis of 393 cases. *Int J Oncol*. (2017) 51:1705–21. doi: 10.3892/ijo.2017.4164
18. Yang X, Deng Y, He RQ, Li XJ, Ma J, Chen G, et al. Upregulation of HOXA11 during the progression of lung adenocarcinoma detected via multiple approaches. *Int J Mol Med*. (2018) 42:2650–64. doi: 10.3892/ijmm.2018.3826

19. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics*. (2012) 16:284–7. doi: 10.1089/omi.2011.0118
20. Damian S, Andrea F, Stefan W, Kristoffer F, Davide H, Jaime HC, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. (2014) 43:D447–52. doi: 10.1093/nar/gku1003
21. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. (2003) 13:2498–504. doi: 10.1101/gr.1239303
22. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res*. (2017) 77:e108–10. doi: 10.1158/0008-5472.CAN-17-0307
23. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. *Biometrics*. (2000) 56:337–44. doi: 10.1111/j.0006-341x.2000.00337.x
24. Tai H, Tabata M, Oike Y. The role of angiopoietin-like proteins in angiogenesis and metabolism. *Trends Cardiovasc Med*. (2008) 18:6–14. doi: 10.1016/j.tcm.2007.10.003
25. Galaup A, Cazes A, Jan SL, Philippe J, Connault E, Coz EL, et al. Angiopoietin-like 4 prevents metastasis through inhibition of vascular permeability and tumor cell motility and invasiveness. *Proc Natl Acad Sci USA*. (2004) 103:18721–6. doi: 10.1073/pnas.0609025103
26. Kersten S. Regulation of lipid metabolism via angiopoietin-like proteins. *Biochem Soc Transact*. (2005) 33:1059. doi: 10.1042/BST20051059
27. Tabata M, Kadomatsu T, Fukuhara S, Miyata K, Ito Y, Endo M, et al. Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. *Cell Metab*. (2009) 10:178–88. doi: 10.1016/j.cmet.2009.08.003
28. Kuo TC, Tan CT, Chang YW, Hong CC, Kuo M-L. Angiopoietin-like protein 1 suppresses SLUG to inhibit cancer cell motility. *J Clin Investig*. (2013) 123:1082–95. doi: 10.1172/JCI64044
29. Zhao T, Liang X, Chen J, Bao Y, Wang A, Gan X, et al. ANGPTL3 inhibits renal cell carcinoma metastasis by inhibiting VASP phosphorylation. *Biochem Biophys Res Commun*. (2019) 516:880–7. doi: 10.1016/j.bbrc.2019.06.120
30. Zhao Y, Wang Y, Zhao E, Tan Y, Geng B, Kang C, et al. PTRF/CAVIN1, regulated by SHC1 through the EGFR pathway, is found in urine exosomes as a potential biomarker of ccRCC. *Carcinogenesis*. (2019) 41:274–83. doi: 10.1093/carcin/bgz147
31. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
32. Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. *Cancer Immunol Res*. (2014) 2:823–30. doi: 10.1158/2326-6066.CIR-14-0112
33. D'ignazio L, Batie M, Rocha S. TNFSF14/LIGHT, a non-canonical NF-κB stimulus, induces the HIF pathway. *Cells*. (2018) 7:102. doi: 10.3390/cells7080102
34. Wu F, Wu S, Gou X. Identification of biomarkers and potential molecular mechanisms of clear cell renal cell carcinoma. *Neoplasma*. (2018) 65:242–52. doi: 10.4149/neo_2018_170511N342
35. Motzer RJ, Figlin RA, Martini JF, Hariharan S, Agarwal N, Li CX, et al. Germline genetic biomarkers of sunitinib efficacy in advanced renal cell carcinoma: results from the RENAL EFFECT trial. *Clin Genitourin Cancer*. (2017) 15:526–33. doi: 10.1016/j.clgc.2017.02.006
36. Jia Z, Zhang Z, Yang Q, Deng C, Li D, Ren L. Effect of IL2RA and IL2RB gene polymorphisms on lung cancer risk. *Int Immunopharmacol*. (2019) 74:105716. doi: 10.1016/j.intimp.2019.105716
37. Coffelt SB, Wellenstein MD, De Visser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer*. (2016) 16:431–46. doi: 10.1038/nrc.2016.52
38. Zhang L, Romero P. Metabolic control of CD8(+) T cell fate decisions and antitumor immunity. *Trends Mol Med*. (2018) 24:30–48. doi: 10.1016/j.molmed.2017.11.005
39. Ocana A, Nieto-Jiménez C, Pandiella A, Templeton AJ. Neutrophils in cancer: prognostic role and therapeutic strategies. *Mol Cancer*. (2017) 16:137. doi: 10.1186/s12943-017-0707-7
40. Engelhardt JJ, Boldajipour B, Beemiller P, Pandurangi P, Sorensen C, Werb Z, et al. Marginating dendritic cells of the tumor microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. *Cancer Cell*. (2012) 21:402–17. doi: 10.1016/j.ccr.2012.01.008
41. Ghatalia P, Gordetsky J, Kuo F, Dulaimi E, Plimack E. Prognostic impact of immune gene expression signature and tumor infiltrating immune cells in localized clear cell renal cell carcinoma. *J Immunother Cancer*. (2019) 7:139. doi: 10.1186/s40425-019-0735-5
42. Wang S, Zheng X, Chen X, Shi X, Chen S. Prognostic and predictive value of immune/stromal-related gene biomarkers in renal cell carcinoma. *Oncol Lett*. (2020) 20:308–16. doi: 10.3892/ol.2020.11574

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Importance of HPV in Chinese Penile Cancer: A Contemporary Multicenter Study

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Objective: To investigate the HPV DNA prevalence and genotype distribution among penile cancer in China. To identify association between HPV prevalence, different histological subtypes, tumor stage, tumor grade, demographics, comorbidity, and phimosis incidence trend. Standardized HPV DNA detection and $p16^{INK4a}$ expression were used in a multi-center series of 340 penile squamous cell carcinomas diagnosed from 2006 to 2017.

Materials and Methods: HPV DNA detection and genotyping were examined by a validated kit for 23 different HPV subtypes (PCR-RDB HPV test). The cases with positive HPV DNA were additional tested for $p16^{INK4a}$ expression to confirm the HPV infection.

Results: Using the PCR-RDB HPV test, overall HPV prevalence was 48.8% (166/340) and that of $p16^{INK4a}$ expression was 45.6%. In this studied population, HPV16 was the most frequent HPV type detected in HPV-positive cancers (76.5%). HPV18 was the second most common type in penile cancers (15.1%). After pathology review, 307 cases were confirmed as invasive penile cancer, and the other 33 were non-invasive caners. The histologic subtypes of warty, basaloid, clear cell papillary, adenosquamaous and pseudohyperplastic were showed high HPV DNA prevalence. Among invasive cancers, no statistically significant differences in prevalence were observed by tumor grade, tumor stage or lymphnode stage at diagnosis. HPV positive penile cancer incidence significantly increase and the phimosis incidence significantly decrease from 2006 to 2017.

Conclusions: About a half of penile cancers were related to HPV infection. Our findings highlight the phimosis related penile cancers have been declining, the HPV related in the development of penile cancer and a fully aware of regional differences in HPV genotype distribution are tasks for penile cancer control and prevention.

Keywords: penile cancer, human papillomavirus, prevalence, genotype, China

INTRODUCTION

Penile cancer is relatively rare in the developed nations of Western Europe and the United States, with an age-standardized incidence of 0.3–1.0 per 100,000 men (1). However, the prevalence of penile cancer in developing areas of Africa, Asia and South America ranges from 6 to 20 per 100,000 men (2). The etiology of penile cancer is multifactorial; it is linked to inflammation, smoking, phimosis, poor personal hygiene, and human papillomavirus (HPV) infection (3). HPV is regarded as the most frequently acquired sexually or not sexually transmitted disease, with more than 6 million new cases transmitted annually in the United States. In addition, HPV is implicated in both benign and malignant diseases, including cervical, head and neck, anal, and penile cancers. There is strong evidence linking the development of penile cancer to infection with high-risk HPV. A new World Health Organization (WHO) classification of penile cancer published in 2016 categorized penile cancer into HPV-related and non-HPV-related tumors (4). Basaloid and warty penile squamous cell carcinomas are considered HPV-related, while non-HPV-related tumors mainly include the usual type, papillary type and verrucous carcinoma.

Recently, Alemany et al. reported the largest epidemiologic study to evaluate the role of HPV infection in penile cancer, examining 1,010 penile cancer specimens from 25 countries (5). One-third to one-quarter of penile cancers were related to HPV infection. The high-risk oncogenic subtypes 16 and 18 accounted for the most frequent subtype, detected in over 70% of HPV-positive specimens. In that study, only 71 Asian samples (7%) were enrolled, and fewer cases in Asian patients (18%) were associated with HPV infection. To date, no substantial data have been reported, and the importance of HPV infection in China remains unclear. China has a huge population and correspondingly a large numbers of penile cancer patients. The best method for combating HPV-related disease is to prevent it with routine vaccination. Therefore, it is important to understand the exact HPV prevalence in China and the specific HPV genotype distribution.

We therefore sought to investigate the HPV DNA prevalence and genotype distribution among penile cancer cases in China. We also intend to identify association between HPV prevalence, different histological subtypes, tumor stage, tumor grade, demographics, comorbidity, and phimosis incidence trend. Standardized HPV DNA detection and $p16^{INK4a}$ expression were used in a multicenter study of 340 penile squamous cell carcinomas.

MATERIALS AND METHODS

Ethics Approval

Informed consent was obtained for all subjects. The patients enrolled signed consent before the treatment for future use the blood or tissue samples for scientific research. The protocols for this study were approved by the Institutional Review Board of Fudan University Shanghai Cancer Center, The Affiliated Hospital of Qingdao University, Hainan General Hospital, Northern Jiangsu People's Hospital, and Affiliated Hospital of Jiangnan University.

Patient Samples and Clinical Data

A multicenter retrospective cross-sectional study was designed and coordinated by the Fudan University Shanghai Cancer Center. In this study, we used 340 formalin-fixed, paraffin-embedded (FFPE) penile cancer specimens diagnosed from 2006 to 2017 that were obtained from 5 level three tertiary hospitals in China (Fudan University Shanghai Cancer Center, The Affiliated Hospital of Qingdao University, Hainan General Hospital, Northern Jiangsu People's Hospital, and Affiliated Hospital of Jiangnan University). Patients with suspiciously invasive penile cancer underwent complete glansectomy, partial or total penectomy. Patients initially diagnosed with pT1b-pT4 or cN1 stage underwent inguinal lymphadenectomy. Patients with more than 2 inguinal lymphnode metastasis, Cloquet's inguinal metastasis, extranodal extension or radiologically suspicious pelvic lymphnode metastasis underwent pelvic dissection. Information about age and year of diagnosis and lymph node metastasis were also obtained from the participating centers. Comorbidities such as smoking, drinking, hypertension, and diabetes were also considered in this study.

Histopathologic Evaluation

The first sections of the FFPE samples were used for histopathologic evaluation after hematoxylin and eosin (HE) staining. All cases were reviewed by a specialized pathologist (Peipei Zhang) from Fudan University Shanghai Cancer Center. The pathological evaluation was performed following the WHO criteria proposed in 2016 (6). All the patients were staged according to the latest published 8th edition American Joint Committee on Cancer TNM system.

Diagnosis included confirmation of penile squamous cell carcinoma, assessment of the subtypes, depth of invasion, lymph node metastasis, extranodal involvement, the proportion of tumor to the whole tissue section, and the adequacy of the sample for HPV testing.

Only the histological confirmation of primary squamous cell penile carcinoma cancer was included. For each tumor tissue sample, sections with the confirmed presence (>70%) of tumor cells were selected for further HPV DNA analysis.

Our pathologist finally confirmed 307 cases were invasive penile cancer. The other 33 were non-invasive caners, most of them were verrucous type (26/33), and 8 of them underwent inguinal lymphadenectomy.

DNA Extraction and Polymerase Chain Reaction (PCR) Conditions

The paraffin samples were processed under strict conditions to avoid potential contamination as described in a previous study. DNA from paraffin-embedded tissue blocks was extracted from 4 to 5 sequential unstained sections, each 4-mm thick (7). Genomic DNA was extracted with the QIAamp DNA FFPE Tissue Kit (Qiagen) following the manufacturer's recommendations. The quality of the extracted DNA was verified using a spectrophotometer (260/280 nm ultraviolet light). The

amplification was tested using the housekeeping gene GAPDH with the same reactions as an internal positive control to ensure the quality of the DNA. A verified HPV multiple infection cervical intraepithelial neoplasia sample was used as a positive control, and distilled water was used as a negative control. To avoid contamination within the lab, all specimens were independently tested in two isolated labs by blind assignment.

HPV DNA Genotyping for the 23 Types

HPV DNA detection was performed using the Human Papillomavirus Genotyping assay for 23 genotypes from Yaneng BIOscience (ShenZhen Co., Ltd., China), which was approved by the China Food and Drug Administration for diagnosis. The system provides a reliable and sensitive clinical reference for HPV detection and has been widely used in cervical cancer screening in China (8). The system can identify 18 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83) and 5 low-risk HPV types (6, 11, 42, 43, and 81).

p16^{INK4a} Expression

Immunohistochemical p16^{INK4a} expression was evaluated in all specimens. Overexpression of p16^{INK4a} can serve as a surrogate marker for transcriptionally active HPV infection due to its strong correlation with high-risk

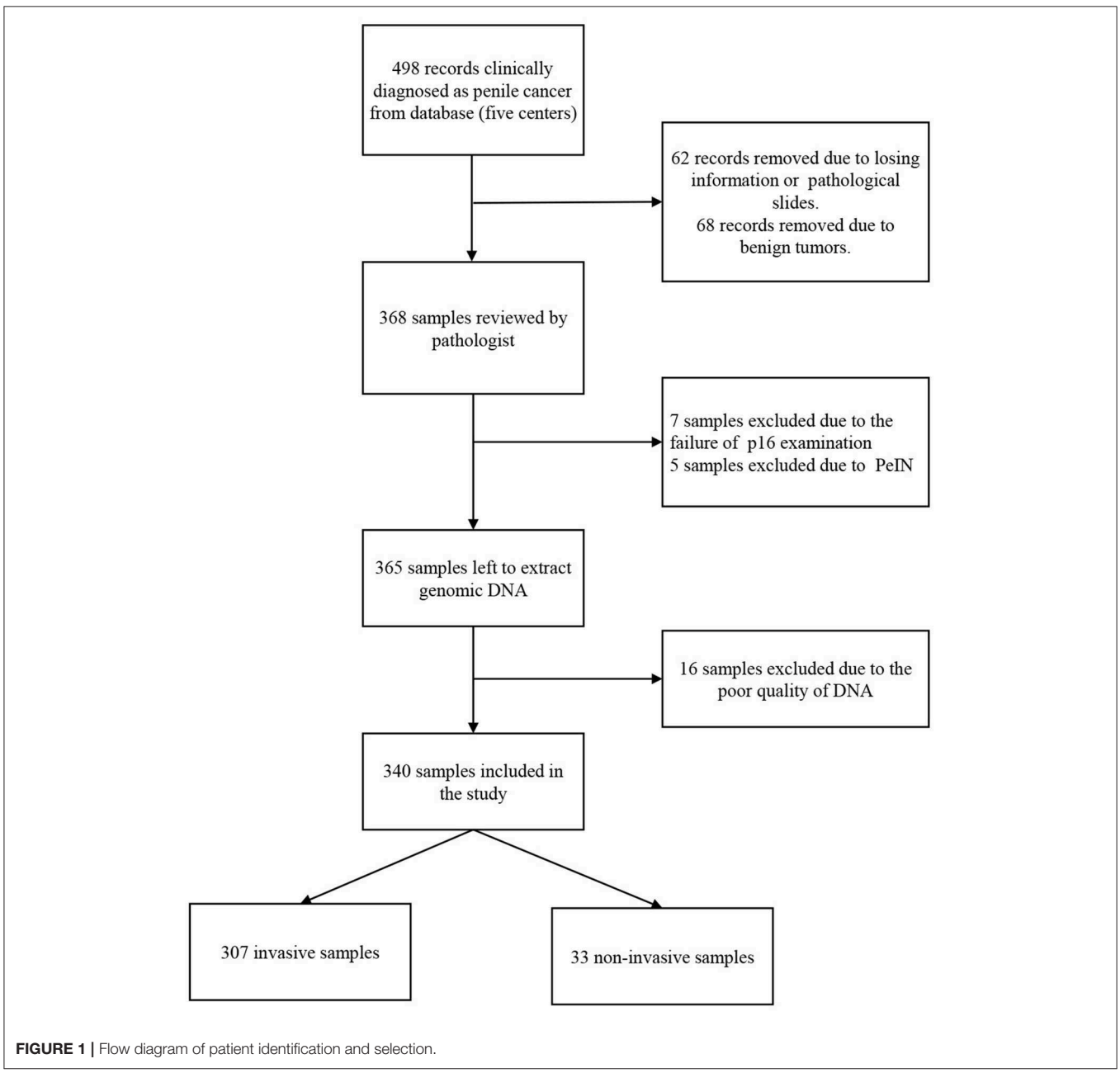


FIGURE 1 | Flow diagram of patient identification and selection.

HPV genotype infection (9). $p16^{INK4a}$ was detected using the $p16^{INK4a}$ kit (Dako, Glostrup, Denmark) following the manufacturer's protocol. A pattern of diffuse nuclear and cytoplasmic staining of >70% of the tumor cells was considered positive.

Statistical Analysis

The HPV DNA prevalence was calculated among HPV DNA-analyzed cases. We analyzed the association between the HPV prevalence, age at diagnosis, histopathological subtypes, tumor grade, tumor stage, nutrition [albumin and body mass index (BMI)], comorbidity (hypertension and diabetes mellitus), cigarette smoking, and alcohol abuse. The HPV type-specific information included single and multiple infections. Differences in categorical variables were assessed using Pearson's Chi square test or Fisher's exact test. Mann-Kendall test was performed to examine the significance of the HPV and phimosis prevalence trends. Data analyses were performed with SPSS software v.20.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at two-sided 0.05.

RESULTS

A total of 340 penile cancers were HPV DNA analyzed and included in the final analyses. **Figure 1** shows the algorithm of the study. The HPV DNA prevalence was 48.8% in penile cancers (**Table 1**), with most HPV infections presenting as high-risk (163/166, 98.2%). HPV16 was the most common type in each histological group (**Table 2**; 129/166, 77.7%). The second most common type was HPV18 (25/166, 15.1%). HPV16 and 18 accounted for ∼88.6% of the HPV positive penile cancers. The positivity of single-type HPV infection was 38.2% (130/340), accounting for 78.3% of the positive population (130/166). The positivity of dual-type infection was 7.1% (24/340), accounting for 14.5% of the positive specimens (24/166). The positivity of triple or more types infection was 3.5% (12/340), accounting for 7.2% of the positive patients (12/166) (**Table 2**).

Among penile cancers, the most frequent diagnosis was usual-type squamous cell carcinoma (74.1%). Less frequently, we identified verrucous (7.6%), warty, sarcomatoid, basaloid, and other types (18.3%). HPV DNA was detected in 128 (50.7%) out of 252 cases of usual type, 29 (72.5%) out of 40 cases of warty and basaloid types, 4 (40%) out of 10 cases of sarcomatoid type, and 5 (41.7%) out of 12 cases of histologically rare cases of penile cancer (clear cell, papillary adenosquamous, and pseudohyperplastic tumors). No HPV infection was detected in cancers with verrucous features (**Table 3**).

TABLE 1 | Patient demographics.

Variables	Statistics
No	340
Age	56 (IQR: 47–65)
BMI	24 (IQR: 21.9–26.2)
HPV infection	166 (48.8%)
Non-invasive cancer	33 (9.7%)
Invasive cancer	307 (90.3%)
Surgery	
Glansectomy	22 (6.5%)
Partial penectomy	270 (79.4%)
Radical penectomy	48 (14.1%)
Inguinal lymph node dissection	197 (57.9%)
Pelvic lymphadenectomy	43 (12.6%)
Phimosis	146 (42.9%)
pT stage	
Ta	33 (9.7%)
T1	126 (37.1%)
T2	67 (19.7%)
T3	111 (32.6%)
T4	3 (0.9%)
pN stage	
N0	226 (66.5%)
N1	47 (13.8%)
N2	35 (10.3%)
N3	32 (9.4%)

TABLE 2 | Human papillomavirus (HPV) type-specific relative contribution among HPV DNA-positive penile cancers.

	Non-invasive penile cancer		Invasive penile cancer	
	Single	Single and Multiple	Single	Single and Multiple
Low risk				
HPV 6	–	–	–	3
HPV 11	–	–	2	5
HPV 42	–	–	–	4
HPV 43	–	–	1	3
HPV 81	–	–	–	2
High risk				
HPV 16	2	2	105	127
HPV 18	–	–	9	25
HPV 31	–	–	2	3
HPV 33	–	–	3	5
HPV 35	–	–	–	1
HPV 39	–	–	–	–
HPV 45	–	–	2	4
HPV 51	–	–	1	1
HPV 52	–	–	–	3
HPV 53	–	–	–	–
HPV 56	–	–	–	4
HPV 58	–	–	–	2
HPV 59	–	–	2	3
HPV 66	–	–	1	2
HPV 68	–	–	–	–
HPV 73	–	–	–	1
HPV 82	–	–	–	–
HPV 83	–	–	–	–

TABLE 3 | Histologic diagnosis and human papillomavirus DNA prevalence in penile cancers.

	Non-invasive penile cancers				Invasive penile cancers			
		HPV DNA positive		*P*		HPV DNA positive		*P*
	n	*n*	%		*n*	*n*	%	
Histology								
HPV related	2	2	100	–	40	29	72.5	0.671
Warty					31	22	70.9	
Basaloid	2	2	100		7	5	71.4	
Clear cell					2	2	100	
Non-HPV related	31	0	–	–	267	135	50.6	0.610
Usual	5	0	–		247	128	51.8	
Verrucous	26	0	–		–	–	–	
Sarcomatoid					10	4	40.0	
Papillary					6	2	33.3	
Adenosquamous					3	1	33.3	
Pseudohyperplastic					1	0	–	
LVI				–				0.864
Yes	0	0	–		64	34	53.1	
No	33	2	6.1		243	132	54.3	

TABLE 4 | Tumor stage (TNM), tumor grade, and human papillomavirus DNA prevalence in invasive penile cancers.

		HPV DNA positive		*P*
	n	*n*	%	
pT stage				0.059
T1	126	76	60.3	
T2	67	30	44.7	
T3-4	114	58	50.9	
pN stage				0.142
N0	193	112	58.0	
N1	47	19	40.4	
N2	35	18	51.4	
N3	32	15	46.8	
Grade				0.901
G1	142	74	52.1	
G2	122	67	54.9	
G3-4	43	23	53.4	

No statistically significant differences in HPV DNA prevalence for invasive penile cancers detected among different tumor stages, lymph node stages, or tumor grade (**Table 4**) The overall HPV prevalence in cases with lymph node metastasis was 45.6%, with a relatively low proportion noted in N1 (40.4%), followed by N3 (46.8%) and N2 (51.4%). Regarding the tumor stage, T2 (44.7%) had a relatively low positivity for HPV DNA compared to T1 (60.3%) and T3-4 (50.9%). For tumor grade, the lowest proportion detected in cases with high differentiation (G1, 52.1%), followed by G3-4 (53.4%), and G2 (54.9%).

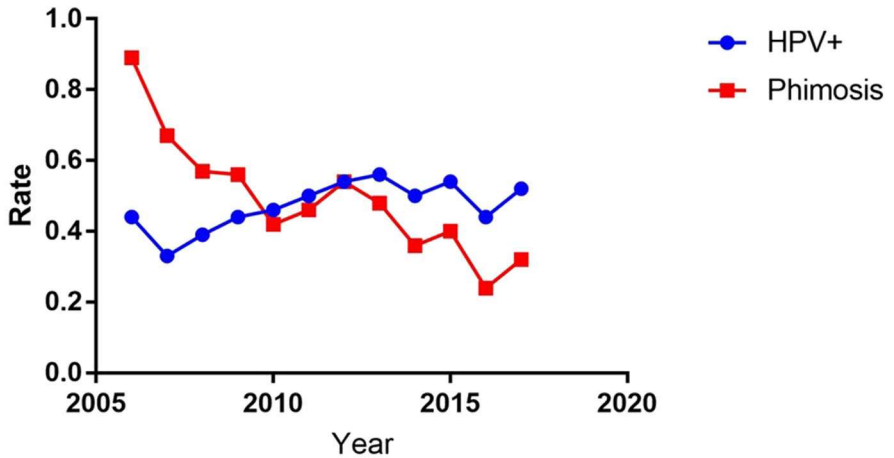
TABLE 5 | Demographics, Smoking, nutrition, comorbidity, and human papillomavirus DNA prevalence in penile cancers.

		HPV DNA positive		*P*
	n	*n*	%	
Age				0.010
<40	40	22	55.0	
40–50	70	38	54.3	
50–60	104	61	59.6	
60–70	76	28	38.1	
≥70	50	17	34.0	
BMI				0.842
<25	214	106	49.5	
≥25	126	61	48.4	
Albumin				0.502
<35	12	7	58.3	
≥35	328	159	48.4	
Heaver smoker				0.073
Yes	127	54	42.5	
No	213	112	52.6	
Diabetes				0.334
Yes	24	14	58.3	
No	316	152	48.1	
Hypertension				0.721
Yes	62	29	46.7	
No	278	137	49.3	
Phimosis				0.144
Yes	146	60	41.1	
No	194	106	56.4	

The HPV infection rates for different age groups were 55.0% (<40 years old), 54.3% (40–50 years old), 59.6% (50–60 years old), 38.1% (60–70 years old), and 34.0% (>70 years old). Thus, penile cancer with HPV infection was more frequently diagnosed in younger patients ($p = 0.010$). No association was observed between HPV type and average age at diagnosis for penile cancers. No statistically significant differences were observed for nutrition status (BMI and albumin), comorbidity (diabetes and hypertension) or lifestyle (cigarette smoking and alcohol drinking) (**Table 5**).

The etiology of penile squamous cell carcinoma has changed dramatically over the past decade. The data showed a significant increase in HPV prevalence (44–52%, $p = 0.039$) and a significant decrease in phimosis (89–32%, $p = 0.001$) from 2006 to 2017 (**Figure 2**).

All the specimens were stained to compare $p16^{INK4a}$ expression and HPV DNA. The overall percentage of $p16^{INK4a}$-positive penile cancer was 45.6%. For non-invasive penile cancers, only two cases (basaloid type) had a both HPV and $p16^{INK4a}$ positivity. HPV-positive invasive penile cancers were found to have a $p16^{INK4a}$ positivity of 82.9% (136/164), while only 17 (17/143, 11.9%) HPV-negative cases were $p16^{INK4a}$ positive. Overall, there was 83.1% (138/166) concordance between the two methods.



Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	p-value
HPV+ (%)	0.44	0.33	0.39	0.44	0.46	0.50	0.54	0.55	0.50	0.54	0.44	0.52	0.039
Phimosis(%)	0.89	0.67	0.57	0.56	0.42	0.46	0.54	0.48	0.36	0.40	0.24	0.32	0.001

FIGURE 2 | The trend of increasing HPV prevalence and decreasing phimosis from 2006 to 2017.

DISCUSSION

Limited epidemiologic data are available for the national incidence of penile cancer in China. We provide reference data for the prevalence of HPV infection and the distribution of HPV genotypes among men with penile cancer. To our knowledge, our data provide the first evidence of a high prevalence of HPV infection in patients with penile cancer in China. Our data indicate that the HPV DNA prevalence (48.8%) in China is as high as that in the United States, European Countries and South America (3, 10). The HPV DNA prevalence presented here for penile cancers is much higher than reported for other Asian countries reported in a recent worldwide study by Laia Alemany (5). The observed differences could be due to the variation of regions and the small number of patients. Although penile cancer is a rare disease, there was a large number of cases of penile cancer in China. Our study indicated that the HPV infection become a more important etiological factor in China nowadays.

HPV prevalence and types have been investigated in fewer studies in Asia compared to Europe, North America and South America. These studies primarily included lesions that were invasive penile cancer and a few cases of intraepithelial neoplasms (11–17) (**Supplementary Table 1**). However, there was a large degree of heterogeneity in PCR primers and sample number (ranging from 16 to 123).

HPV 16 and HPV 18 were the two most common types, with relative contributions of 76.5 and 15.1%, respectively. The combination of HPV types 16 and 18 greatly increased the relative contribution of HPV to penile cancer to 88.6%. Together, the HPV types 16, 18 and 33 further increased the relative contribution to 91.0%, and the results of this study strongly support the restricted genotype contribution in the pathogenesis of penile cancer in China. Our data also confirm the dominant roles of HPV16 and 18 in all types of penile cancers. According to our data, the 2-valent HPV vaccine, approved by the Chinese Food and Drug Administration, indicated a combined prevalence among HPV DNA-positive cases of 88.6% for penile cancers. Recently, the new 9-valent HPV vaccine has been approved, and 95.2% of patients might receive protection from penile cancer.

It has been reported that HPV-related tumors are diagnosed in younger patients than non-HPV-related cancers, as has been reported for female genitalia cancers and head and neck cancers (7). Our findings also indicated that age might be an important factor related to high-risk HPV infection in penile cancers, which is in agreement with previous studies. Previous studies showed that the age at diagnosis was much younger for women with invasive cervical cancers with specific HPV types, such as HPV 45 (7). However, we could not identify these significant associations, with the possible explanation of the majority of patients with positive HPV 16 infection.

The etiology of penile squamous cell carcinoma has shifted over the past decade. Our results indicated a significant increase in HPV prevalence and a significant decrease in phimosis. One explanation may be related to increase awareness of early detection of phimosis and prevention of phimosis. The other explanation was the sharp increased population floating in the past decade in China, and HPV are more likely to spread.

Our study indicated that HPV DNA may be associated with aggressive tumors, which was similar with a previous report (18). HPV DNA prevalence tends to be associated with high-grade tumors (HPV prevalence 53.4% for G3-4 vs. 43.4% for G1). However, no statistically significant differences were detected among different tumor, lymph node stages, or tumor grade. Our study did not analyze the association between the prognosis and HPV prevalence, because the median follow-up time was too short. A recent published international multicenter study including 230 patients in our study found HPV status could separate the prognosis of pN2-3 patients, and pN2-3 high

risk HPV negative group was associated with only 32% 5-year survival (19).

The success of HPV testing in clinical practice is largely dependent on its high sensitivity and negative predictive value. A previous study showed that the PCR-RDB HPV test is a reliable, sensitive, and accurate method for cervical cancer screening (8, 20). In addition, to avoid false-negative results, an internal quality control was used with the PCR-RDB method to evaluate the occurrence of false negatives. Our results also indicated a good concordance rate between the PCR-RDB HPV test and immunohistochemistry for $p16^{INK4a}$ expression, which further confirms the PCR-RDB HPV test as a reliable and easy-to-use detection method. This concordance is in agreement with a previous global study (5).

We adopted the new 2016 WHO classification and applied it to our large cohort. The updated subtypes and grades were applied to 340 cases. We confirmed the HPV differences between the two general groups and found grade discrepancies between pathological subtypes. The connection between subtype and grade may indicate a corresponding HPV positivity rate and warrants further research to determine an individualized treatment approach.

To our knowledge, our study is the first large multicenter examination of HPV prevalence and the distribution of HPV genotypes in China. The strengths of the study include the use of the same protocol for specimen collection, central pathological review, and classification by an experienced pathologist, and well-standardized virus detection in a single central laboratory using two different markers of viral presence at the levels of viral DNA and $p16^{INK4a}$ ed information on HPV positivity according to the recent WHO update of pathological subtypes and grades. This study, however, has some potential limitations. The included cases were obtained from five level three tertiary hospitals, mainly on the eastern coast of China, which may represent the developed region in China. Thus, bias could arise due to unbalanced regional development.

In conclusion, our study provided information regarding HPV infection in penile cancers in China using robust methods. Approximately half of the penile cancer cases were related to HPV infection, as detected with a PCR-RDB HPV test. As demonstrated in our study population, genotypes 16, 18, and 33 are the predominant HPV types in invasive penile cancer. Our findings highlight the phimosis related penile cancers have been declining. Regional differences in HPV genotype distribution and the carcinogenic impact of HPV indicate the need to be mindful of HPV control and prevention.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Fudan University Shanghai Cancer Center, The Affiliated Hospital of Qingdao University, Hainan General Hospital, Northern Jiangsu People's Hospital, and Affiliated Hospital of Jiangnan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DY, YZ, and WG designed research and wrote the manuscript. PZ reviewed the pathology slides. BW, SJ, and YW performed the HPV DNA genotype analysis and immunohistochemical analysis. GZ, JZ, XD, and QW performed data collection and processing of data. All authors approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.01521/full#supplementary-material

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. (2016) 66:7–30. doi: 10.3322/caac.21332
2. Parkin DM, Muir CS. Cancer incidence in five continents. Comparability and quality of data. *IARC Sci Publ.* (1976) 120:45–173. doi: 10.1007/978-3-642-85851-2
3. Sarkar FH, Miles BJ, Plieth DH, Crissman JD. Detection of human papillomavirus in squamous neoplasm of the penis. *J Urol.* (1992) 147:389–92. doi: 10.1016/S0022-5347(17)37245-2
4. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO classification of tumours of the urinary system and male genital organs-part A: renal, penile, and testicular tumours. *Eur Urol.* (2016) 70:93–105. doi: 10.1016/j.eururo.2016.02.029
5. Alemany L, Cubilla A, Halec G, Kasamatsu E, Quiros B, Masferrer E, et al. Role of human papillomavirus in penile carcinomas worldwide. *Eur Urol.* (2016) 20:513–8. doi: 10.1016/j.eururo.2015.12.007
6. Cubilla AL, Velazquez EF, Amin MB, Epstein J, Berney DM, Corbishley CM, et al. The world health organization 2016 classification of penile carcinomas: a review and update from the International Society of Urological

Pathology expert driven recommendations. *Histopathology.* (2017) 72:893–904. doi: 10.1111/his.13429
7. De Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.* (2010) 11:1048–56. doi: 10.1016/S1470-2045(10)70230-8
8. Sun P, Song Y, Ruan G, Mao X, Kang Y, Dong B, et al. Clinical validation of the PCR-reverse dot blot human papillomavirus genotyping test in cervical lesions from Chinese women in the Fujian province: a hospital-based population study. *J Gynecol Oncol.* (2017) 28:e50. doi: 10.3802/jgo.2017.28.e50
9. Mannweiler S, Sygulla S, Winter E, Regauer S. Two major pathways of penile carcinogenesis: HPV-induced penile cancers overexpress $p16^{ink4a}$, HPV-negative cancers associated with dermatoses express p53, but lack $p16^{ink4a}$ overexpression. *J Am Acad Dermatol.* (2013) 69:73–81. doi: 10.1016/j.jaad.2012.12.973
10. Miralles-Guri C, Bruni L, Cubilla AL, Castellsague X, Bosch FX, De Sanjose S. Human papillomavirus prevalence and type distribution in penile carcinoma. *J Clin Pathol.* (2009) 62:870–8. doi: 10.1136/jcp.2008.063149
11. Iwasawa A, Kumamoto Y, Fujinaga K. Detection of human papillomavirus deoxyribonucleic acid in penile carcinoma by polymerase chain reaction and in situ hybridization. *J Urol.* (1993) 149:59–63. doi: 10.1016/S0022-5347(17)35999-2
12. Chan KW, Lam KY, Chan AC, Lau P, Srivastava G. Prevalence of human papillomavirus types 16 and 18 in penile carcinoma: a study of 41 cases using PCR. *J Clin Pathol.* (1994) 47:823–6. doi: 10.1136/jcp.47.9.823
13. Suzuki H, Sato N, Kodama T, Okano T, Isaka S, Shirasawa H, et al. Detection of human papillomavirus DNA and state of p53 gene in Japanese penile cancer. *Jpn J Clin Oncol.* (1994) 24:1–6. doi: 10.1093/oxfordjournals.jjco.a039667
14. Senba M, Kumatori A, Fujita S, Jutavijittum P, Yousukh A, Moriuchi T, et al. The prevalence of human papillomavirus genotypes in penile cancers from northern Thailand. *J Med Virol.* (2006) 78:1341–6. doi: 10.1002/jmv.20703
15. Yanagawa N, Osakabe M, Hayashi M, Tamura G, Motoyama T. Detection of HPV-DNA, p53 alterations, and methylation in penile squamous cell carcinoma in Japanese men. *Pathol Int.* (2008) 58:477–82. doi: 10.1111/j.1440-1827.2008.02259.x
16. Senba M, Mori N, Wada A, Fujita S, Yasunami M, Irie S, et al. Human papillomavirus genotypes in penile cancers from Japanese patients and HPV-induced NF-kappaB activation. *Oncol Lett.* (2010) 1:267–72. doi: 10.3892/ol_00000047
17. Mu S, Hlaing Myat T, Khin Saw A, Aye Aye M, Mya T, Khin Shwe M, et al. Determination of oncogenic human papillomavirus (HPV) genotypes in anogenital cancers in myanmar. *Acta Med Okayama.* (2016) 70:103–10. doi: 10.18926/AMO/54189
18. Djajadiningrat RS, Jordanova ES, Kroon BK, Van Werkhoven E, De Jong J, Pronk DT, et al. Human papillomavirus prevalence in invasive penile cancer and association with clinical outcome. *J Urol.* (2015) 193:526–31. doi: 10.1016/j.juro.2014.08.087
19. Wang B, Gu W, Wan F, Wei Y, Xiao W, Lu X, et al. Prognosis of the 8th TNM staging system for penile cancer and refinement of prognostication by incorporating high risk human papillomavirus status. *J Urol.* (2020) 203:562–9. doi: 10.1097/JU.0000000000000584
20. Zhao J, Guo Z, Wang Q, Si T, Pei S, Wang C, et al. Human papillomavirus genotypes associated with cervical precancerous lesions and cancer in the highest area of cervical cancer mortality, Longnan, China. *Infect Agent Cancer.* (2017) 12:8. doi: 10.1186/s13027-017-0116-y

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Copy Number Analysis Reveal Genetic Risks of Penile Cancer

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Objectives: To evaluate copy number alterations (CNAs) in genes associated with penile cancer (PeC) and determine their correlation and prognostic ability with PeC.

Methods: Whole-exome sequencing was performed for tumor tissue and matched normal DNA of 35 patients diagnosed with penile squamous cell carcinoma from 2011 to 2016. Somatic CNAs were detected using the Genome Analysis Toolkit (GATK). Retrospective clinical data were collected and analyzed. All the data were statistically analyzed using SPSS 16.0 software. The cancer-specific survival rates were estimated by Kaplan-Meier curves and compared with the log-rank test.

Results: CNAs in the *MYCN* gene was detected in 19 (amplification: 54.29%) patients. Other CNAs gene targets were *FAK* (amplification: 45.72%, deletion: 8.57%), *TP53* (amplification: 2.86%, deletion: 51.43%), *TRKA* (amplification: 34.29%, deletion: 2.86%), *p75NTR* (amplification: 5.71%, deletion: 42.86%), *Miz-1* (amplification: 14.29%, deletion: 20.00%), *Max* (amplification: 17.14%, deletion: 2.86%), *Bmi1* (amplification:14.29%, deletion: 48.57%), and *MDM2* (amplification: 5.71%, deletion: 45.72%). The CNAs in *MYCN* and *FAK* correlated significantly with patient prognosis ($P<0.05$). The 3-year Recurrence-free survival rate was 87.10% among patients followed up. The 5-year survival rate of patients with *MYCN* amplification was 69.2%, compared to 94.4% in the non-amplification group. The 5-year survival rate of patients with *FAK* amplification was 65.6%, compared to 94.7% in the non-amplification group. The PPI network showed that *TP53* and *MYCN* might play meaningful functional roles in PeC.

Conclusion: *MYCN* and *FAK* amplification and *TP53* deletion were apparent in PeC. *MYCN* and *TP53* were hub genes in PeC. *MYCN* and *FAK* amplification was also detected and analyzed, and the findings indicated that these two genes are predictors of poor prognosis in PeC.

Keywords: penile cancer, copy number alterations, *MYCN*, *FAK*, *TP53*

INTRODUCTION

Penile cancer (PeC) is a rare and aggressive malignant tumor that accounts for less than 1% of carcinomas in males in the United States (1). The regional differences in incidence are significant, with the high incidence in the developing countries (2.8–6.8 per 100,000), where the low rate of neonatal circumcision and socioeconomic conditions make the patients vulnerable to a variety of risk factors. Various have been identified, including lack of circumcision, phimosis, smoking, balanitis, obesity, lichen sclerosus, and psoralen UV-A phototherapy, contributing to the courses of PeC. Moreover, human papilloma virus (HPV) has been linked to nearly 40%–50% of cases (2), and the molecular mechanism. Several studies have detected somatic changes that arise in (3–5), but few of them were based on whole-exome sequencing (5). Our previous study has performed a whole-exome sequencing analysis of PeC, and identified recurrent mutations in 11 genes (6).

Copy number alterations (CNAs) are somatic changes that cause the amplification or deletion of DNA fragment (7, 8), and represent the most common alterations of cancer cells (7, 9, 10). They contribute to both onset and progression of cancer by inappropriate activation of proto-oncogenes and/or inactivation of tumor suppressor genes (11, 12). Similar to genome-wide association studies (GWAS) that help find single nucleotide polymorphisms associated with disease phenotypes, GWAS can be extended to CNAs to help find structural variations associated with human traits and diseases (13). To date, the CNAs of PIK3CA, IL-22 and MYC have been reported in PeC. PIK3CA copy number amplification was found to have no prognostic value for cancer-specific survival (14). The function of IL-22 copy number amplifications in PeC is not clear (15). However, MYC amplification increases during PeC progression (16–18).

MYCN, an MYC family member, is a proto-oncogene that is mainly expressed in primarily neuronal cell lineages during embryogenesis and could be involved in tumorigenesis when uninhibited (19). The *MYCN* oncogene is amplified in approximately 20% of neuroblastomas (NBs) (20). *MYCN* belongs to the Myc/Max/Mad/Mnt network of proteins that regulate proliferation, apoptosis, and differentiation (21), which were amplified in neuroblastoma, small-cell lung cancer (22) and hepatic cancer (23), respectively.

Because knowledge of the pathogenesis and carcinogenesis of PeC remains limited. In the present study, we performed a whole-exome sequencing analysis of PeC in Han Chinese patients to search for the relationship between CNAs and clinical characters in penile cancer.

MATERIALS AND METHODS

Sample Source and Clinical Characteristics

Fresh PeC and blood samples from 35 PeC patients diagnosed from 2011 to 2016 were collected in the Affiliated Hospital of Qingdao University and frozen at constant temperature of −80°C. The patients were followed up by telephone and outpatient service to assess their health. All the tumor samples used for DNA extraction and exome sequencing were confirmed to have 80% tumor content by an experienced pathologist. PSCC diagnoses were made according to the clinical history, physical examination, and biopsy results. Primary treatment for PSCC included partial or radical penectomy with concomitant inguinal lymph node dissection (ILD), which included ipsilateral or ilioinguinal lymphadenectomy *via* contralateral superficial inguinal or ilioinguinal dissection according to the clinical condition. Recurrence-free survival was defined as the period from the time of present surgery in our hospital to tumor recurrence (or last follow-up visit).

Clinical information for 35 patients, including gender, age, patient number, sample acquisition method, tumor size, pathological subtype, differentiated degree, local infiltration status, WHO grade, and follow-up results (recurrence and survival), was collected and summarized in **Table S1**. Informed consent was obtained from all human subjects, and our study was approved by the Affiliated Hospital of Qingdao University Ethics Committee.

Whole-Exome Sequencing

In order to capture the tumor and blood exonic region, library preparation was performed with Agilent SureSelect Human All Exon V5+UTR Kits (Santa Clara, CA) according to the manufacturer's guidelines. Samples were deeply sequenced on the Illumina HiSeq2500 platform. The mean per-target depth of coverage across all targets was 92, with 89% of targets sequenced to an average of 10× or greater.

CNAs Detection

Raw reads from each library were quality controlled with FastQC, trimmed using Trimmomatic and then mapped to the reference human genome (NCBI build 38, hg 38) by a Burrows Wheeler Alignment (BWA) tool v 0.7.17 (24) with the BWA-maximal exact match algorithm and default parameters. PCR duplicates were flagged with Picard, and the outputs were locally realigned using the Indel Realignment tool of the Genome Analysis Toolkit (GATK) version 4.1.2.0 (25). After local realignment, the BaseRecalibrator tool from GATK was used for recalibration. CNAs in normal samples were compared to matching tumor samples using a relative coverage method performed in GATK. GISTIC version 2.0 (http://archive.broadinstitute.org/cancer/cga/gistic) analysis was performed to identify significantly recurrent copy number amplification and deletion at focal level. A log2 ratio above 0.1 was considered as "amplification," and a log2 ratio below -0.1 was considered as "deletion." (https://gatkforums.broadinstitute.org/firecloud/discussion/8254/gistic2-0)

KEGG Pathway Analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database can be seen as a set of orthologue group tables including category pathways, subcategory pathways and secondary pathways, which are often encoded by positionally coupled genes on the chromosome and much meaningful in predicting

gene functions (3). To analyze the possible relationship between related genes and PeC, we characterized somatic mutations of this pathway for PeC, which was absent in KEGG. One canonical signaling pathway, *MYCN*/Max, was found to be altered at varying frequencies in the different cancer types analyzed by KEGG (https://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map05202&keyword=MYCN).

Analysis of the PPI Network

The STRING database (http://string-db.org/) is a precomputed global resource for predicting functional associations between proteins. In this paper, the STRING online tool was applied to analyze the protein-protein interactions (PPIs) of the CNA genes in PeC with the threshold of combined score >0.4.

Statistical Analysis

All the data collected were statistically analyzed by SPSS 16.0 software. We divided the patients into two groups based on the CNA results: the normal target gene group and the abnormal target gene amplification group. Patient status information was obtained through outpatient or telephone follow-up. Chi-square analysis and Kaplan-Meier survival curves were used to compare the five-year survival rates among different groups, and significant differences were determined using the log-rank test. All *P* values were two-sided, and a *P* value < 0.05 was considered to indicate statistical significance. Figures were edited with Origin 8 software.

RESULTS

Clinical Characteristics and Gene Variations

The median age of the 35 patients participating in exome sequencing analysis was 63 years (range 27–86 years). Twenty-three patients (65.71%) had redundant prepuce, 9 patients (25.71%) had phimosis, and nobody had been circumcised. All patients were diagnosed with penile squamous cell carcinoma. The majority of primary lesions presented on the corpus cavernosum (n = 16; 45.71%) and occasionally involved the adjacent structures (submucosa [n = 5; 14.29%], dartos [n = 1; 2.86%], subcutaneous soft tissue [n = 1; 2.86%]), and 5 specimens could not be defined. Sixteen (45.71%) patients were diagnosed with pT stage expressing T2 disease. The proportion of histologically well to high and moderately differentiated cases were 57.14% and 31.43%, separately. The proportion of samples with low differentiation was 8.82%, and 1 case was unknown. Partial penectomy was performed in the majority of cases (n = 29; 82.86%). Five patients also underwent inguinal lymph node dissection in addition to surgical removal of the lesion, and the result showed that 2 cases were positive. The median follow-up time was 60 months, and the number of patients lost to follow-up was 4. Recurrence occurred in four patients within 3 years after surgery and The 3-year Recurrence-free survival rate was 87.10%. The overall 3- and 5-year cancer-specific survival (CSS) rates were 87.10% and 83.87%, respectively.

For HPV detection, only 30 samples were tested for HPV and five were not. Among 30 PSCC samples, six were found to be HPV-positive. Moreover, five were HPV type 16 and one appeared HPV types 18 and 81. Clinicopathologic data were obtained from electronic clinical medical records and are shown in Supporting Information **Table S1**.

The average sequencing depth was 120× for the tumor samples and 70× for matched normal blood samples, with ≥10× coverage for 89.0% of the target regions in tumor samples and 88.7% of the target regions in blood samples (**Figure 1** and **Table S1**).

Mutational Features and Pathway Alterations Relative to PeC

The mutational features of the PeC sample were also assessed. These features can be indicative of specific mutagenic mechanisms promoting tumorigenesis. A gene set analysis was performed with KEGG to determine pathways associated with PeC. In our PeC

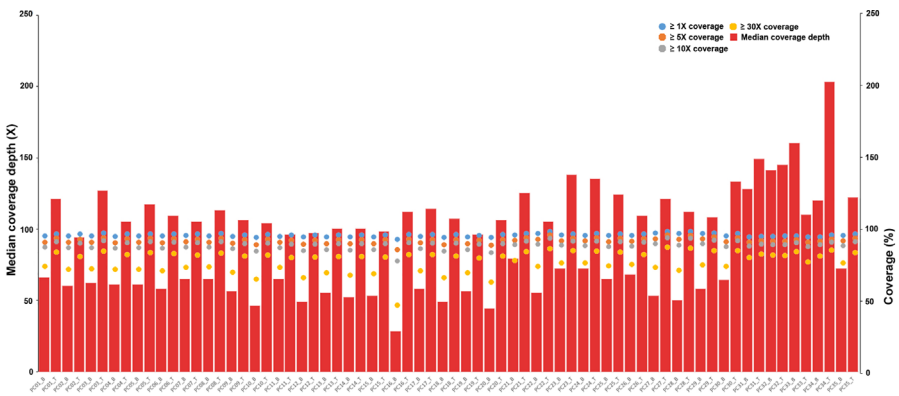

FIGURE 1 | Depth and coverage of 35 paired samples. The histograms represent the average sequencing depth of each sample, and the specific values are shown on the left axis. The scatter plot shows the distribution of all samples under coverage values of 1×, 5×, 10× and 30×, and the specific values refer to the right axis.

samples, this pathway had at least one alteration, and most alterations were found in 19 tumors (54.29% of samples). The relative pathway targets involve 10 kinds of proteins (**Figure 2A**). Nine target proteins were detected in PeC samples, except for sp-1 in the known pathway. Most mutations caused amplification of the affected genes, including *MYCN, Max, FAK, SP-1* and *TRKA*, while *MDM2, Bmi1,TP53*, *p75NTR*, and *Miz-1were more likely to* exhibits downregulation levels.

The copy number amplification of *MYCN* was 19 (54.29%), which is the largest number of mutations in the analysis. The remaining targets were *FAK* (amplification: 45.72%, deletion: 8.57%), *TP53* (amplification: 2.86%, deletion: 51.43%), *TRKA* (amplification: 34.29%, deletion: 2.86%), *p75NTR* (amplification: 5.71%, deletion: 42.86%), *Miz-1* (amplification: 14.29%, deletion: 20.00%), *Max* (amplification: 17.14%, deletion: 2.86%), *Bmi1* (amplification:14.29%, deletion: 48.57%), and *MDM2* (amplification: 5.71%, deletion: 45.72%). Therein, the *TP53* and *p75NTR* mutations were inactivating, and the remaining targets were amplified. Detailed information about the samples for the exome sequencing analysis can be found in Supporting Information **Table 1** and **Figure 2B**.

Correlation and Prognosis Between CNAs and PeC

The 35 patients' follow-up information was obtained through outpatient and telephone visits. The rate of lost follow-up was 11.4%, and four patients could not be contacted. Finally, only 31 patients were included to analyze the correlation between prognosis and gene mutations. According to the results of the Kaplan-Meier survival curve, the five-year survival rates of the *MYCN* amplification and *MYCN* non-amplification groups were 69.2%

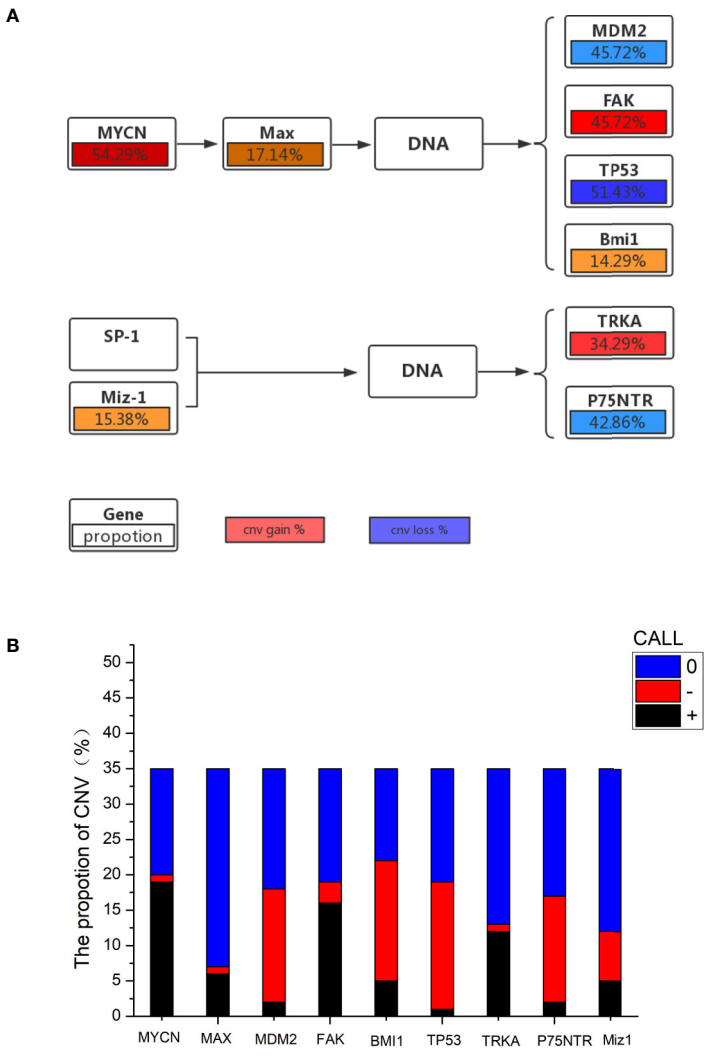

FIGURE 2 | **(A)** Proportion of gene copy number variation in the KEGG pathway. **(B)** Proportion of gene copy number variation: 0: normal gene number; –: gene copy number deletion. +: gene copy number amplification.

TABLE 1 | Proportion of gene copy number alterations.

Gene	CNA	Number	Total	Proportion	Variation Proportion
MYCN	+	19	35	54.29%	57.14%
	–	1		2.86%	
	0	15		42.86%	
Max	+	6	35	17.14%	20.00%
	–	1		2.86%	
	0	28		80.00%	
MDM2	+	2	35	5.71%	51.43%
	–	16		45.72%	
	0	17		48.57%	
PTK2(FAK)	+	16	35	45.72%	54.29%
	–	3		8.57%	
	0	16		45.72%	
Bmi1	+	5	35	14.29%	62.86%
	–	17		48.57%	
	0	13		37.14%	
TP53	+	1	35	2.86%	54.29%
	–	18		51.43%	
	0	16		45.71%	
NTRK1(TRKA)	+	12	35	34.29%	37.14%
	–	1		2.86%	
	0	22		62.86%	
NGFR(p75NTR)	+	2	35	5.71%	48.57%
	–	15		42.86%	
	0	18		51.43%	
ZBTB17(Miz1)	+	5	35	14.29%	34.29%
	–	7		20.00%	
	0	23		65.71%	

and 94.4%, respectively. Simultaneously, the P value from the log-rank test was 0.047<0.05, which means that the difference between the groups with significantly correlation and *MYCN* was an independent prognostic factor of PeC (**Figure 3A**). Analogously, the five-year survival rates of the *FAK* non-amplification group and the *FAK* amplification group were 94.7% and 65.6%, respectively. The P value from the log-rank test was 0.032<0.05, which means that the difference between the groups was statistically significant, and *FAK* amplification could act as an independent prognostic factor of PeC (**Figure 3B**). However, the five-year survival rates of the normal *TP53* group and *TP53* inactivation group were 88.9% and 76.9%, respectively. The P value from the log-rank test was 0.329<0.05, which means that the difference between the groups without correlation and *TP53* inactivation was not the prognostic value of PeC (**Figure 3C**).

PPI Network Constructed for CNA Genes in PeC

We constructed a PPI network of proteins encoded by CNA genes in PeC based on the PPI network, and the present study identified the top 2 hub genes: *TP53* and *MYCN* (**Figure 4**). These two genes might play meaningful functional roles in PeC.

DISCUSSION

PeC is a rare malignancy in the developed world but is much more common in developing countries. The genetic and molecular basis of PeC is still poorly understood (5), and further understanding of these aspects is important to improve our ability to diagnose, treat and prevent PeC. In our previous study (6), we characterized the PSCC genomic landscape using whole-exome sequencing. Of the 30 paired blood and tumor samples, recurrent mutations were identified in 11 genes; we also observed the frequently altered pathways for potential. The present study was based on the previous study in the samples and another five samples was added to the present study but not done HPV detection. SNPs have been reported in the previous study (6), so we're not going to repeat it here.

Overall survival and recurrence-free survival in our study were higher than in Jong Won' s study (26), the possible reason for which was that the pathological stage of the patients in our study were all≤T2, while the pathological stage ≥T3 accounts for 30.2% in Jong Won' s study resulting to the lower OS and RFS. The patients in our study had earlier stages of pathology, perhaps because modern people in our region are more concerned about their health.

Currently, significant poor prognosticator in patients with penile cancer include lymph node positivity (27), metastatic nodes≥ 4 (28), AJCC stage ≥ III disease (26), pathologic stage of the primary tumor (29), histologic grade<G1 (30, 31), a tumor thickness≥ 5 mm (32), vertical growth pattern (33), age<53, Lactate Dehydrogenase (LDH)<188.5 U/L and Platelet-lymphocyte Ratio (PLR)<133.5 (34), p53 positivity (31), Human papillomavirus infection (35). However, there are few studies on the prognosis of penile cancer at the gene level, so our study also studied the gene types of prognostic factors later.

Our results provide the first detection of a *MYCN* CNA in the somatic mutant spectrum of PeC in Chinese men through whole-exome sequencing. In our sample, the *MYCN* amplification detection rate was 54.29%; *MYCN* is the type of gene in which we detected the highest mutation rate. In addition, the CNAs associated with *FAK* amplification and *TP53* deletion were found in more than one-third of the total samples tested. Furthermore, variations in other genes, such as *Max*, *Miz-1*, *Bmi1*, and *p75NTR*, were found in more than 10% of the samples.

The *MYCN* oncogene plays an important role in human tumorigenesis and has been proven to bind to gene promoters for various oncogenes involved in multiple life activities (36) and to increase the expression of many downstream targets. Previous data have indicated that the primary function of *MYCN* is as a transcription factor known to specifically bind the DNA E-box sequence CACGTG (37). A later study supports a dual role for *MYCN*. Murphy et al. showed that *MYCN* binds more often to the CATGTG E-box sequence, and *MYCN* binding is associated with DNA hypermethylation and can therefore also serve as an intermediary for chromatin structure-mediated regulation of various cellular processes, including cell growth, cell proliferation and cell differentiation (38). As a hub gene identified by our PPI study, *MYCN* plays an important regulatory role in its related pathways.

A previous study (39) found that *MYCN* amplification is one of the most significant prognostic indicators of neuroblastoma and is associated with rapid tumor progression and poor

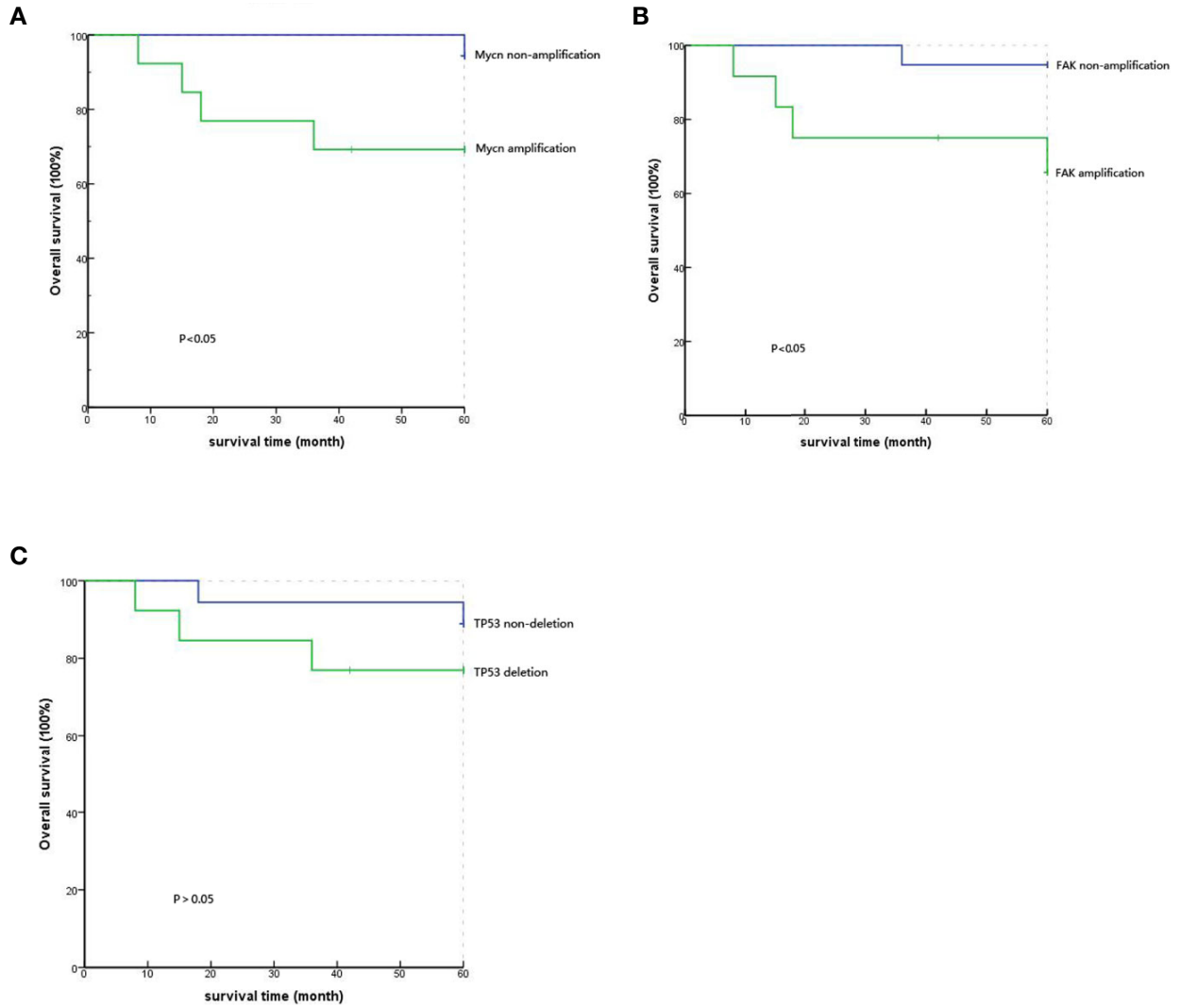

FIGURE 3 | Comparison of the 5-year survival rate with different gene variations by Kaplan-Meier analysis. **(A)** Comparison of the 5-year survival rate between *MYCN* amplification and *MYCN* non-amplification showed significant difference (P=0.047). **(B)** Comparison of the 5-year survival rate between *FAK* amplification and *FAK* non-amplification showed a significant difference (P=0.032). **(C)** Comparison of the 5-year survival rate between *TP53* deletion and *TP53* nondeletion showed no significant difference (P=0.329).

prognosis. Similarly, our data analysis found that *MYCN* was correlated with the prognosis of PeC, *MYCN* amplification indicated a poor prognosis. Lloveras B's study (40) found that the *MYC* copy number amplification was significantly associated with poor outcome (mortality, node metastasis and/or recurrence) in PeC. As a member of the *MYC* family of proto-oncogenes, *MYCN* is more likely to appear as an independent prognostic indicator of PeC if the sample size is increased.

Studies (36) found that in neuroblastoma, the relationship between *MYCN* amplification and cell activity and aggressiveness suggests a potential relationship between focal adhesion kinase (*FAK*) and *MYCN*, since *FAK* is a key protein involved in cell activity.

FAK is a nonreceptor protein tyrosine kinase that targets focal adhesion and controls multiple cellular signaling pathways, including proliferation and survival (41). The inhibition of *FAK* activation has been found to affect a number of cellular pathways (36). *FAK* overexpression has also been shown in human sarcoma and melanoma tumors (42). A previous study (43) found that *MYCN* binds to the *FAK* promoter *in vitro* and *in vivo*, resulting in upregulation of *FAK* with electrophoretic mobility shift, chromatin immunoprecipitation (ChIP), and dual luciferase assays. Therefore, it is well proven that *MYCN*-regulated *FAK* intervention affects the prognosis of patients, which is consistent with our finding that *FAK* amplification could be an independent prognostic factor of PeC.

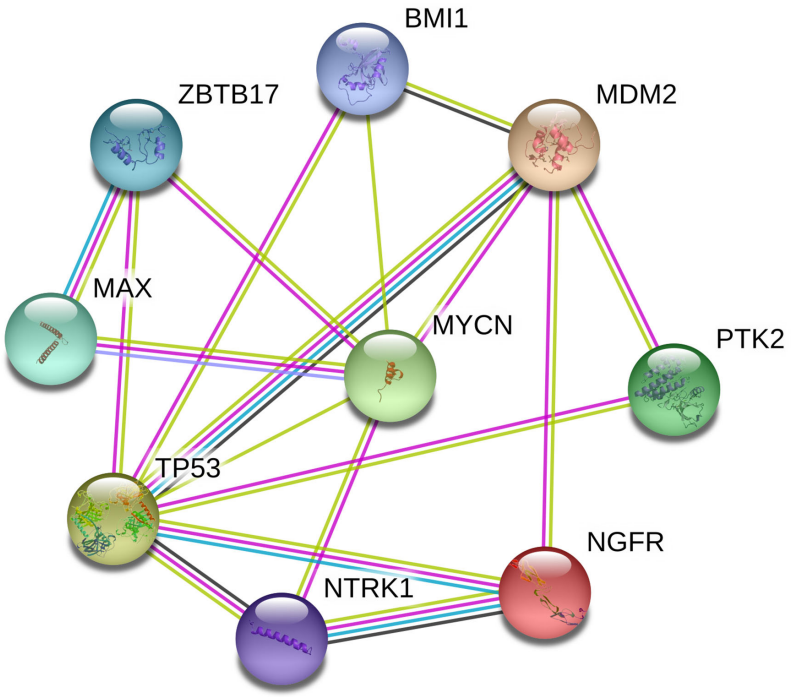

FIGURE 4 | Protein-protein interaction network of copy number alteration (CNA) genes in penile cancer (PeC). Circles represent genes, lines represent the interaction of proteins encoded by the genes, and the results within the circle represent the structures of the proteins. The line color represents evidence of the interaction between the proteins.

Cloning and evaluation of the *FAK* promoter has shown that it has many binding sites for various oncogenes, such as *TP53* (44). The tumor protein *TP53* (*TP53* or *TTP53*) was the first tumor suppressor gene, discovered in 1979, and is the guardian of the genome (45). *TP53* is the most widely studied tumor suppressor gene, playing an important role in inhibiting tumor development. The function of the *TP53* gene is to inhibit cell proliferation in response to DNA damage. By regulating target genes, *TP53* induces a variety of cellular responses, including growth arrest, senescence, and apoptosis (46, 47). However, the mutated *TP53* protein loses its protection against genomic functions, including the ability to inhibit cell proliferation and induce apoptosis, when mutated (48). Mutations in the *TP53* gene occur in most malignant tumors, such as lung cancer (49) and breast cancer (50). At the genetic level, carcinogenesis is a multistep process in which both oncogene activation and tumor suppressor gene inactivation are involved. Examination of the samples revealed a large number of SNP mutations in *TP53* in PeC, and the most common mutation classifications were missense mutation and nonsense mutation. These may account for the role of *TP53* in the molecular etiology of PeC.

In this study, a large number of gene variants of the samples were found in the *MYCN/Max* pathway in PeC, especially in *MYCN*. In addition, the *MYCN* and *FAK* CNA is associated with the prognosis of PeC, and its high expression level indicates a poor prognosis. However, mutations in *TP53* were not found to be related to prognosis, perhaps because our sample size was insufficient; it is necessary to carry out relevant tests with a larger sample size in the future.

DATA AVAILABILITY STATEMENT

According to national legislation/guidelines, specifically the Administrative Regulations of the People's Republic of China on Human Genetic Resources (http://www.gov.cn/zhengce/content/2019-06/10/content_5398829.htm, http://english.www.gov.cn/policies/latest_releases/2019/06/10/content_281476708945462.htm), no additional raw data is available at this time. Data of this project can be accessed after an approval application to the China National Genebank (CNGB, https://db.cngb.org/cnsa/). Please refer to https://db.cngb.org/, or email: CNGBdb@cngb.org for detailed application guidance. The accession code CNP0001368 should be included in the application.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Affiliated Hospital of Qingdao University Ethics

Committee. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YY, CG, and ZL contributed conception and design of the study. YY and YC organized the database. MW performed the statistical analysis. YY wrote the first draft of the manuscript. CG, YC, MW, and JZ wrote sections of the manuscript. XM, SL, and HY read and revised the manuscript. HN provided approval for publication of the content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.596261/full#supplementary-material

REFERENCES

1. Douglawi A, Masterson TA. PeC epidemiology and risk factors: a contemporary review. *Curr Opin Urol* (2018) 29(2):1. doi: 10.1097/MOU.0000000000000581
2. Yu YB, Wang YH, Yang XC, Zhao Y, Niu HT. The relationship between human papillomavirus and PeC over the past decade: a systematic review and meta-analysis. *Asian J Androl* (2019) 21(4):375–80. doi: 10.4103/aja.aja_39_19
3. Andersson P, Kolaric A, Windahl T, Kirrander P, Söderkvist P, Karlsson MG. PIK3CA, HRAS and KRAS gene mutations in human penile cancer. *J Urol* (2008) 179(5):2030–4. doi: 10.1016/j.juro.2007.12.040
4. Annunziata C, Buonaguro L, Losito S. Somatic mutations of STK11 gene in human papillomavirus positive and negative penile cancer. *Infect Agents Cancer* (2013) 8:6. doi: 10.1186/1750-9378-8-2
5. Feber A, Worth DC, Chakravarthy A, De WP, Shah K, Arya M, et al. CSN1 Somatic Mutations in Penile Squamous Cell Carcinoma. *Cancer Res* (2016) 76 (16):4720–7. doi: 10.1158/0008-5472.CAN-15-3134
6. Wang Y, Wang K, Chen Y, Zhou J, Liang Y, Yang X, et al. Mutational landscape of penile squamous cell carcinoma in a Chinese population. *Int J Cancer* (2019) 145(5):1280–9. doi: 10.1002/ijc.32373
7. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. *Nature* (2010) 463(7283):899–905. doi: 10.1038/nature08822
8. Redon R. Global variation in copy number in the human genome. *Nature* (2006) 444(7118):444–54. doi: 10.1038/nature05329
9. Kim TM, Xi CH, Luquette LJ, Park RW, Johnson MD, Park PJ. Functional genomic analysis of chromosomal aberrations in a compendium of 8000 cancer genomes. *Genome Res* (2013) 23(2):217–27. doi: 10.1101/gr.140301.112
10. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* (2015) 518(7540):495–501. doi: 10.1038/nature14169
11. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* (2012) 489 (7417):519–25. doi: 10.1038/nature11404
12. Nik-Zainal S, Van Loo P, Wedge DC, Alexandrov LB, Greenman CD, Lau KW, et al. The Life History of 21 Breast Cancers. *Cell* (2012) 149(5):14. doi: 10.1016/j.cell.2012.04.023
13. Larsen SJ, do Canto LM, Rogatto SR, Jan B. CoNVaQ: a web tool for copy number variation-based association studies. *BMC Genomics* (2018) 19(1):369. doi: 10.1186/s12864-018-4732-8
14. Adimonye A, Stankiewicz E, Touche S, Kudahetti S, Tinwell B, Corbishley C, et al. The Prognostic Value of PIK3CA Copy Number Gain in PeC. *Urology* (2018) S0090-4295(18)30560-0. doi: 10.1016/j.urology.2018.03.056
15. Marchi FA, Martins DC, Barros-Filho MC, Kuasne H, Busso LA, Brentani H, et al. Multidimensional integrative analysis uncovers driver candidates and biomarkers in penile carcinoma. *Sci Rep* (2017) 7:11. doi: 10.1038/s41598-017-06659-1
16. Kayes O, Minhas S. Biomarker discovery in PeC. *J Urol* (2012) 188(5):1660–1. doi: 10.1016/j.juro.2012.08.056
17. Masferrer E, Ferrándiz-Pulido C, Lloveras B, Masferrer-Niubò M, Espinet B, Salido M, et al. MYC Copy Number Gains are Associated with Poor Outcome in Penile Squamous Cell Carcinoma. *J Urol* (2012) 188(5):1965–71. doi: 10.1016/j.juro.2012.07.003
18. Busso-Lopes AF, Marchi FA, Kuasne H, Scapulatempo-Neto C, Trindade-Filho JCS, De Jesus CMN, et al. Genomic Profiling of Human Penile Carcinoma Predicts Worse Prognosis and Survival. *Cancer Prev Res* (2015) 8(2):149–56. doi: 10.1158/1940-6207.CAPR-14-0284
19. Lovén J, Zinin N, Wahlström T, Muller I, Brodin P, Fredlund E, et al. *MYCN*-regulated microRNAs repress estrogen receptor-alpha (ESR1) expression andneuronal differentiation in human neuroblastoma. *Proc Natl Acad Sci USA* (2010) 107(4):1553–8. doi: 10.1073/pnas.0913517107
20. Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, et al. Neuroblastoma. *Nat Rev Dis Primers* (2016) 2:16078. doi: 10.1038/nrdp.2016.78
21. Smith AG, Popov N, Imreh M, Axelson HK, Henriksson M. Expression and DNA-binding activity of *MYCN*/*Max and* Mnt/*Max* during induced differentiation of human neuroblastoma cells. *J Cell Biochem* (2004) 92 (6):1282–95. doi: 10.1002/jcb.20121
22. Ruizpérez MV, Albihn A, Arsenianhenriksson M. *MYC Oncogene*. In: Schwab M, editor. *Encyclopedia of Cancer*. Berlin, Heidelberg:Springer Berlin Heidelberg (2017). p.2976–82.
23. Yasukawa K, Liew LC, Hagiwara K, Hironaka-Mitsuhashi A, Qin XY, Furutani Y, et al. MicroRNA-493-5p-mediated repression of the *MYCN*oncogene inhibits hepatic cancer cell growth and invasion. *Cancer Sci* (2020) 111(3):869–80. doi: 10.1111/cas.14292

24. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheelertransform. *Bioinformatics* (2009) 25(14):1754–60. doi: 10.1093/bioinformatics/btp324
25. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* (2010) 20(9):1297–303. doi: 10.1101/gr.107524.110
26. Kim JW, Kim YS, Ko WJ, Choi YD, Hong SJ, Chung BH, et al. Prognostic Factors of Penile Cancer and the Efficacy of Adjuvant Treatment after Penectomy: Results from a Multi-institution Study. *J Korean Med Sci* (2018) 33(37):e233. doi: 10.3346/jkms.2018.33.e233
27. Lopes A, Hidalgo GS, Kowalski LP, Torloni H, Rossi BM, Fonseca FP. Prognostic Factors in Carcinoma of the Penis: Multivariate Analysis of 145 Patients Treated with Amputation and Lymphadenectomy. *J Urol* (2015) 156 (5):1637. doi: 10.1016/S0022-5347(01)65471-5
28. Pandey D, Mahajan V, Kannan RR. Prognostic factors in node-positive carcinoma of thepenis. *J Surg Oncol* (2010) 93(2):133–8. doi: 10.1002/jso.20414
29. Lont AP, Kroon BK, Horenblas S, Gallee MPW, Berkhof J, Meijer CJLM, et al. Presence of high-risk human papillomavirus DNA in penile carcinoma predicts favorable outcome in survival. *Int J Cancer* (2010) 119(5):1078–81. doi: 10.1002/ijc.21961
30. Ficarra V, Zattoni F, Cunico SC, Galetti TP, Luciani L, Fandella A, et al. Lymphatic and vascular embolizations are independent predictive variables of inguinal lymph node involvement in patients with squamous cell carcinoma of the penis. *Cancer* (2010) 103(12):2507–16. doi: 10.1002/cncr.21076
31. Lopes A, Bezerra ALR, Pinto CAL, Serrano SV, De Mello CA, Villa LL, et al. p53 as a New Prognostic Factor for Lymph Node Metastasis in Penile Carcinoma: Analysis of 82 Patients Treated with Amputation and Bilateral Lymphadenectomy. *J Urol* (2002) 168(1):81–6. doi: 10.1016/S0022-5347(05)64836-7
32. Slaton JW, Morgenstern N, Levy DA, Santos MJ, Tamboli P, Ro JY, et al. Tumor Stage, Vascular Invasion and The Percentage of Poorly Differentiated Cancer: Independent Prognosticators for Inguinal Lymph Node Metastasis In Penile Squamous Cancer. *J Urol* (2001) 165(4):1138–42. doi: 10.1016/S0022-5347(05)66450-6
33. Villavicencio H, Rubio-Briones J, Regalado R, Chéchile G, Palou J. Grade, local stage and growth pattern as prognostic factors in carcinoma of the penis. *Eur Urol* (1997) 32(4):442–7. doi: 10.1159/000480804
34. Hu C, Bai Y, Li J, Zhang G, Yang L, Bi C, et al. Prognostic value of systemic inflammatory factors NLR, LMR, PLR and LDH in penile cancer. *BMC Urol* (2020) 20(1):57. doi: 10.1186/s12894-020-00628-z
35. Bezerra AL, Lopes A, Santiago GH, Ribeiro KC, Latorre MR, Villa LL. Human papillomavirus as a prognostic factor in carcinoma of thepenis. *Cancer* (2001) 91(12):2315–21. doi: 10.1002/1097-0142(20010615)91:12<2315::AID-CNCR1263>3.0.CO;2-C
36. Megison ML, Stewart JE, Nabers HC, Gillory LA, Beierle EA. *FAK* inhibition decreases cell invasion, migration and metastasis in *MYCN* amplified neuroblastoma. *Clin Exp Metastasis* (2012) 30(5):555–68. doi: 10.1007/s10585-012-9560-7
37. Yang J, Sung E, Donlin-Asp PG, Corces VG. A subset of Drosophila Myc sites remain associated with mitotic chromosomes colocalized with insulator proteins. *Nat Commun* (2013) 4:1464. doi: 10.1038/ncomms2469
38. Murphy DM, Buckley PG, Bryan K, Sudipto D, Leah A, Foley NH, et al. Global *MYCN* transcription factor binding analysis in neuroblastoma reveals association with distinct E-box motifs and regions of DNA hypermethylation. *PLoS One* (2009) 4(12):e8154. doi: 10.1371/journal.pone.0008154
39. Fulda S, Poremba C, Berwanger B, Hacker S, Eilers M, Christiansen H, et al. Loss of Caspase-8 Expression Does Not Correlate with *MYCN* Amplification, Aggressive Disease, or Prognosis in Neuroblastoma. *Cancer Res* (2006) 66 (20):10016–23. doi: 10.1158/0008-5472.CAN-05-4079
40. Masferrer E, Ferrándiz-Pulido C, Lloveras B, Masferrer-Niubò M, Espinet B, Salido M, et al. MYC Copy Number Gains are Associated with Poor Outcome in Penile Squamous Cell Carcinoma. *J Urol* (2012) 188(5):1965–71. doi: 10.1016/j.juro.2012.07.003
41. Dunty JM, Schaller MD, Gabarra-Niecko V. *FAK* regulates biological processes important for the pathogenesis of cancer. *Cancer Metastasis Rev* (2003) 22(4):359–74. doi: 10.1023/A:1023725029589
42. Hanks SK, Ryzhova L, Shin NY, Brabek J. Focal adhesion kinase signaling activities and their implications in the control of cell survival and motility. *Front Biosci* (2003) 8(4):d982–96. doi: 10.2741/1114
43. Beierle EA, Trujillo A, Nagaram A, Kurenova EV, Finch R, Ma X, et al. N-MYC regulates focal adhesion kinase expression in human neuroblastoma. *J Biol Chem* (2007) 282(17):12503–16. doi: 10.1074/jbc.M701450200
44. Beierle EA. *MYCN*, neuroblastoma and focal adhesion kinase (*FAK*). *Front Biosci (Elite Ed)* (2011) 3(2):421–33. doi: 10.2741/e257
45. Saha MN, Qiu L, Chang H. Targeting *TP53*by small molecules in hematological malignancies. *J Hematol Oncol* (2013) 6(1):23. doi: 10.1186/1756-8722-6-23
46. Ma JT, Han CB, Zhao JZ, Jing W, Zhou Y, Huang L-T, et al. Synergistic cytotoxic effects of recombinant human adenovirus *TP53*and radiation at various time points in A549 lung adenocarcinoma cells. *Oncol Lett* (2012) 4 (3):529–33. doi: 10.3892/ol.2012.747
47. Guan Y-s, Liu Y, Zou Q, He Q, La Z, Yang L, et al. Adenovirus-mediated wild-type *TP53*gene transfer in combination with bronchial arterial infusion for treatment of advanced non-small-cell lung cancer, one year follow-up. *J Zhejiang Universityence B* (2009) 10(5):331–40. doi: 10.1631/jzus.B0820248
48. Rousseau B, Jacquot C, Palabe JL, Malleter M, Roussakis C. *TP53*transcription factor for the NEDD9/HEF1/Cas-L gene: potential targets in Non-Small Cell Lung Cancer treatment. *Sci Rep* (2015) 5:10356. doi: 10.1038/srep10356
49. Liu K, Gao W, Lin J. Effect of the *TP53*α gene on thechemosensitivity of the H1299 human lung adenocarcinoma cell line. *Oncol Lett* (2017) 14(2):1411–8. doi: 10.3892/ol.2017.6356
50. Kaur RP, Vasudeva K, Kumar R, Kumar R, Munshi A, et al. Role of *TP53*Gene in Breast Cancer: Focus on Mutation Spectrum and Therapeutic Strategies. *Curr Pharm Des* (2018) 24(30):3566–75. doi: 10.2174/1381612824666180926095709

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CASE REPORT
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Case Report: Two Cases of Chemotherapy Refractory Metastatic Penile Squamous Cell Carcinoma With Extreme Durable Response to Pembrolizumab

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Background: Penile squamous cell carcinoma (PSCC) is a rare malignancy, and those patients with metastatic disease have limited treatment options. Treatment is largely comprised of platinum-based chemotherapy; however, patients progressing after initial chemotherapy have a median overall survival (OS) of less than 6 months. Based on a high percentage of PD-L1 expression in patients with PSCC, and its biological similarities to other squamous cell carcinomas, we present two patient cases treated with pembrolizumab with extraordinary durable treatment response far beyond treatment with standard therapy.

Main Body: The first patient is a 64 year old male with PSCC who was treated with neoadjuvant chemotherapy, partial penectomy, and adjuvant radiation prior to developing metastatic disease. He had a high TMB (14 mutations/Mb) and was started on pembrolizumab with a complete response, which has been maintained for 38 months. The second patient is an 85 year old male with PSCC who was treated with partial penectomy and adjuvant chemotherapy and radiation prior to developing metastatic disease. He had positive PD-L1 expression CPS 130) and was started on pembrolizumab with a partial response, which has been maintained for 18 months after starting treatment.

Conclusions: These two cases of extreme durable response with pembrolizumab (with molecular data including TMB and PD-L1 status) represent a significant clinical benefit in this patient population. With limited treatment options that result in a median OS of less than 6 months, along with the toxicity profile of chemotherapy which may not be tolerated

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in elderly patients with comorbidities, this survival benefit with pembrolizumab, along with advances in tumor sequencing and clinical trials shows that there is a potentially significant benefit with novel therapies in this patient population.

Keywords: immunotherapy, penile cancer, durable response, pembrolizumab, metastatic

INTRODUCTION

Penile squamous cell carcinoma (PSCC) is a very rare malignancy, accounting for 0.12% of malignancies in the United States in 2020 but 0.73% of cancer deaths (1). Nevertheless, in 2018 around 35,000 new cases of PSCC were reported (2, 3). Between 30% and 50% of penile cancers are related to human papillomavirus (HPV), particularly subtypes 6, 16, and 18 (4, 5). Despite HPV positive status being an increased risk for the development of penile carcinoma, those with penile carcinoma with a positive HPV status have improved outcomes (6). The treatment of PSCC is largely dependent on the stage of the tumor, with initial management of localized disease usually being surgical resection (7). Lymphatic involvement has been shown to be a strong prognostic factor for survival, as patients with no involved lymph nodes have 5-year survival of 96%, with survival decreasing incrementally with more involved nodal groups (80% for N1, 66% with N2, and 37% with N3) (8). Unfortunately, patients with locally advanced penile carcinoma are likely to recur, as 70% of patients will have recurrent disease despite neoadjuvant chemotherapy with combination chemotherapy with cisplatin, paclitaxel and ifosfamide followed by surgical lymph node dissection (9). Also, for patients presenting with visceral or metastatic disease, treatment options are largely based on performance status (10) and outcomes are generally dismal and estimated to < 5 months. For those with adequate performance status, potential regimens include different chemotherapy regimens including combinations of paclitaxel/ifosfamide/cisplatin (10), cisplatin/fluorouracil (11), cisplatin/irinotecan (12), paclitaxel/carboplatin (13), and monotherapy with the EGFR inhibitor cetuximab in some patients. Paclitaxel monotherapy showed a median PFS of 11 weeks (95% CI, 7–30) and median OS of 23 weeks (95% CI, 20–48), and was well tolerated with the most common grade 3/4 side effect neutropenia in 7 patients (28%) (14). Unfortunately, patients who progress following initial chemotherapy have a median overall survival (OS) of less than 6 months (15) with no currently accepted standard of care. Given the poor survival following progression as well as the lack of treatment options in this patient population, further investigation of novel therapeutical approaches is necessary.

Immune check point inhibitors (ICB) have changed the treatment landscape in many solid tumors over the past decade with multiple FDA drug approval, specifically in other advanced solid tumors with squamous cell histology. The lack of durable response to conventional chemotherapy including taxanes and platinums has led to numerous investigative efforts exploring the role of immunotherapy. Cemiplimab, approved for the treatment of patients with advanced cutaneous squamous-cell carcinoma, induced a response in approximately half of patients (16). Also, for patients with metastatic or recurrent squamous cell carcinoma of the head and neck following progression on platinum-based therapy, nivolumab is approved (17). The molecular and viral similarities between PSCC and these SCCs can be the rationale for investigation of these therapies in patients with advanced PSCCs (18–20). In addition, 62% of patients with metastatic penile cancer were shown to be positive (≥5%) for PD-L1 expression, along with a strong correlation of PD-L1 expression in primary and metastatic samples (21). It has also been shown that 32% to 62% of squamous cell penile cancers test positive for PD-L1 expression, and thus may have a role as a predictive biomarker of response to immunotherapy (21–25).

Another important potential marker of ICB response in solid cancers is the tumor mutational burden (TMB). A recent paradigm-shifting study has resulted in the FDA approving pembrolizumab in patients with unresectable or metastatic solid tumors which harbor a high tumor mutational burden (TMB) in those who have either progressed on prior therapy or have no alternative treatment options, and is of note an approval despite the site of the primary tumor (26).

Based on the above rationale, we present 2 cases of patients with metastatic penile carcinoma with high tumor mutational burden and PDL-1 expression in penile tumor tissue who were treated with pembrolizumab with excellent response.

CASE PRESENTATION A (PATIENT 1)

The first patient is a 64-year-old male with no past medical history who was originally diagnosed with moderate to poorly differentiated keratinizing squamous cell carcinoma of the penis in 2015. He underwent 4 cycles of neoadjuvant chemotherapy with TIP (paclitaxel, ifosfamide, and cisplatin) and then underwent partial penectomy with lymph node dissection and was found to have bilateral inguinal lymph node metastases with extranodal extension, along with a positive right obturator pelvic lymph node metastasis (stage IV TxN3M0) (**Figure 1A**). HPV status was unknown. Thereafter, he received adjuvant external beam radiation therapy (EBRT) with 57.4 Gy in 28 fractions concurrently with weekly cisplatin. Surveillance imaging May of 2017 revealed increasing hypermetabolic right pelvic adenopathy (biopsy-proven, largest lymph node measuring 5.6 × 4.3 cm) along with new bone metastasis in the right anterior acetabulum (**Figure 1B**). Foundation One molecular testing of an inguinal lymph node revealed 7 alterations in clinically relevant genes, including PTCH1 S1203fs*52 (variant allele frequency [VAF] 19.2%), EP300 N419fs*12 (VAF 20.3%), FAT1 S1669* (VAF 33.1%),

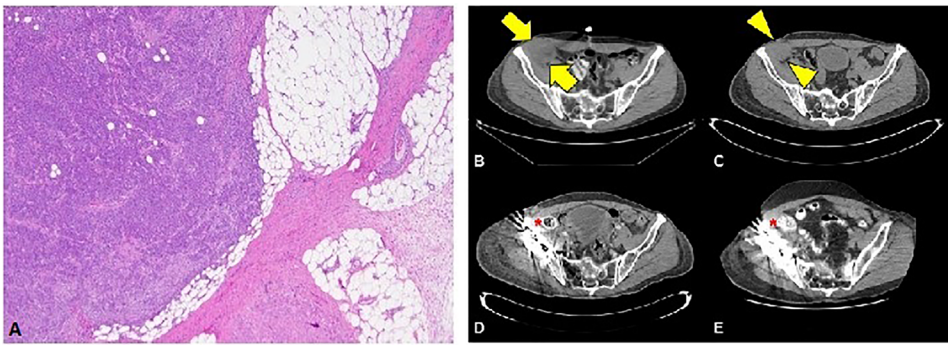

FIGURE 1 | **(A)** Squamous cell carcinoma with perinodal extension into fat (4X magnification). **(B)** Cross section through the CT portion of the PET/CT prior to the initiation of immunotherapy. The largest lymph node measures 5.6 x 4.3 cm transverse (arrows). **(C)** Noncontrast CT of the pelvis obtained approximately 1 month after initiation of immunotherapy reveals decreasing lesion size (arrowheads) now measuring 4.2 x 3.8 cm (arrowheads). Subsequent contrast enhanced CTs at 3 months **(D)** and 36 months **(E)** after initiation of immunotherapy show no evidence of residual nodal disease.

HSD3B1 G171R (VAF 1.2%), MLL2 L4921fs*74 (VAF 21.9%), MLL2 P2354fs*30 (VAF 22.9%), and QKI K134fs*14 (VAF 24.4%). There were also 17 variants of unknown significance. The microsatellite status could not be assessed but the tumor mutational burden (TMB) was high at 14 mutations/megabase, (**Table 1**). His PD-L1 status was not assessed. Given the fact that he had refractory disease to platinum-based chemotherapy and chemoradiation, with the noted high TMB and the high prevalence of PD-L1 expression in penile SCC, he was started on pembrolizumab 7/2017 and subsequently underwent a wide resection hemipelvectomy with acetabular reconstruction and total hip arthroplasty the following month. Imaging 3 months after starting pembrolizumab showed complete resolution of lymph node metastasis with no evidence of metastatic disease (**Figures 1C, D**). Surveillance scans have continued every 2 months to show no evidence of metastatic disease 38 months after starting pembrolizumab with last follow-up 8/2020 (**Figure 1E**). He has tolerated the pembrolizumab well with no grade 3 or higher AEs, his only immune related adverse event being grade 2 hypothyroidism for which he was started on levothyroxine with normalization of thyroid function.

CASE PRESENTATION B (PATIENT 2)

The second patient is an 85-year-old male with no past medical history who was diagnosed with moderately differentiated squamous cell carcinoma of the penis in 2017. He underwent partial penectomy and glansectomy and shortly after the procedure was found to have a 1.5-cm palpable right inguinal lymph node, for which biopsy confirmed metastatic squamous cell carcinoma (no extranodal extension or lymphovascular/perineural invasion). HPV testing was negative (subtypes 6, 11, 16, 18, 31, 33, 45, and 58 were not detected). He underwent bilateral inguinal lymph node dissection, which showed a positive right inguinal lymph node with extranodal extension (**Figure 2A**). Final surgical staging stage IV TxpN3M0. He underwent four cycles of adjuvant chemotherapy with cisplatin and 5-FU. Following chemotherapy, he underwent adjuvant radiation to the bilateral inguinal lymph nodes (45 Gy in 25 fractions) and a right inguinal lymph node boost with 2160 cGy in 12 fractions. He was followed with surveillance scans and 5 months following completion of radiation, was found to have avid lymphadenopathy in the subcarinal region and right hilum of the chest, along with progression of adenopathy in the

TABLE 1 | Clinicopathologic characteristic of patients with metastatic prostate cancer treated with immunotherapy.

	Response	Duration of Follow up	Adverse Events (AEs)	HPV status	PDL-1 status	MSI status	TMB	Molecular mutations
Patient 1	CR	38 months	Hypothyroidism	Unknown	Unknown	Ambiguous	High (14 mutations/Mb)	PTCH1 S1203fs*52 (VAF 19.2%) EP300 N419fs*12 (VAF 20.3%) FAT1 S1669* (VAF 33.1%) HSD3B1 G171R (VAF 1.2%) MLL2 L4921fs*74 (VAF 21.9%) MLL2 P2354fs*30 (VAF 22.9%) QKI K134fs*14 (VAF 24.4%)
Patient 2	PR	18 months	N/A	Negative	Positive (CPS 130)	Stable	Low (3 mutations/Mb)	MYD88 L265P (VAF 1.5%) NFE2L2 W24R (VAF 36.4%) SMARCA4 M1233I (VAF 8.9%) TERT promoter 146C>T (VAF 18.7%) TP53 R280G (VAF 18.3%)

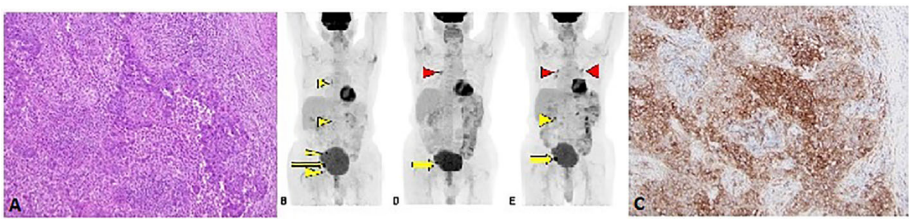

FIGURE 2 | **(A)** H&E stain from lymph node metastasis (10X magnification). **(B)** F18-FDG PET/CT Coronal MIP projection obtained prior to initiation of immunotherapy. The yellow arrow indicates the dominant lesion, while the yellow arrowheads indicate additional nontarget and/or suspicious lesions. **(C)** PD-L1 stain showing strong membranous staining in the tumor cells (10X magnification). Follow-up PET/CT **(D)** approximately 3 months after initiation of immunotherapy reveals resolution or near resolution of the nontarget lesions with decrease in dominant lesion. There is new focus of radiotracer accumulation in the right hilum, which is of unclear clinical significance (red arrowhead). The six months follow-up PET/CT **(E)** reveals grossly symmetric FDG avid hilar regions (red arrowheads) suggesting possible development of sarcoidosis of sarcoid-like reaction, an uncommon but reported finding in immunotherapy (27). There has been interval enlargement of the dominant lesion and re-emergence of some suspicious lymph nodes (yellow arrow and arrowhead).

retroperitoneum and pelvis (**Figure 2B**), of which biopsy confirmed chemotherapy and radiation refractory metastatic squamous cell carcinoma. Analysis of his FoundationOne CDx molecular testing of an inguinal lymph node revealed 5 mutations, including MYD88 L265P (VAF 1.5%), NFE2L2 W24R (VAF 36.4%), SMARCA4 M1233I (VAF 8.9%), TERT promoter 146C>T (VAF 18.7%), and TP53 R280G (VAF 18.3%). There were also 6 variants of unknown significance. The microsatellite status was stable and the tumor mutational burden was low at 3 mutations/megabase. His PD-L1 was markedly positive with a CPS (combined positive score) of 130 (**Figure 2C**). Based on the tumor strongly positive's PD-L1 status, he was started on pembrolizumab 3/2019 and surveillance scans 3 months thereafter showed marked improvement in adenopathy (**Figure 2D**). Imaging 10 months after starting pembrolizumab showed some progression of adenopathy but overall his disease has been controlled since starting pembrolizumab 18 months ago (**Figure 2E**), consistent with partial response (PR) and disease control from stabilization of an otherwise deadly tumor within months. This 85-year-old gentleman tolerated pembrolizumab without any reported AEs and continued to have clinical benefit with significant improvement in daily activity level in comparison to the time of recurrence diagnosis.

DISCUSSION

We present two cases of patients treated with pembrolizumab for refractory metastatic penile cancer with disease progression after multiple lines of chemotherapy and radiation therapy with excellent response and very durable clinical benefit. The rationale to treat these patients with this rare cancer with single agent ICB was noting a high TMB in one patient and markedly positive PDL-1 expression on IHC staining in the other patient. As Wang et al. showed, patients progressing following initial chemotherapy have a median OS of less than 6 months (15). Many established treatment options are toxic and may not be tolerated well in elderly patients with comorbidities or decreased performance status. This report highlights the potential clinical benefit of testing ICB response and therapy with single agent ICB in patients with PSCC when enrollment on clinical trials is not possible. A recent Phase II trial examining the combination of nivolumab with ipilimumab enrolled 6/56 patients with penile carcinoma, where 2/6 had stable disease, 3/6 progressive disease, and 1 was not evaluated at a median follow up of 9.9 months (28). The TMB, PDL-1 expression and other markers of response to therapy from the patients tissue on this trial were not presented as part of the preliminary data presentation. In contrast, the two patients that we report on had markers of immunotherapy response have had tumor response to therapy and excellent clinical benefit 38 and 18 months following initiation of treatment with pembrolizumab. This is a very significant clinical impact, as with standard therapies these two patients would survive for a very short time and with significant toxicity-related side effects as a result of treatment).

The recent FDA-approval for use of pembrolizumab in solid tumors harboring a high TMB portends a potential promising outcome for this patient cohort (26) and thus all patients with refractory PSCC should receive genomic testing for TMB. It has also been shown that 21% of penile cancer patients had an increased TMB of more than 10 mutations per Mb, thereby portending a potential increased response to immunotherapy (19). There are currently two phase II trials examining immunotherapy with pembrolizumab (NCT028307042) and avelumab (NCT03391479) in patients with advanced penile cancer, the results of which are eagerly anticipated. The optimal biomarker for directing immune checkpoint inhibitor therapy is unclear and may be histology specific in certain circumstances, though evolving research across solid tumors has elucidated the value of TMB and PD-L1 positivity, as well as more specific measurements of immune cell infiltration into the tumor and related microenvironment (29). Mutation burden has been shown to be a surrogate marker for neoantigens which have been associated with response to immune checkpoint inhibitors, including ipilimumab in melanoma and pembrolizumab in lung cancer. Based on these observations across malignancies and the results of the KEYNOTE-158 trial, pembrolizumab was granted accelerated approval for the

treatment of patients with unresectable or metastatic solid tumors with a TMB ≥ 10 who have progressed on prior therapy (26). Recent data has also shown that immunotherapy combined with targeted therapeutics may have a better outcome, but given that PD-L1 expression is not correlated with HPV status, both HPV-positive and negative patients can be treated with combination immunotherapy and targeted therapies (27).

In the evolving era of molecular medicine, next-generation sequencing (NGS) genomic profiling assays can help to identify potentially targetable therapy options and can be especially valuable for cancers with limited effective treatment options. Both of the aforementioned patients underwent large panel NGS molecular testing, the findings discussed above. While the targetability of specific mutations was limited to the inactivating PTCH1 alteration in the first patient case, NGS can also provide information on additional biomarkers, such as TMB and microsatellite status that can also identify additional treatment options. Both TMB and microsatellite instability (MSI-high) are recognized as histology agnostic FDA approved biomarkers for pembrolizumab. While at this time our limited understanding of numerous genomic alterations precludes definitive treatment options for patients with these mutations, the further development of clinical trials will help provide more information, and the fact that these patients were treated with pembrolizumab with treatment response far exceeding the current standards of care is important in providing prognostic and therapeutic information in this patient population.

The evolving landscape of treatment of metastatic penile cancer is a subject of great research focus, with multiple clinical trials seeking to enroll patients with the aim to improve clinical outcomes. There was a phase II trial of pembrolizumab for advanced penile squamous cell carcinoma following prior systemic chemotherapy but unfortunately this was terminated due to poor patient accrual (NCT02837042), illustrating the challenge of assessing novel therapy options in patients with rare malignancies such as penile cancer and further supporting the value of case series evidence. Recent work by our group with a PSCC mouse model has demonstrated a potential role of combinatory strategies with immunotherapy and targeted therapies (29), these could lay the foundation for future clinical trials in refractory PSCC.

The lack of data for new approaches to treat metastatic penile cancer serves as a conundrum for clinicians with patients with advanced disease. While we realize our limited case series of two patients treated with pembrolizumab for their metastatic penile cancer does not provide a level of evidence to change treatment paradigms, the authors feel that it is of the utmost importance to report on these two patients with extraordinary responses and durable benefit from immunotherapy with pembrolizumab for their refractory metastatic disease. While penile carcinoma is a rare disease, it is still a significant cause of mortality, as it is responsible for 383 deaths yearly in North America and over 15,000 deaths yearly in the world (2). This report aims to highlight the importance of genomic testing in refractory PSCC and the potential clinical benefit of ICB in specific patients when clinical trials are not available. We look forward to future innovations in the field starting with basic science work to further improve our understanding of this disease, as well as multi-institutional collaborative efforts in clinical trials with support from industry and advocacy groups to improve the survival of patients with PSCC.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors have approved this submission to Frontiers in Oncology. All persons listed as authors have contributed to preparing the manuscript and no person(s) other than the authors have contributed significantly to its preparation. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA: Cancer J Clin* (2020) 70(1):7–30. doi: 10.3322/caac.21590
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin* (2018) 68(6):394–424. doi: 10.3322/caac.21492
3. Montes Cardona CE, García-Perdomo HA. Incidence of penile cancer worldwide: systematic review and meta-analysis. *Rev Panam Salud Public* (2017) 41:e117. doi: 10.26633/RPSP.2017.117
4. Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, et al. Penile cancer: importance of circumcision, human papillomavirus and smoking in in situ and invasive disease. *Int J Cancer* (2005) 116(4):606–16. doi: 10.1002/ijc.21009
5. Olesen TB, Sand FL, Rasmussen CL, Albieri TB, Toft BG, Norrild B, et al. Prevalence of human papillomavirus DNA and p16INK4a in penile cancer and penile intraepithelial neoplasia: a systematic review and meta-analysis. *Lancet Oncol* (2019) 20(1):145–58. doi: 10.1016/S1470-2045(18)30682-X
6. Zhang J, Zhang H, Xiu Y, Cheng H, Gu M, Song N. Prognostic Significance of P16INK4a Expression in Penile Squamous Cell Carcinoma: A Meta-Analysis with Trial Sequential Analysis. *BioMed Res Int* (2018) 2018:8345893. doi: 10.1155/2018/8345893
7. Philippou P, Shabbir M, Malone P, Nigam R, Muneer A, Ralph DJ, et al. Conservative surgery for squamous cell carcinoma of the penis: resection

margins and long-term oncological control. *J Urol* (2012) 188(3):803–8. doi: 10.1016/j.juro.2012.05.012
8. Djajadiningrat RS, Graafland NM, van Werkhoven E, Meinhardt W, Bex A, van der Poel HG, et al. Contemporary management of regional nodes in penile cancer-improvement of survival. *J Urol* (2014) 191(1):68–73. doi: 10.1016/j.juro.2013.07.088
9. Pagliaro LC, Williams DL, Daliani D, Williams MB, Osai W, Kincaid M, et al. Neoadjuvant paclitaxel, ifosfamide, and cisplatin chemotherapy for metastatic penile cancer: a phase II study. *J Clin Oncol: Off J Am Soc Clin Oncol* (2010) 28 (24):3851–7. doi: 10.1200/JCO.2010.29.5477
10. Dickstein RJ, Munsell MF, Pagliaro LC, Pettaway CA. Prognostic factors influencing survival from regionally advanced squamous cell carcinoma of the penis after preoperative chemotherapy. *BJU Int* (2016) 117(1):118–25. doi: 10.1111/bju.12946
11. Di Lorenzo G, Buonerba C, Federico P, Perdona S, Aieta M, Rescigno P, et al. Cisplatin and 5-fluorouracil in inoperable, stage IV squamous cell carcinoma of the penis. *BJU Int* (2012) 110(11 Pt B):E661–6. doi: 10.1111/j.1464-410X.2012.11453.x
12. Theodore C, Skoneczna I, Bodrogi I, Leahy M, Kerst JM, Collette L, et al. A phase II multicentre study of irinotecan (CPT 11) in combination with cisplatin (CDDP) in metastatic or locally advanced penile carcinoma (EORTC PROTOCOL 30992). *Ann Oncol: Off J Eur Soc Med Oncol* (2008) 19(7):1304–7. doi: 10.1093/annonc/mdn149
13. Bermejo C, Busby JE, Spiess PE, Heller L, Pagliaro LC, Pettaway CA. Neoadjuvant chemotherapy followed by aggressive surgical consolidation for metastatic penile squamous cell carcinoma. *J Urol* (2007) 177(4):1335–8. doi: 10.1016/j.juro.2006.11.038
14. Wang J, Pettaway CA, Pagliaro LC. Treatment for Metastatic Penile Cancer After First-line Chemotherapy Failure: Analysis of Response and Survival Outcomes. *Urology* (2015) 85(5):1104–10. doi: 10.1016/j.urology.2014.12.049
15. Di Lorenzo G, Federico P, Buonerba C, Longo N, Carteni G, Autorino R, et al. Paclitaxel in Pretreated Metastatic Penile Cancer: Final Results of a Phase 2 Study. *Eur Urol* (2011) 60(6):1280–84. doi: 10.1016/j.eururo.2011.08.028
16. Migden MR, Rischin D, Schmults CD, Guminski A, Hauschild A, Lewis KD, et al. PD-1 Blockade with Cemiplimab in Advanced Cutaneous Squamous-Cell Carcinoma. *N Engl J Med* (2018) 379(4):341–51. doi: 10.1056/NEJMoa1805131
17. Harrington KJ, Ferris RL, Blumenschein G, Colevas AD, Fayette J, Licitra L, et al. Nivolumab versus standard, single-agent therapy of investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck (CheckMate 141): health-related quality-of-life results from a randomised, phase 3 trial. *Lancet Oncol* (2017) 18(8):1104–15. doi: 10.1016/S1470-2045(17)30421-7
18. Chahoud J, Pickering CR, Pettaway CA. Genetics and penile cancer: recent developments and implications. *Curr Opin Urol* (2019) 29(4):364–70. doi: 10.1097/MOU.0000000000000640
19. Chahoud J, McCormick BZ, Netto F, Rao P, Pickering CR, Pettaway CA. Penile squamous cell carcinoma is genomically similar to other HPV-driven tumors. *J Clin Oncol* (2019) 37(7):505. doi: 10.1200/JCO.2019.37.7_suppl.505
20. Jacob JM, Ferry EK, Gay LM, Elvin JA, Verglio J, Ramkissoon S, et al. Comparative Genomic Profiling of Refractory and Metastatic Penile and Nonpenile Cutaneous Squamous Cell Carcinoma: Implications for Selection of Systemic Therapy. *J Urol* (2018) 201(3):541–8. doi: 10.1016/j.juro.2018.09.056
21. Udager AM, Liu TY, Skala SL, Magers MJ, McDaniel AS, Spratt DE, et al. Frequent PD-L1 expression in primary and metastatic penile squamous cell carcinoma: potential opportunities for immunotherapeutic approaches. *Ann Oncol* (2016) 27(9):1706–12. doi: 10.1093/annonc/mdw216
22. Cocks M, Taheri D, Ball MW, Bezerra SM, Rodriguez MD, Ricardo BF, et al. Immune-checkpoint status in penile squamous cell carcinoma: a North American cohort. *Hum Pathol* (2017) 59:55–61. doi: 10.1016/j.humpath.2016.09.003
23. Deng C, Li Z, Guo S, Chen P, Chen X, Zhou Q, et al. Tumor PD-L1 expression is correlated with increased TILs and poor prognosis in penile squamous cell carcinoma. *Oncoimmunology* (2017) 6(2):e1269047. doi: 10.1080/2162402X.2016.1269047
24. Ottenhof SR, Djajadiningrat RS, de Jong J, Thygesen HH, Horenblas S, Jordanova ES, et al. Expression of Programmed Death Ligand 1 in Penile Cancer is of Prognostic Value and Associated with HPV Status. *J Urol* (2017) 197(3 Part 1):690–7. doi: 10.1016/j.juro.2016.09.088
25. Jacob JM, Ferry EK, Gay LM, Elvin JA, Verglio J, Ramkissoon S, et al. PD-L1 Expression in Men with Penile Cancer and its Association withClinical Outcomes. *Eur Urol Oncol* (2018) 3(0). doi: 10.1016/j.euo.2018.07.010
26. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, Jesus-Acosta A, Delord J, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* (2020) 38(1):1–10. doi: 10.1200/JCO.19.02105
27. Huang T, Cheng X, Chahoud J, Sarhan A, Tamboli P, Rao P, et al. Effective combinatorial immunotherapy for penile squamous cell carcinoma. *Nat Commun* (2020) 11(1):2124. doi: 10.1038/s41467-020-15980-9
28. McGregor BA, Campbell MT, Xie W, Farah S, Asim B, Sonpavde G, et al. Phase II study of nivolumab and ipilimumab for advanced rare genitourinary cancers. 2020 ASCO Virtual Scientific Program. *J Clin Oncol* (2020) 38 suppl: abstr 5018. doi: 10.1200/JCO.2020.38.15_suppl.5018
29. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Cancer* (2019) 19(3):133–50. doi: 10.1038/s41568-019-0116-x

Conflict of Interest: PS: NCCN bladder and penile cancer panel vice-chair, and President of the Global Society of Rare GU Tumors. CW: Consultant for the Molecular Tumor Boards of Intermountain Healthcare and Jackson Genetic Laboratories, PRN paid employee of HCA Mission Hospital. RJ: Advisory board —Pfizer, Seattle Genetics; Speakers bureau—Astellas/Seattle Genetics.

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ORIGINAL RESEARCH
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Development and Validation of a Nomogram for the Prediction of Inguinal Lymph Node Metastasis Extranodal Extension in Penile Cancer

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Purpose: To determine whether a clinicopathologic and laboratory-based nomogram is capable of predicting the risk of lymph node extranodal extension (ENE) in patients with penile cancer.

Materials and Methods: From June 2006 to January 2021, 234 patients who underwent bilateral inguinal lymph node dissection (ILND) surgery were included in the analysis. A Lasso regression model was utilized to select the most useful predictive features from among 46 laboratory variables. Then, a logistic regression analysis was used to develop the prediction model. Calibration curves, concordance index (C-index) and Areas under the receiver-operating characteristic curves (AUCs) were performed to evaluate the performance of the nomogram. We also investigated model fit using changes in Akaike Information Criteria (AICs). Decision curve analyses (DCAs) were applied to assess the clinical usefulness of this nomograms. Its internal validation was confirmed.

Results: Among the 234 patients, 53 were confirmed to have ENE. The platelet-lymphocyte ratio (PLR) and Squamous cell carcinoma antigen (SCC-Ag) were significantly associated with ENE ($P<0.05$). The individualized prediction nomogram, including the PLR, SCC-Ag, lymphovascular invasion (LVI), and pathologic tumor stage (pT-stage), showed good discrimination, with a C-index of 0.817 (95% CI, 0.745 to 0.890) and good calibration. Clinical-laboratory nomogram (AIC, 180.034) become the best-fitting model. DCA findings revealed that the clinical-laboratory nomogram was more clinically useful than the pT-stage or tumor grade.

Conclusions: This study presents a clinicopathologic and laboratory-based nomogram that incorporates PLR, SCC-Ag, lymphovascular invasion (LVI), and pT-stage, which can be conveniently utilized to facilitate the individualized prediction of lymph node metastasis ENE in patients with penile cancer.

Keywords: risk assessment, risk model, nomogram, extranodal extension, penile cancer

INTRODUCTION

Penile squamous cell carcinoma (PSCC) is an uncommon malignancy, describing 0.4% to 0.6% of all malignant disease among men in the United States and Europe (1). Its incidence is higher among men in developing regions (2).

One of the most unfavorable prognostic factors in penile cancer for poor prognosis is lymph node metastasis (LNMs). Extranodal extension (ENE) is defined as extension of the tumor through the lymph node capsule into the perinodal fibrous-adipose tissue and is an independent adverse prognostic factor in PSCC (3, 4).

In the 8th American Joint Committee on Cancer Tumor Node Metastasis (AJCC TNM) staging1, both ENE and pelvic lymph node metastasis (PLNM) are staged as pN3, suggesting that ENE is an adverse pathological characteristic. Johnson and colleagues (5) reported that 5-year survival is reduced by approximately half with lymph node involvement(LNI). Extranodal extension (ENE) of LNMs portends an even worse prognosis. ENE has similarly been implicated in worse outcomes in carcinoma of the bladder, breast, pancreas, stomach, and cervix (6–8).

According to the National Comprehensive Cancer Network (NCCN) guidelines, patients who are pathologically diagnosed with ENE should receive neoadjuvant therapies, and it is reasonable to give 4 courses of (Paclitaxel + Ifosfamide + Cisplatin) TIP in the adjuvant setting if it was not given preoperatively. Nevertheless, the prognosis of the disease remains poor due to a high rate of recurrence. Pelvic lymph node dissection (PLND) should be considered at the time or following inguinal lymph node dissection (ILND) in patients with ≥2 positive inguinal nodes on the ipsilateral ILND site or in the presence of ENE on final pathologic review.

From the above, ENE is an extremely important feature, especially for predicting the outcomes of treatment. In this study, patients who underwent ILND were included, and an individualized prediction model was established and validated.

MATERIALS AND METHODS

Patient Population

The ethics committee of Sun Yat-Sen University Cancer Hospital approved the retrospective data analysis. We retrospectively reviewed 234 patients who underwent bilateral ILND for curative purposes from June 2006 to January 2021. To be eligible for analysis, patients must have met the following criteria: (i) PSCC was their primary disease, (ii) Immediate or delayed ILND, (iii) detailed clinical and pathologic data available, and (iv) no known distant metastasis.

In all patients, variables extracted from their medical records included age, chronic disease (hypertension, heart disease and diabetes), surgical management of the primary tumor (including partial penectomy, lesionectomy and phallectomy), immediate or delayed ILND, perineural invasion (PNI), lymphovascular invasion (LVI), pT-stage, TNM stage, tumor grade, unilateral or bilateral inguinal LNM, number of LNMs and ENE status. Patients were divided into no LNM group, 1 LNM group, 2 LNMs group, 3 LNMs group, and ≥4 LNMs group depend on LNMs status. Laboratory tests were performed within 1 week before the surgery.

Laboratory Tests

Serum specimens were collected before the bilateral inguinal lymph node dissections. Various routine blood indexes, routine biochemical tests (33 source indicators) and coagulation tests of hemostasis were tested by LABOSPECT 008 AS and Sysmex CS5100, respectively. The SCC-Ag was detected by immunodetection (Cobas e801).

Construction and Validation of the Nomogram

We incorporated clinicopathological and laboratory indicators as predicted factors into the design of the nomogram. We used the least absolute shrinkage and selection operator (LASSO) regression with 10-fold cross-validation to select variables that were predictive of ENE. Then, a logistic regression model was adapted to screen out the significant ($P<0.05$) predictors of ENE from the clinicopathological features and the candidate laboratory indicators. Then, we developed a nomogram to predict the probability of ENE.

Areas under the receiver-operating characteristic curves (AUCs) and Harrell's C-index was used to describe performance and accuracy of nomogram. Model fitting is conducted using AIC and calibration curve, accompanied by the Hosmer-Lemeshow test. Higher AUCs indicated better discrimination and lower AICs indicated superior model-fitting. The calibration curves were assessed by reviewing the predicted versus actual probabilities. Clinical usefulness and net benefit were estimated with decision curve analysis.

Statistics

Statistical analyses were performed defining a two-sided $P<0.05$ as significant. Models, statistics, and figures were prepared using SAS 9.4 software version (Cary, NC) and R 3.5.1 (http://www.cran.r-project.org).

RESULTS

Patient Characteristics

A total of 234 PSCC patients (median age: 54.7 years; IQR: 46-64) were eligible, including 79 (33.9%)≤T1, 49 (21.0%) in T2, 99 (42.5%) in T3, and 6 (2.6%) in T4 tumor stage. The clinicopathologic characteristics and treatment option of patients with penile cancer are shown in **Table 1**. Inguinal lymph metastasis occurred in 103 (44.0%) patients, and 53 (22.6%) patients were confirmed to have ENE.

Abbreviations: ENE, Extranodal extension; ILND, Inguinal lymph node dissection; PLR, Platelet-lymphocyte ratio; SCC-Ag, Squamous cell carcinoma antigen; PSCC, Penile squamous cell carcinoma; LNMs, Lymph node metastasis; PLND, Pelvic lymph node dissection; pT-stage, Pathologic tumor stage; LNI, Lymph node involvement; PNI, Perineural invasion; PLNM, Pelvic lymph node metastasis; LVI, Lymphovascular invasion.

In our study, the T staging and tumor stage of the ENE+ group were significantly higher than those of LNM+ ENE- and patients with no LNM (p<0.0001, <0.001, respectively). In addition, a higher percentage of patients were treated with post-operative adjuvant therapy in the ENE+ group (**Table 2**).

Feature Selection

For the development of the nomogram, we incorporated 46 laboratory tests as predictive features. All of these parameters were reduced to the 2 most useful potential predictors for ENE, with nonzero coefficients in the LASSO regression model (**Figure 1**). As shown in **Figure 2**, the nomogram indicates that platelet-lymphocyte ratio (PLR) has the strongest correlation with ENE and LNM.

TABLE 1 | Clinical characteristics of 234 patients with penile cancer.

Characteristic	No. of patients (%) (n = 234)
Age, yr, median (IQR)	55.0 (45.8-64.0)
pT-stage	
≤pT1	79 (33.9)
pT2	49 (21.0)
pT3	99 (42.5)
pT4	7 (2.6)
pN-stage	
pN0	131 (56.0)
pN1	14 (6.0)
pN2	24 (10.3)
pN3	65 (27.8)
M stage	
M0	234 (100.0)
M1	0 (0.0)
Grade	
G1	123 (52.6)
G2	93 (39.7)
G3	18 (7.7)
No. of positive inguinal lymph nodes	
No positive	131 (56.0)
1 Positive	18 (7.7)
2 Positive	38 (16.2)
3 Positive	13 (5.6)
≥4 Positive	34 (14.5)
Inguinal LNM	
Absent	131 (56.0)
Present	103 (44.0)
Unilateral inguinal LNM	61 (26.1)
Bilateral inguinal LNM	42 (17.9)
Primary tumor surgery and ILND	
Simultaneous	182 (77.8)
Nonsimultaneous	52 (22.2)
Primary tumor surgery	
PPA	180 (76.9)
TPA	41 (17.5)
LC	13 (5.6)
Lymph node ENE[a]	
Positive	53 (22.6)
Negative	181 (77.4)
Adjuvant therapy	
Yes	72 (30.8)
NAC	0 (0)
AC	68 (29.1)
AC + AR	4 (1.7)

RT, radiotherapy; pT-stage, pathology tumor stage; pN-stage, pathology lymph node metastasis stage; IQR, interquartile range; M stage, distant metastasis stage; G, tumor grade; ENE, extranodal extension; AC, adjuvant chemotherapy; AC + AR, adjuvant chemotherapy + radiotherapy; ILND, inguinal lymph node dissection; ILNM, inguinal lymph node metastasis; LC, lesionectomy; LNM, lymph node metastasis; NAC, neoadjuvant chemotherapy; PPA, partial penile amputation; TPA, total penile amputation.
[a]ENE, Extranodal extension was defined as extension of the tumor through the lymph node capsule into the perinodal fibrous-adipose tissue.

Nomogram Development and Internal Validation

Univariable analysis was performed initially, followed by multivariate analysis (Variables with P < 0.05 on univariate analysis were included in the multivariate model (**Table 3**). Tumor stage, PLR, serum Squamous cell carcinoma antigen (SCC-Ag), tumor grade, PNI and LVI were significant Inguinal lymph node ENE predictors at the initial screening. On multivariate analysis, we found that tumor stage (P=0.006), PLR (P<0.001), SCC-Ag (P=0.001) and LVI (P=0.017) remained independent predictive factors (**Table 3**).

Derived from the four independent predictive factors, a model that incorporated the above predictors was developed and presented as a nomogram (**Figure 2**). According to the score table, each variable has a corresponding score. We get the total score by calculating the score of each variable. Next, by plotting the total score on the probability scale, the ENE probability of lymph nodes can be estimated at the predicted risk points (**Figure 2**).

The calibration curve of the nomogram for the probability of lymph node ENE demonstrated good agreement between the prediction and observation in the cohort (**Figure 3**). The Hosmer-Lemeshow test yielded a nonsignificant statistic (P =0.340>0.05), which suggested that there was no departure from a perfect fit. The C-index for the prediction nomogram was 0.817 (95% CI, 0.745 to 0.890) for the cohort (**Figure 4**), which was confirmed to be 0.864 by bootstrapping validation.

The prediction model after the addition of PLR and SCC-Ag is shown in **Table 4**. The highest C-index (0.817; 95% CI, 0.745 to 0.890) and the lowest AICs (180.034) was observed for the model with PLR and SCC-Ag integrated into the cohort. Comparison of Clinical Usefulness between Nomogram and some risk factors or EAU risk model, and the nomogram showed the best net benefit (**Figure 5**).

DISCUSSION

ENE is one of the most important predictors of unfavorable outcomes in PSCC and determines TNM staging and therapeutic options. However, there is no unified institutional clinical practice guidelines or established method for the diagnosis of ENE. We successfully developed and validated a predictive model, a new nomogram to predict lymph node ENE in PSCC patients. Here, we describe the first successful establishment of a prediction model for ENE. Incorporating laboratory and clinical factors into an easy-to-use nomogram for the prediction of lymph node ENE.

PSCC with lymph node ENE has a low survival rate. As early as 1987, Srinivas et al. (9) reported that lymph node metastasis-positive PSCC with ENE was related to a higher mortality than patients without lymph node ENE. Lughezzani et al. (10)

TABLE 2 | Patient characteristics and descriptive statistics between different and lymph node status.

Variable	LNM		no LNM n = 131	P
	with ENE[a] n = 53	without ENE[a] n = 50		
Age, yr, median (IQR)	55.0 (46.0-65.0)	58.5 (48.8-70.0)	54.0 (44.0-62.0)	0.0502
pT-stage				p<0.0001
≤pT1	3 (5.7)	12 (24.0)	64 (48.9)	
pT2	14 (26.4)	9 (18.0)	26 (19.8)	
pT3	31 (58.5)	27 (54.0)	41 (31.3)	
pT4	5 (9.4)	2 (4.0)	0 (0.0)	
Grade				p<0.001
G1	16 (30.2)	16 (51.7)	91	
G2	29 (54.7)	29 (39.7)	35	
G3	8 (15.1)	5 (7.7)	5	
Adjuvant therapy				p<0.001
Yes	44 (83.0)	28 (56.0)	0	
NAC	0	0	0	
AC	42 (79.2)	26 (52.0)	0	
AC + AR	2 (3.8)	2 (4.0)	0	

RT, radiotherapy; pT-stage, pathology tumor stage; pN-stage, pathology lymph node metastasis stage; IQR, interquartile range; M stage, distant metastasis stage; G, tumor grade; ENE, extranodal extension.

[a]*ENE, Extranodal extension was defined as extension of the tumor through the lymph node capsule into the perinodal fibrous-adipose tissue.*

identified ENE (OR, 8.01; P <.001) as a strong, independent predictor of PLNM. Niels et al. (11) reported that in patients without ENE, the five-year survival can be as high as 80% compared to a 5-year cancer-specific survival of 42% in patients with ENE.

In 2020, the NCCN guidelines recommended that PLND should be performed at the time or following ILND in the presence of ENE on final pathologic review (2). In addition, adjuvant external beam radiation therapy (EBRT) or chemoradiotherapy can be considered for patients with ENE. This means that patients with ENE are recommended to receive subsequent PLND and postoperative adjuvant therapy. Therefore, the prediction of lymph node ENE prior to ILND is important for selecting the most appropriate surgical procedure and postoperative adjuvant therapy. For patients with lower-risk or high-risk tumors who didn't received immediate ILND, we recommend active surveillance and partially patients may experience an inguinal nodal recurrence during follow-up. Some patients underwent secondary inguinal lymph node dissection after primary surgery. For this patient group, if any patients that have predicted ENE according to their nomogram, we suggest 4 courses of neoadjuvant TIP and stable or responding disease should then undergo a PLND together with ILND and thereby avoid secondary procedures.

The pT-stage of the primary tumor is a strong predictor of high cancer-specific mortality (CSS) (12). Previous studies confirmed that patients with LVI seem to have systemic disease and is related to an addition risk of invasion and metastasis and was a significant independent predictor of a shorter OS (1, 2, 11, 12). As everyone knows, the infiltration of

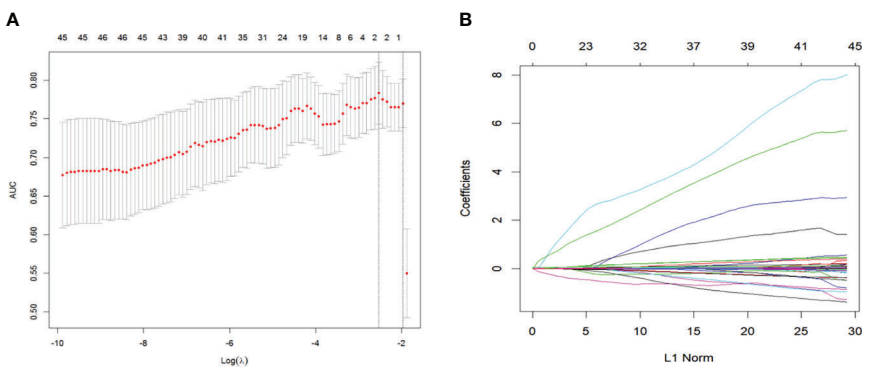

FIGURE 1 | Texture feature selection using the LASSO binary logistic regression model. **(A)** By selecting a 10-fold cross-validation in the LASSO model with minimum standards. The binomial deviance was plotted versus log (λ). Dotted vertical lines were drawn at the optimal λ values based on the minimum criteria and 1 standard error of the minimum standards and the optimal λ was 0.069. **(B)** The LASSO logistic regression algorithm was used to screen out 2 features with non-zero coefficients out of 46 features. LASSO, least absolute shrinkage and selection operator.

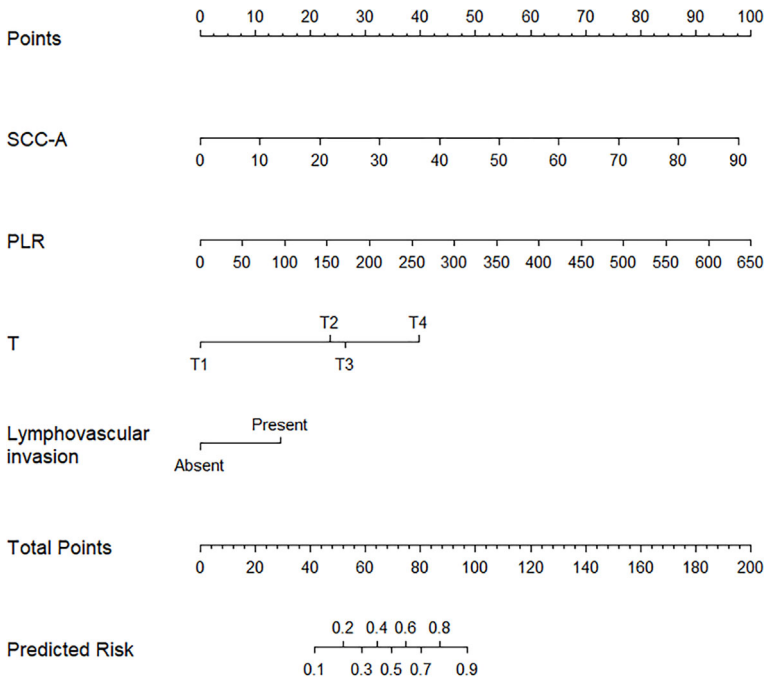

FIGURE 2 | Predicted nomogram for PCCS patients: a line was drawn straight down to predict the risk of ENE. T, Pathology T stage; PLR, platelet-lymphocyte ratio; SCC-Ag, Squamous cell carcinoma antigen.

tumor cells into lymphatic vessels or blood vessels is the committed step of tumor diffusion.

A recent study (13, 14) investigated the prognostic value of the preoperative tumor marker SCC-Ag and systemic inflammatory factors in penile cancer. Interestingly, similar to our results, we found that SCC-Ag and PLR are highly correlated with the presence of ENE. SCC-Ag levels have been validated to predict LNM and have prognostic significance for disease-free survival(DFS) in patients with penile cancer treated with surgery (15). The predictive value of PLR has been investigated in various cancers (16, 17). The pretreatment PLR has been demonstrated to be a significant predictor in patients with cervical (18–20), colon, and colorectal cancer (21). The precise molecular mechanisms underlying the repercussion of PLR in PSCC remain unknown.

TABLE 3 | Univariable and multivariable analyses.

Characteristic	Univariable		Multivariable	
	Odds Ratio (95% CI)	P[a] value	Odds Ratio (95% CI)	P[a] value
SCC-Ag	1.123 (1.072-1.177)	<0.001	1.090 (1.035-1.148)	0.001
PLR	1.014 (1.008-1.020)	<0.001	1.012 (1.006-1.019)	<0.001
pT-stage				
≤pT1	reference		reference	
pT2	6.205 (1.909-20.161)	0.002	6.522 (1.716-24.791)	0.006
pT3	9.667 (3.233-28.900)	<0.001	8.077 (2.322-28.103)	0.001
pT4	52.500 (7.680-358.906)	<0.001	23.258 (2.431-222.560)	0.006
Grade				
G1	reference			
G2	3.871 (1.896-7.902)	<0.001		
G3	5.026 (1.682-15.02)	0.004		
PNI	2.424 (1.213-4.845)	0.012		
LVI	5.773 (2.736-12.181)	<0.001	3.205 (1.227-8.371)	0.017

CI, Confidence Interval; OR, odds ratio; PLR, platelet-lymphocyte ratio; SCC-Ag, squamous cell carcinoma antigen; pT-stage, pathology tumor stage; IQR, interquartile range; G, tumor grade; PNI, Perineural invasion; LVI, Lymphovascular invasion.

[a]*P values were calculated using Logistic regression model.*

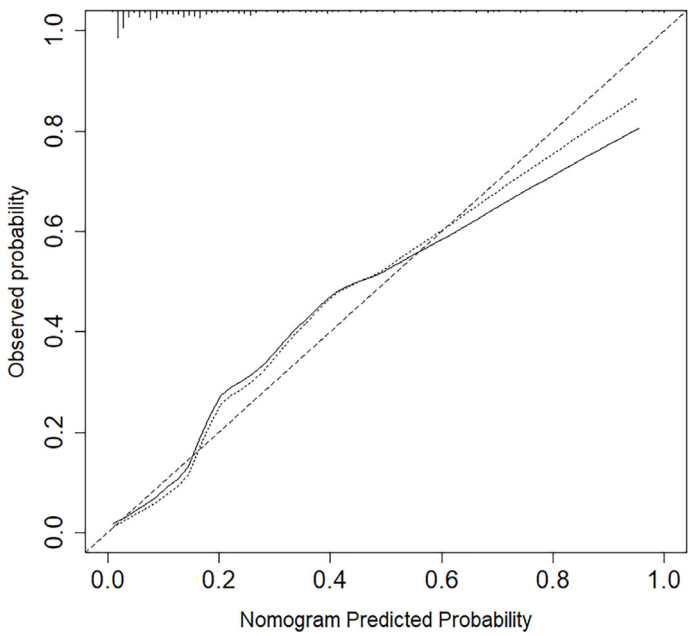

FIGURE 3 | Nomogram calibration between the predicted risk and observed incidence. Calibration curves depict the calibration of models in terms of the agreement between the predicted risks of ENE and observed outcomes of ENE. The y-axis represents the actual ENE rate. The x-axis represents the predicted ENE risk. The diagonal dotted line represents a perfect prediction by an ideal model.

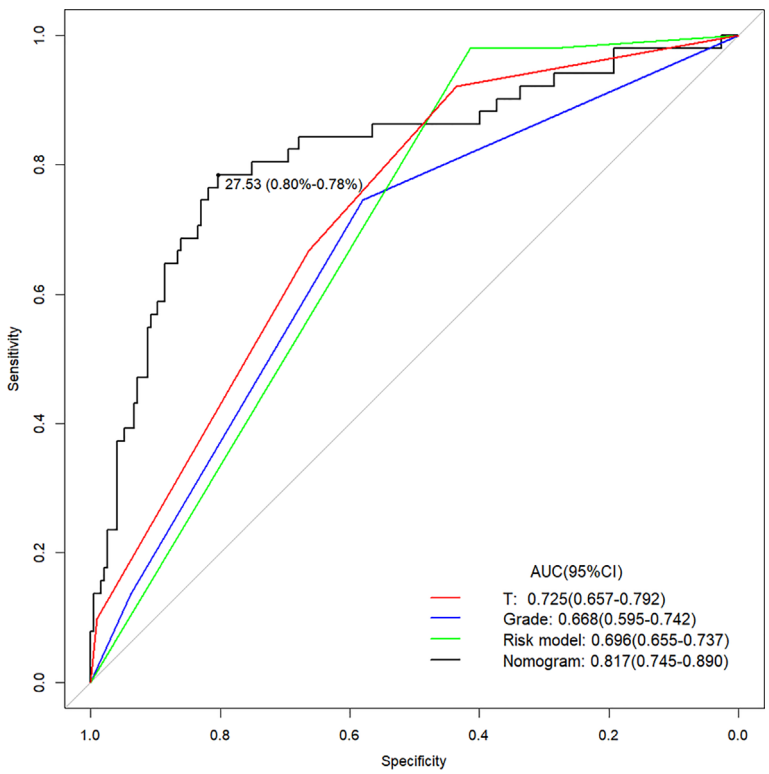

FIGURE 4 | The Area Under Curve (AUC) of the prediction nomogram on T, Grade, Risk model and nomogram. T, tumor stage.

TABLE 4 | Comparisons of different predictive models of Lymph Node ENE in Penile cancer.

Intercept and Variable	Clinical-laboratory nomogram		Model 2		Model 3		Model 4	
	Odds Ratio (95% CI)	P[a]	Odds Ratio (95% CI)	P[a]	Odds Ratio (95% CI)	P[a]	Odds Ratio (95% CI)	P[a]
Intercept		50						
SCC-Ag	1.088 (1.035-1.143)	0.001	NA	NA	1.095(1.042-1.152)	<0.001	NA	NA
PLR	1.013 (1.006-1.019)	<0.001	1.013 (1.007-1.02)	<0.0001	NA	NA	NA	NA
pT-stage	2.385(1.488-3.823)	0.006	2.481 (1.574-3.912)	<0.0001	2.549 (1.628-3.991)	<0.0001	2.661(1.74-4.069)	<0.0001
LVI	3.077 (1.193-7.938)	0.017	4.976 (2.1-11.789)	<0.001	2.642 (1.067-6.539)	0.036	4.892 (2.19-10.925)	<0.001
C-index	0.817(0.745-0.890)		0.799 (0.724-0.874)		0.781(0.709-0.853)		0.640 (0.570-0.710)	
AIC	180.034		189.824		197.480		211.036	

A higher C-index indicates better discrimination and a lower AIC indicates superior model-fitting.

Clinical-laboratory nomogram, variables included, SCC-Ag, PLR, pT-stage, and LVI. Model 2, variables included, PLR, pT-stage, and LVI. Model 3, variables included, SCC-Ag, pT-stage, and LVI. Model 4, variables included, pT-stage, and LVI.

CI, Confidence Interval; OR, odds ratio; PLR, platelet-lymphocyte ratio; SCC-Ag, squamous cell carcinoma antigen; pT-stage, pathology tumor stage; LVI, Lymphovascular invasion.

[a]*P values were calculated using Logistic regression model.*

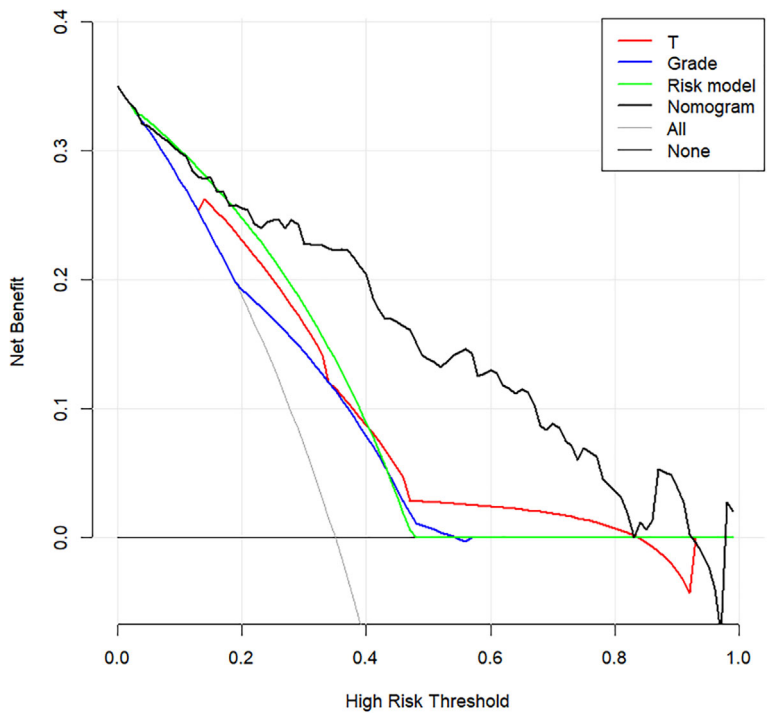

FIGURE 5 | Decision curve analysis to assess the clinical usefulness of the nomogram, T stage, grade, Risk model and ENE. T stage, tumor stage; ENE, extranodal extension.

Platelets, as a critical source of cytokines, bind FGF, PDGF, VEGF, and TGF-β family proteins, permitting plts to serve as a reservoir for secreted growth factors that promote tumorigenesis and the development of metastasis (22–24). Lymphocytes act a pivotal part in withstanding cancer cells by inducing cytotoxic cell death and inhibiting tumor cell proliferation and migration. Tumor infiltrating lymphocytes (TILs) are vital immune cells found in tumors, eligible for anti-tumor immune response (25). Taken together, PLR combined with the effects of platelets and lymphocytes may predict the presence of lymph node ENE.

The generalisability of these results suffer from several limitations. First, there was an inevitable selection bias, as the study was retrospectively designed. Secondly, imaging features were not included in our analysis. We see this investigation as an exploratory study and our aim is to provide clinicians with a good predictive tool which can serve as effective adjunctive tools with anatomic imaging, instead of contain imaging features. We believe that the present analysis or others which including imaging features variables will be important in future validation studies of larger and multicenter cohorts. Another limitation may be the smaller proportion of ENE-positive patients (22.6% [53/234]), although this study had a relatively large sample size. However, as our study included all patients who underwent bilateral ILND (including prophylactic ILND), the relatively small proportion who were ENE-positive could thus be explained. Fourth, we didn't test our data with an independent external validation set.

CONCLUSION

This study presents a clinicopathologic and laboratory-based nomogram that incorporates PLR, SCC-Ag, lymphovascular invasion (LVI), and pT-stage, which can be easily utilized to promote the individualized prediction of lymph node ENE in patients with PSCC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HH had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design: CW, ZL, and HH. Acquisition of data: CW, ZL, and HH. Analysis and interpretation of data: All. Drafting of the manuscript: CW and ZL. Critical revision of the manuscript for important intellectual content: All. Statistical analysis: CW, ZL, and HH. Obtaining funding: ZL and HH. Administrative, technical, or material support: FZ and HH. Supervision: HH. Other (specify): None. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.675565/full#supplementary-material.

REFERENCES

1. Pettaway CA, Lance RS, Davis JW. Tumors of the Penis. (2012). Elsevier Saunders.
2. Clark PE, Spiess PE, Agarwal N, Biagioli MC, Ho M. Penile Cancer: Clinical Practice Guidelines in Oncology. *J Natl Compr Cancer Netw: JNCCN* (2013) 11(5):594–615. doi: 10.6004/jnccn.2013.0195
3. Ravi R. Correlation Between the Extent of Nodal Involvement and Survival Following Groin Dissection for Carcinoma of the Penis. *Br J Urol* (1993) 72:817–9. doi: 10.1111/j.1464-410x.1993.tb16273.x
4. Protzel C, Alcaraz A, Horenblas S, Pizzocaro G, Zlotta A, Hakenberg OW. Lymphadenectomy in the Surgical Management of Penile Cancer. *Eur Uro* (2009) 55(5):1075–88. doi: 10.1016/j.eururo.2009.02.021
5. Johnson J, Barnes E, Myers E, Schramm V, Borochovitz D, Sigler B. The Extracapsular Spread of Tumors in Cervical Node Metastasis. *Arch Otolaryngol* (1981) 107(12):725–9. doi: 10.1001/archotol.1981.00790480001001
6. Fleischmann A, Thalmann G, Markwalder R, Studer U. Extracapsular Extension of Pelvic Lymph Node Metastases From Urothelial Carcinoma of the Bladder Is an Independent Prognostic Factor. *J Clin Oncol* (2005) 23 (10):2358–65. doi: 10.1200/jco.2005.03.084
7. Alakus H, Hölscher A, Grass G, Hartmann E, Schulte C, Drebber U, et al. Extracapsular Lymph Node Spread: A New Prognostic Factor in Gastric Cancer. *Cancer* (2010) 116(2):309–15. doi: 10.1002/cncr.24764
8. Prenzel KL, H?Lscher AH, Drebber U, Bollschweiler E, Gutschow CA, Stippel DL, et al. Extracapsular Lymph Node Spread as a Negative Prognostic Factor of Adenocarcinoma of the Pancreas and Cancer of the Papilla of Vater. *Pancreas* (2013) 43(1):64–8. doi: 10.1097/MPA.0b013e3182a44a91
9. Srinivas V, Morse MJ, Herr HW, Sogani PC, Whitmore WF. Penile Cancer: Relation of Extent of Nodal Metastasis to Survival. *J Urol* (1987) 137(5):880–1. doi: 10.1016/S0022-5347(17)44281-9
10. Lughezzani G, Catanzaro M, Torelli T, Piva L, Biasoni D, Stagni S, et al. The Relationship Between Characteristics of Inguinal Lymph Nodes and Pelvic Lymph Node Involvement in Penile Squamous Cell Carcinoma: A Single Institution Experience. *J Urol* (2014) 191(4):977–82. doi: 10.1016/j.juro.2013.10.140
11. Graafland NM, van Boven HH, van Werkhoven E, Moonen LMF, Horenblas S. Prognostic Significance of Extranodal Extension in Patients With Pathological Node Positive Penile Carcinoma. *J Urol* (2010) 184(4):1347–53. doi: 10.1016/j.juro.2010.06.016
12. Cubilla A. The Role of Pathologic Prognostic Factors in Squamous Cell Carcinoma of the Penis. *World J Urol* (2009) 27(2):169–77. doi: 10.1007/s00345-008-0315-7
13. Touloupidis S, Zisimopoulos A, Giannakopoulos S, Papatsoriset AG, Kalaitzis C, Thanos A, et al. Clinical Usage of the Squamous Cell Carcinoma Antigen in Patients With Penile Cancer. *Int J Urol* (2007) 14:174–6. doi: 10.1111/j.1442-2042.2007.01694.x
14. Hu C, Bai Y, Li J, Zhang G, Yang L, Bi C, et al. Prognostic Value of Systemic Inflammatory Factors NLR, LMR, PLR and LDH in Penile Cancer. *BMC Urol* (2020) 20(1):57. doi: 10.1186/s12894-020-00628-z
15. Li Z-S, Yao K, Li Y-H, Chen J-P, Deng C-Z, Zhao Q, et al. Clinical Significance of Preoperative C-Reactive Protein and Squamous Cell Carcinoma Antigen Levels in Patients With Penile Squamous Cell Carcinoma. *Bju Int* (2016) 118 (2):272–8. doi: 10.1111/bju.13379
16. Kuo Y, Wang J, Hung C, Rau K, Wu I, Chen C, et al. Albumin-Bilirubin Grade Predicts Prognosis of HCC Patients With Sorafenib Use. *J Gastroenterol Hepatol* (2017) 32(12):1975–81. doi: 10.1111/jgh.13783
17. Yu X, Wen Y, Lin Y, Zhang X, Chen Y, Wang W, et al. The Value of Preoperative Glasgow Prognostic Score and the C-Reactive Protein to Albumin Ratio as Prognostic Factors for Long-Term Survival in Pathological T1N0 Esophageal Squamous Cell Carcinoma. *J Cancer* (2018) 9(5):807–15. doi: 10.7150/jca.22755

18. Chen L, Zhang F, Sheng X, Zhang S, Chen Y, Liu BJM. Peripheral Platelet/Lymphocyte Ratio Predicts Lymph Node Metastasis and Acts as a Superior Prognostic Factor for Cervical Cancer When Combined With Neutrophil: Lymphocyte. *Med (Baltimore)* (2016) 95(32):e4381. doi: 10.1097/md.0000000000004381
19. Kozasa K, Mabuchi S, Komura N, Yokoi E, Hiromasa K, Sasano T, et al. Comparison of Clinical Utilities of the Platelet Count and Platelet-Lymphocyte Ratio for Predicting Survival in Patients With Cervical Cancer: A Single Institutional Study and Literature Review. *Oncotarget* (2017) 8(33):55394–404. doi: 10.18632/oncotarget.19560
20. Zhang W, Liu K, Ye B, Liang W, Ren Y. Pretreatment C-Reactive Protein/Albumin Ratio Is Associated With Poor Survival in Patients With Stage IB-IIA Cervical Cancer. *Cancer Med* (2018) 7(1):105–13. doi: 10.1002/cam4.1270
21. Kwon H-C, Kim SH, Oh SY, Lee S, Lee JH, Choi H-J, et al. Clinical Significance of Preoperative Neutrophil-Lymphocyte Versus Platelet-Lymphocyte Ratio in Patients With Operable Colorectal Cancer. *Biomarkers* (2012) 17(3):216–22. doi: 10.3109/1354750x.2012.656705
22. Wakefield L, Smith D, Flanders K, Sporn M. Latent Transforming Growth Factor-Beta From Human Platelets. A High Molecular Weight Complex Containing Precursor Sequences. *J Biol Chem* (1988) 263(16):7646–54. doi: 10.1016/S0021-9258(18)68547-3
23. Banks R, Forbes M, Kinsey S, Stanley A, Ingham E, Walters C, et al. Release of the Angiogenic Cytokine Vascular Endothelial Growth Factor (VEGF) From Platelets: Significance for VEGF Measurements and Cancer Biology. *Br J Cancer* (1998) 77(6):956–64. doi: 10.1038/bjc.1998.158
24. Gay LJ, Felding-Habermann B. Contribution of Platelets to Tumour Metastasis. *Nat Rev Cancer* (2011) 11(2):123–34. doi: 10.1038/nrc3004
25. Man Y, Stojadinovic A, Mason J, Avital I, Bilchik A, Bruecher B, et al. Tumor-Infiltrating Immune Cells Promoting Tumor Invasion and Metastasis: Existing Theories. *J Cancer* (2013) 4(1):84–95. doi: 10.7150/jca.5482

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Impact of Examined Lymph Node Count and Lymph Node Density on Overall Survival of Penile Cancer

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Background: Few studies have explored the optimal examined lymph node count and lymph node density cutoff values that could be used to predict the survival of patients with penile cancer. We further clarify the prognostic value of lymph node density and examined lymph node count in penile cancer.

Methods: The Surveillance, Epidemiology, and End Results (SEER) database was explored to recruit penile cancer patients from 2010 to 2015. A retrospective analysis of penile cancer patients' data from the First Affiliated Hospital of Anhui Medical University was performed for verification (2006–2016). The cutoff values of examined lymph node count and lymph node density were performed according to the ROC curve. Kaplan-Meier survival analysis was used to compare survival differences among different groups. Univariate and multivariate Cox proportional hazard regression analyses were used to determine the significant variables. On the basis of Cox proportional hazards regression model, a nomogram was established and validated by calibration plot diagrams and concordance index (C-index).

Results: A total of 528 patients in the Surveillance, Epidemiology, and End Results cohort and 156 patients in the Chinese cohort were included in this study. Using the ROC curve, we found that the recommended cutoff values of ELN and LND were 13 and 9.3%, respectively (P <0.001). Kaplan–Meier curves suggested the significant differences of overall survival among different examined lymph nodes and lymph node density. Multivariate analysis indicated ELN and LND were independent prognostic factor for OS of penile cancer patients. Nomogram showed the contribution of ELN and LND to predicting OS was large. The C-index at 3-, and 5-year were 0.744 for overall survival (95% CI 0.711–0.777).

Conclusions: The more lymph nodes examined, the lower the density of lymph nodes, and the higher the long-term survival rate of penile cancer. We recommended 13 examined lymph nodes and lymph node density >9.3% as the cutoff value for evaluating the prognosis of penile cancer patients.

Keywords: lymph node, examined lymph node count, lymph node density, overall survival, penile cancer

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INTRODUCTION

Penile cancer (PeCa) is a rare disease, but its incidence has been rising slowly in recent years. According to the 2020 Cancer Research UK (CRUK) report, the incidence rate has increased by 15% over the past decades (1).

As we all know, PeCa is an aggressive urological malignancy, which follows the pattern of gradual invasion from the primary tumor site to inguinal lymph nodes (LNs) before its systemic spread (2, 3). Previous studies have shown that nodal involvement is the most important prognostic factor in PeCa (4). Patients with pN2 and pN3 stages have a 5-year cancer specific survival ranging from 17 to 60% and 0–17%, respectively (5). Although according to the current research on the TNM staging of PeCa, the number of positive LNs can predict the overall survival (OS), like other tumors, the resection quantity of LN metastasis is affected by various factors in survival analysis, such as LN resection method, pathologist's evaluation and individual physiological changes, these mask the true degree of LN involvement to a certain extent (6–9). Therefore, a more optimized variable is needed to evaluate the OS.

From the previous studies we have known that examined lymph node (ELN) count and lymph node density (LND) are the percentage of positive LNs, which have been used as a prognostic factor for other tumors, such as esophageal cancer, non-small-cell lung cancer and bladder cancer (2, 10–14). Unfortunately, these were rarely studied in PeCa. A study conducted by Li et al. determined the prognostic value of ELN in patients with PeCa, but the number of patients was relatively small (6). Additionally, Pettaway et al. first reported the significance of LND for PeCa in 2009 and also, the European Urological Association (EAU) recommended LND for the first time to predict the prognosis of PeCa patients in 2014 (15, 16). However, they didn't calculate the exact optimal cutoff value.

Nomogram, a statistical forecasting tool, has the advantages of low cost and strong reliability, which is used to quantify individual risks according to forecasting factors (4, 17). However, nomogram for predicting the survival of penile cancer patients is rarely constructed. Zheng et al. developed a nomogram that incorporated age, N classification, and log odds of positive LNs which could be conveniently used to predict the long-term OS of patients with penile squamous cell carcinoma (18). However, the variable of ELN and LND was not included in their study.

Therefore, in the current study, we analyzed the effect of ELN and LND on OS in patients with PeCa and evaluated the extent of this effect. Moreover, we included the variable ELN and LND to create an accurate and personalized prognostic nomogram for predicting OS in patients with PeCa, in order to further clarify the prognostic value of ELN and LND in PeCa.

MATERIALS AND METHODS

Study Design and Data Source

This is a retrospective study, using the clinical data of two groups of people diagnosed with PeCa: one from the Surveillance, Epidemiology, and End Results (SEER) database as the training cohort (1975–2016) and the other from Blinded for peer review of China as the validation cohort (2006–2016). All patients in both cohorts underwent radical lymphoadenectomy in addition to surgery of primary tumor site. In patients with nonpalpable nodes, a superficial dissection above the fascia lata was performed. In cases with palpable adenopathy or suspicious nodes encountered during superficial dissection, a deep dissection was performed. Pelvic lymphadenectomy was performed in patients with positive deep inguinal lymph nodes or with enlarged pelvic lymph nodes on cross sectional imaging. The demographic information of age at diagnosis, marital status at diagnosis, ELN, LND, surgery of primary site and tumor characteristics of differentiation grade, histological type, T-stage, N-stage, M-stage and tumor size were collected. Incompletely documented variables such as primary surgical site, grade, TNM stage, marital status, tumor size, ELN, and positive lymph nodes were excluded from this study. In the calculation of "examined lymph node count" and "lymph node density", inguinal and pelvic lymph nodes were included.

OS is defined as the time from diagnosis to original death, whatever the reasons. TNM staging and histopathological grading of PeCa were determined according to the American Joint Committee on Cancer (AJCC) 6th edition staging system and SEER cancer grading system, respectively.

The SEER database is a publicly available, federally funded cancer reporting system and also the largest publicly available cancer data set. Institutional review committees and ethics committees allow the use of public database data without patient identity information (19). Additionally, this study was approved by our University Research Subject Review Board.

Statistical Methods

All statistical analyses were performed using SPSS version 20.0 (SPSS Inc, Chicago, IL, USA). Chi-square, Pearson's chi-square, and Fisher's exact tests were used to determine the significance of differences between continuous variables and categorical variables. Kaplan–Meier analysis was used to estimate survival and compare different variables, namely, average survival time, median survival time and 95% confidence interval (95% CI). Based on Cox proportional hazard regression analysis, multivariate and univariate survival analyses were conducted. As for the evaluation of the model performance and the verification of the accuracy of the new scoring system, we use the Harrell concordance index (C-index) and calibration curve, respectively. Moreover, the receiver operating characteristic (ROC) curve was used to evaluate the effectiveness of the nomogram. P <0.05 values were considered statistically significant for all.

RESULTS

Cutoff Values of ELN and LND

At present, in clinical diagnostic trials, an ROC curve is used to select the critical value reasonably. The curve area under the

optimal critical point is the largest, its sensitivity and specificity are relatively high, and the number of misdiagnosis and missed diagnosis is also the smallest. Using the ROC curve, we found that the recommended cutoff values of ELN and LND were 13 [sensitivity, 50.9; specificity, 64.4; AUC (area under the ROC curve), 0.59; P <0.001] and 9.3% [sensitivity, 59.6; specificity, 78.4; AUC, 0.717; P <0.001], respectively (**Figure 1**).

Patient Characteristics

After screening, 528 patients in the SEER cohort and 156 patients in the China cohort were included in this study. As shown in **Table 1**, all variables had no statistical difference between the training group and the validation group (P >0.05 for all).

Relationship Between LND and Demographics/Clinicopathologic Characteristics

With the cutoff value obtained by ROC curve, we divided all the patients of the training group into two groups: LND ≤rain and LND >9.3%, the numbers were 328 (62.1%) and 200 (37.9%), respectively. The connection is displayed in **Table 2**. LND wasn't significantly correlated with marital status (P = 0.6); however, the association between LND and age at diagnosis (P = 0.003), grade (P <0.001), T-stage (P = 0.001), N-stage (P <0.001), M-stage (P = 0.002), histological type (P = 0.007), ELN (P <0.001), tumor size (P = 0.012) and surgery of primary site (P = 0.012) were significant.

Comparison of Oncology Features of Patients With Different LND

Patients were divided into groups according to LND, and the oncology characteristics of each group were compared (shown in **Figure 2**). There are significant differences in the distribution of T-, N-, and M-stages, histological type, tumor grade and size among different LND patients (P <0.05 for all). Generally speaking, LND is closely related to the pathological features of tumors.

Distribution and Correlation of Clinicopathological Features of Patients

The distribution and correlation of clinical and pathological characteristics of patients in the training group were represented by the mosaic plot to which area of the nested matrix is proportional to the unit frequency, and the frequency is the frequency in the multi-dimensional contingency table. The residual value of fitted model are represented by color and shading. Patients with LND >9.3% have the characteristics of higher tumor grade, more prone to distant metastasis, higher clinical tumor stage and larger tumor size. Also, their histopathological types are significantly different from LND (**Figure 3**).

Univariate and Multivariate Analyses and Identification of Predictors of OS

Univariate risk factors of OS are shown in **Table 3**. We can see that age at diagnosis, marital status, grade, N- and M-stages, surgery of primary site, tumor size, ELN and LND were significant prognostic factors. Besides, as indicated by multivariate analysis, age at diagnosis, N- and M-stages, ELN and LND were independent prognostic factors for OS.

Kaplan–Meier Survival Analysis for Different LND/ELN

In order to evaluate the OS of PeCa patients with different LND/ELN, the Kaplan–Meier survival analysis was performed on all patients. As shown in **Figure 4**, the significant differences of OS were seen among different LND/ELN (P < 0.001 for all). Patients with LND ≤9.3% had the highest OS (median OS and 95%CI undefined), followed by LND >9.3% (median OS = 23, 95%CI = 16.565–29.435). Similarly, patients with ELN >13 have the highest survival rate (median OS = 114, 95%CI = 88.966–139.034), followed by ELN ≤39 (median OS = 58, 95%CI = 36.546–79.454).

Construct and Validate Nomogram

On the basis of Cox proportional hazards regression model, age, N- and M-stages, ELN and LND were selected as variables to

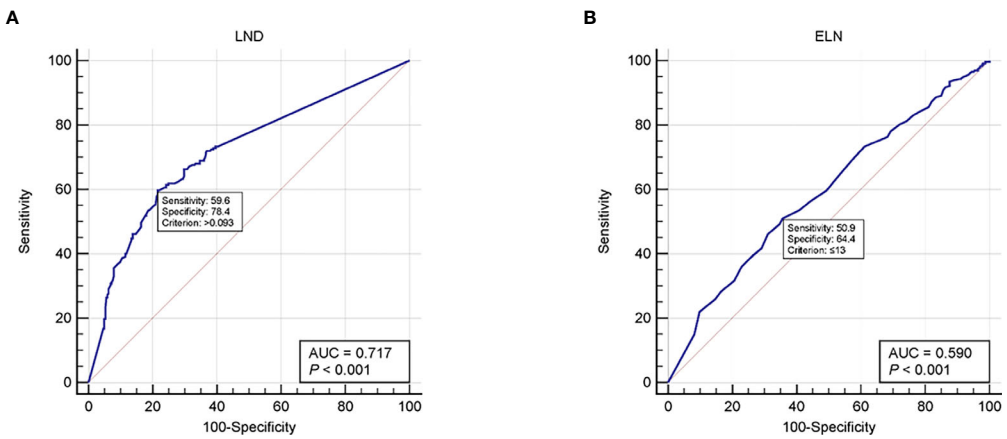

FIGURE 1 | ROC curves for **(A)** LND and **(B)** ELN. ROC, Receiver operating characteristic; LND, Lymph node density; ELN, Examined lymph node.

TABLE 1 | Demographics and clinicopathological characteristics of patients in training cohort and validation cohort.

Demographics and clinicopathologic characteristics	Training set (n = 528)		Validation set (n = 156)		*P*-value
	No. of patients	%	No. of patients	%	
Age at diagnosis (year)					0.825
<50	96	18.2	25	16.0	
50–69	271	51.3	82	52.6	
≥70	161	30.5	49	31.4	
Marital status					0.877
Married	306	60.0	85	54.5	
Divorced	64	12.1	21	13.5	
Widowed	39	7.4	14	9.0	
Single	101	19.1	32	20.5	
Unknown	18	1.4	4	2.5	
Grade					0.763
G1	73	13.8	23	14.7	
G2	283	53.6	82	52.6	
G3	142	26.9	46	29.5	
G4	7	1.3	1	0.6	
Unknown	23	4.4	4	2.6	
T-stage					0.656
T1	138	26.1	41	26.3	
T2	219	41.5	59	37.8	
T3 + T4	171	32.4	56	35.9	
N-stage					0.793
N0	238	45.1	75	48.1	
N1	115	21.8	30	19.2	
N2	115	21.8	36	23.1	
N3	60	11.3	15	9.6	
M-stage					0.477
M0	510	96.6	149	95.5	
M1	18	3.4	7	4.5	
Histological type					0.804
SCC	491	93.0	144		
PC	15	2.8	5		
LC	18	3.4	7		
BCC	1	0.2	0	92.3	
TCC	3	0.6	0	7.7	
ELN					0.854
≤13	221	41.9	67	42.9	
>13	307	58.1	89	57.1	
LND					0.707
≤9.3%	328	62.1	100	64.1	
>9.3%	200	37.9	56	35.9	
Tumor size					0.467
≤3.5 cm	285	54.0	79	50.6	
>3.5 cm	243	46.0	77	49.4	
Surgery of primary site					0.927
LTE	60	11.4	18	11.5	
SS	432	81.8	126	80.8	
RS	36	6.8	12	7.7	

SCC, Squamous cell carcinoma; PC, Papillary carcinoma; LC, Lymphoepithelial carcinoma; BCC, Basal cell carcinoma; TCC, Transitional cell carcinoma; LTE, Local tumor excision; SS, Simple/partial surgical removal of primary site; RS, Radical surgery; ELN, Examined lymph node; LND, Lymph node density.

TABLE 2 | Relationship between LND and demographics/clinicopathologic characteristics.

Demographics/ clinicopathologic characteristics	LND (n = 528)				*P*-value
	≤9.3% (n = 328) No. of patients	%	>9.3% (n = 200) No. of patients	%	
Age at diagnosis (year)			35	17.5	0.003
<50	61	18.6	87	43.5	
50–69	184	56.1	78	39.0	
≥70	83	25.3			
Marital status					0.6
Married	189	57.6	117	58.5	
divorced	35	10.7	29	14.5	
widowed	25	7.6	14	7.0	
single	68	20.7	33	16.5	
unknown	11	3.4	7	3.5	
Grade					0.000
G1	61	18.6	12	6.0	
G2	182	55.5	101	50.5	
G3	67	20.4	75	37.5	
G4	2	0.6	5	2.5	
Unknown	16	4.9	7	3.5	
T-stage					0.001
T1	93	28.4	45	22.5	
T2	148	45.1	71	35.5	
T3 + T4	87	26.5	84	42.0	
N-stage					0.000
N0	238	72.6	0	0.0	
N1	52	15.9	63	31.5	
N2	27	8.2	88	44.0	
N3	11	11.3	49	24.5	
M-stage					0.002
M0	323	98.5	187	93.5	
M1	5	1.5	13	6.5	
Histological type					0.007
SCC	300	91.5	191	95.5	
PC	13	4.0	2	1.0	
LC	15	4.5	3	1.5	
BCC	0	0.0	1	0.5	
TCC	0	0.0	3	1.5	
ELN					0.000
≤13	109	33.2	112	56.0	
>13	219	66.8	88	44.0	
Tumor size					0.012
≤3.5 cm	191	58.2	94	47.0	
>3.5 cm	137	41.8	106	53.0	
Surgery of primary site					0.012
LTE	38	11.6	22	11.0	
SS	276	84.1	156	78.0	
RS	14	4.3	22	11.0	

SCC, Squamous cell carcinoma; PC, Papillary carcinoma; LC, Lymphoepithelial carcinoma; BCC, Basal cell carcinoma; TCC, Transitional cell carcinoma; LTE, Local tumor excision; SS, Simple/partial surgical removal of primary site; RS, Radical surgery; ELN, Examined lymph node; LND, Lymph node density.

construct nomogram (**Figure 5**). Each variable has a corresponding score from 0 to 100 according to its contribution to the result variable. Then add the scores to get the total score at the bottom, and finally calculate the predicted value of the individual outcome event through the functional transformation relationship between the total score and the probability of occurrence of the outcome event. From the nomogram, we know the selected factors had varying degrees of influence on OS. The nomogram scoring system is displayed in **Table 4**.

As shown in **Figure 6A**, the ability of the model to predict the 3- and 5-year OS of PeCa patients was verified by the calibration

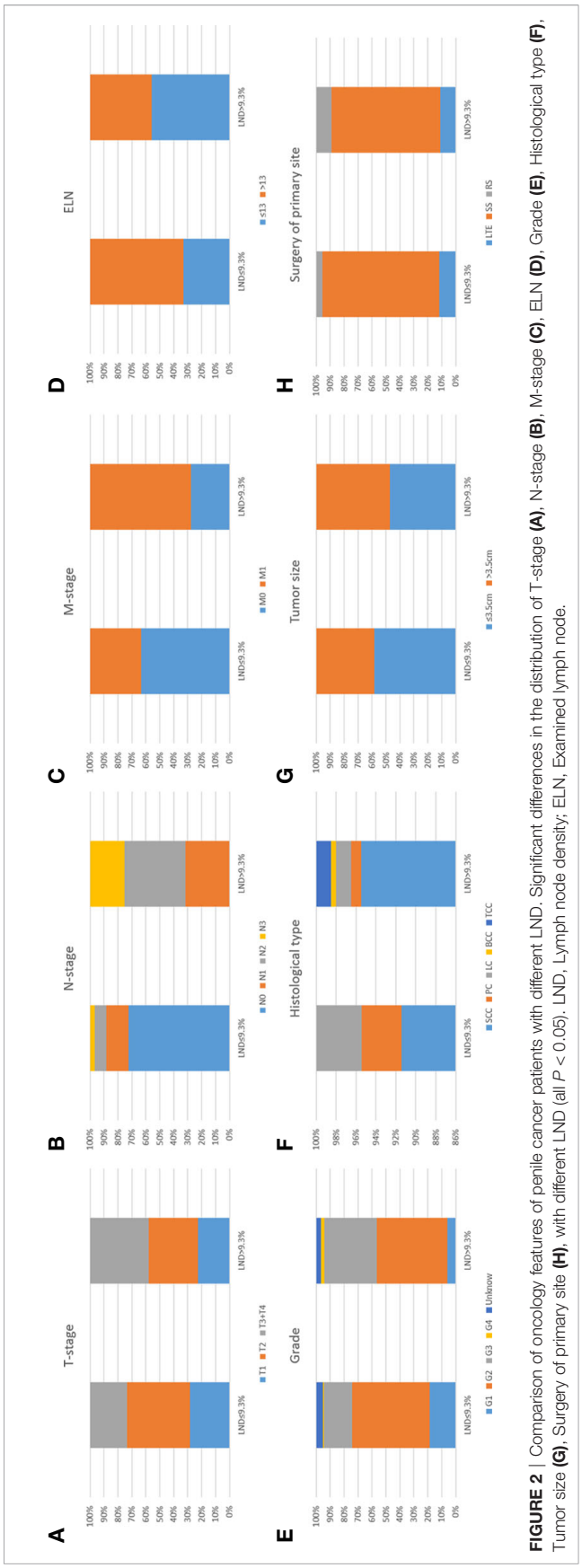

FIGURE 2 | Comparison of oncology features of penile cancer patients with different LND. Significant differences in the distribution of T-stage **(A)**, N-stage **(B)**, M-stage **(C)**, ELN **(D)**, Grade **(E)**, Histological type **(F)**, Tumor size **(G)**, Surgery of primary site **(H)**, with different LND (all $P < 0.05$). LND, Lymph node density; ELN, Examined lymph node.

curve (C-index value: 0.744 >0.7, suggesting that our nomogram is suitable for patients with PeCa). To further validate the performance of the model, the ROC curve was plotted for the nomogram (**Figure 6B**), and the AUC of the nomogram was large, which shows that the accuracy of nomogram was good.

Verified by External Population

On the basis of the nomogram, we drew 3- and 5-year calibration curves and ROC curves from our single center population for independent verification, and the results of the curves were in high agreement with the results of our training group (**Figures 6C, D**).

DISCUSSION

Previous studies have shown that LN status is the most important prognostic factor of PeCa, and its influence on the prognosis of the disease is more significant than that of the tumor grade, general appearance, morphology or microscopic pattern of the primary tumor (20–23). ELN and LND are two basic aspects to determine the status of LNs, which are considered to be predictive factors for the survival of patients with other types of cancer (7, 24). However, up to now, there is no suggestion about ELN count in the National Comprehensive Cancer Network (NCCN) PeCa Guide, although some studies tried to set a benchmark, and the results are not satisfactory (16, 25–27). Recently, Mao et al. used multivariate Cox regression analysis to show that ≥ RLNs removed indicates lower all-cause mortality, PeCa-specific mortality, and lower 5-year mortality, but they had no data to indicate why the cutoff value of the removed LN was 8 (28). Another study conducted by Li et al. reported that the removal of at least 16 lymph nodes in PeCa patients is related to the significant prolongation of disease-specific survival rate, however, they did not have any data on the correlation between the number of LNs removed and OS (6).

Of note, as illustrated in our study, we not only show that ELN is an independent predictor of survival of PeCa, but also that OS with ELN >13 are significantly higher than OS with ELN ≤13. The key point is that we calculate the appropriate threshold for ELN is 13. This shows from another perspective that the more LNs are examined, the less positive LNs are not detected, and this may lead to more thorough removal of remnants to improve long-term survival. Therefore, in PeCa patients with positive and negative LN status, the more the number of LNs examined, the higher the OS, and there is a consistent positive correlation between them.

Additionally, previous studies have shown that the burden of LNs expressed by the number of positive LNs is related to poor prognosis (29, 30). Compared with the number of positive LNs, LND is a more optimized index in the prognosis of PeCa, which can reflect both the degree of LN dissection and the disease burden of LNs (2, 9). The significance of the LND for PeCa was first reported by Pettaway et al. in 2009. In their study, they proved that LND is a better index to predict the disease specific survival of PeCa than the TNM LN staging system (15). Subsequently, in 2014, LND was first recommended by EAU to

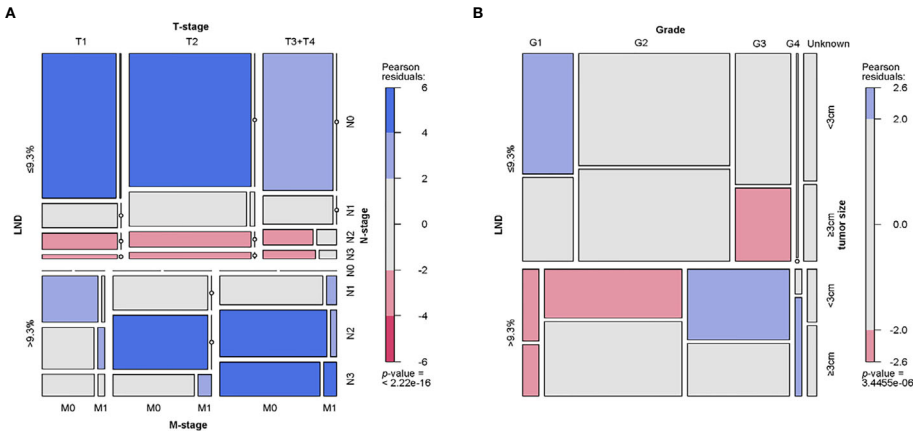

FIGURE 3 | Mosaic plot. **(A)** Distribution and relationship of LND, T-stage, N-stage and M-stage. **(B)** Distribution and relationship of LND, tumor grade, and tumor size. LND, Lymph node density.

TABLE 3 | Univariate and multivariate analysis of the training cohort.

Variables	Univariate analysis		Variables	Multivariate analysis	
	HR (95% CI)	p-value		HR (95% CI)	p-value
Statistically significant factors			**Statistically significant factors**		
Age at diagnosis (years)			Age at diagnosis (years)		
<50 *vs.* 50–59	1.009 (0.686–1.486)	0.961	<50 *vs.* 50–59	1.093 (0.733–1.631)	0.661
<50 *vs.* ≥70	1.702 (1.144–2.531)	0.009	<50 *vs.* ≥70	1.637 (1.066–2.514)	0.024
Marital status at diagnosis			N-stage		
Married *vs.* divorced	1.332 (0.899–1.975)	0.152	N0 *vs.* N1	1.904 (1.182–3.069)	0.008
Married *vs.* widowed	1.663 (1.066–2.596)	0.024	N0 *vs.* N2	1.960 (1.143–3.362)	0.014
Married *vs.* single	1.198 (0.847–1.693)	0.305	N0 *vs.* N3	4.045 (2.303–7.103)	<0.001
Married *vs.* unknown	0.419 (0.133–1.317)	0.136	M-stage		
Grade			M0 *vs.* M1	2.154 (1.212–3.826)	0.009
G1 *vs.* G2	1.197 (0.792–1.809)	0.391	ELN		
G1 *vs.* G3	1.740 (1.121–2.700)	0.013	≤13 *vs.* >13	0.718 (0.524–0.983)	0.039
G1 *vs.* G4	1.421 (0.431–4.679)	0.562	LND		
G1 *vs.* Unknown	0.772 (0.350–1.700)	0.521	≤9.3% *vs.* >9.3%	1.903 (1.218–2.974)	0.005
N-stage			**Statistically non-significant factors**		
N0 *vs.* N1	2.874 (2.010–4.109)	<0.001	Marital status at diagnosis		
N0 *vs.* N2	3.081 (2.148–4.418)	<0.001	Married *vs.* divorced	1.197 (0.797–1.798)	0.387
N0 *vs.* N3	5.851 (3.955–8.654)	<0.001	Married *vs.* widowed	1.443 (0.903–2.306)	0.125
M-stage			Married *vs.* single	1.165 (0.810–1.677)	0.410
M0 *vs.* M1	3.558 (2.062–6.138)	<0.001	Married *vs.* unknown	0.457 (0.142–1.472)	0.190
Surgery of primary site			Grade		
LTE *vs.* SS	1.1040 (0.721–1.691)	0.649	G1 *vs.* G2	0.760 (0.489–1.181)	0.222
LTE *vs.* RS	2.207 (1.260–3.867)	0.006	G1 *vs.* G3	0.813 (0.496–1.333)	0.412
ELN			G1 *vs.* G4	0.696 (0.203–2.380)	0.563
≤13 *vs.* >13	0.644 (0.470–0.836)	0.001	G1 *vs.* Unknown	0.493 (0.221–1.103)	0.085
LND			Surgery of primary site		
≤9.3% *vs.* >9.3%	0.261 (0.200–0.342)	<0.001	LTE *vs.* SS	1.028 (0.644–1.640)	0.909
Tumor size			LTE *vs.* RS	1.467 (0.807–2.668)	0.209
≤3.5 cm *vs.* >3.5 cm	1.421 (1.095–1.844)	0.008	Tumor size		
Statistically non-significant factors			≤3.5 cm *vs.* >3.5 cm	1.237 (0.936–1.636)	0.135
Histological type					
SCC *vs.* PC	0.386 (0.123–1.205)	0.101			
SCC *vs.* LC	0.812 (0.360–1.829)	0.615			
SCC *vs.* BCC	0 (0.000–7.615E+102)	0.943			
SCC *vs.* TCC	0.867 (0.121–6.195)	0.887			
T-stage					
T1 *vs.* T2	0.847 (0.609–1.179)	0.326			
T1 *vs.* T3 + T4	1.251 (0.898–1.744)	0.184			

SCC, Squamous cell carcinoma; PC, Papillary carcinoma; LC, Lymphoepithelial carcinoma; BCC, Basal cell carcinoma; TCC, Transitional cell carcinoma; LTE, Local tumor excision; SS, Simple/partial surgical removal of primary site; RS, Radical surgery; ELN, Examined lymph node; LND, Lymph node density.

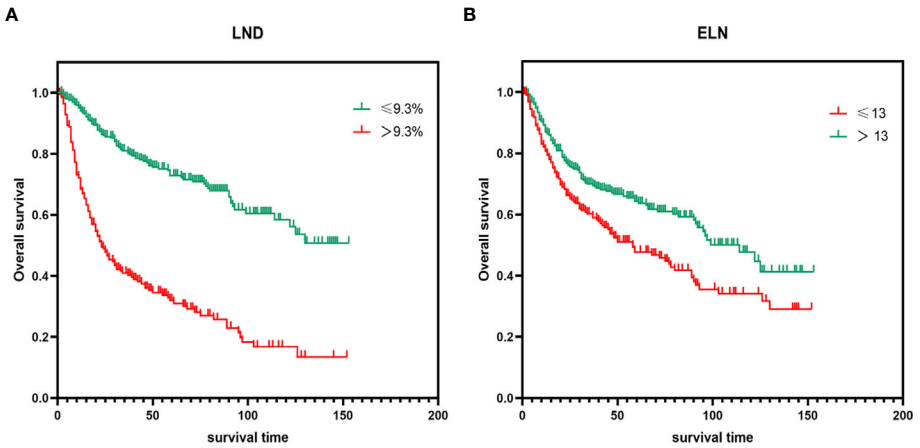

FIGURE 4 | Kaplan–Meier survival analysis for different **(A)** LND and **(B)** ELN ($P < 0.001$ for all). LND, Lymph node density; ELN, Examined lymph node.

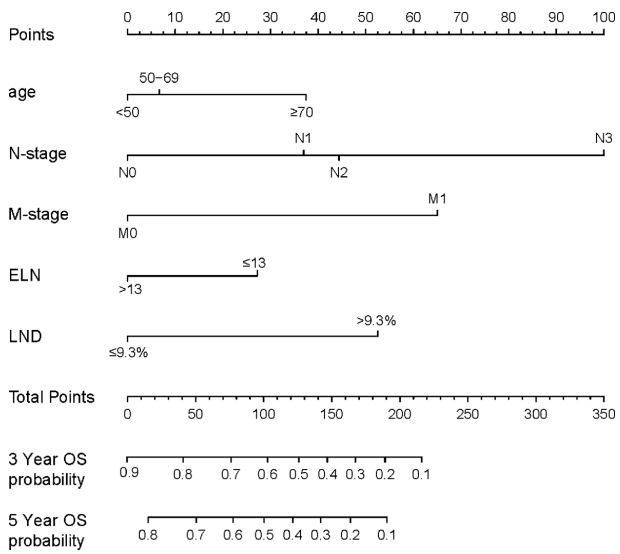

FIGURE 5 | A nomogram for predicting the OS. In order to use the nomogram, the value of each predicted value is determined by drawing a line up to the point reference line, these points are summed, and drawing a line down from the total point line to find the predicted probability of OS. OS, Overall survival; LND, Lymph node density; ELN, Examined lymph node.

TABLE 4 | Nomogram scoring system.

Variables	Points	Variables	Points
Age at diagnosis (years)		M-stage	
<50	0	M0	0
50–69	7	M1	65
≥70	38	ELN	
N-stage		≤13	28
N0	0	>13	0
N1	38	LND	
N2	45	≤9.3%	0
N3	100	>9.3%	53
3-Year OS probability	Points	5-Year OS probability	Points
0.1	218	0.1	191
0.2	190	0.2	164
0.3	169	0.3	142
0.4	148	0.4	121
0.5	128	0.5	100
0.6	102	0.6	78
0.7	78	0.7	50
0.8	41	0.8	28
0.9	0		

ELN, Examined lymph node; LND, Lymph node density; OS, Overall survival.

predict the prognosis of PeCa patients (16). However, in limited studies, the critical value of optimal LND varies widely, ranging from 6.7 to 33% (6, 10, 31). Unlike previous studies, in our study, we not only conformed that LND is a predictor of PeCa, but also, we determined that the recommended cutoff value for LND is 9.3%. More significantly, we found that LND has a good predictive significance for OS in the nomogram and it is verified by external data.

In recent years, nomogram, as a statistical model, shows high reliability in predicting tumor progression (32). Zheng et al. established a simple nomogram for predicting OS for the first time by using the cohort of contemporary penile squamous cell carcinoma patients from the SEER database, in which only three variables were integrated, including age, nitrogen classification and log odds of positive LNs in 2020 (18). Svatek et al. also conducted similar research; they stratified survival outcomes simply according to its median LND of 6.7%, which limits its clinical applicability (15). So far, no studies have included ELN and LND to build nomogram to predict OS of PeCa. Our research indicates that the following five factors are independently related to OS of PeCa patients, including age, N- and M-stages, ELN and LND. All the above factors are included in the construction of the nomogram. As seen in our nomogram, LND contributes more to prognosis than ELN, suggesting that LND has better prognostic value than ELN.

To our knowledge, our study was the first to thoroughly examine the prognostic role of ELN and LND in PeCa and to

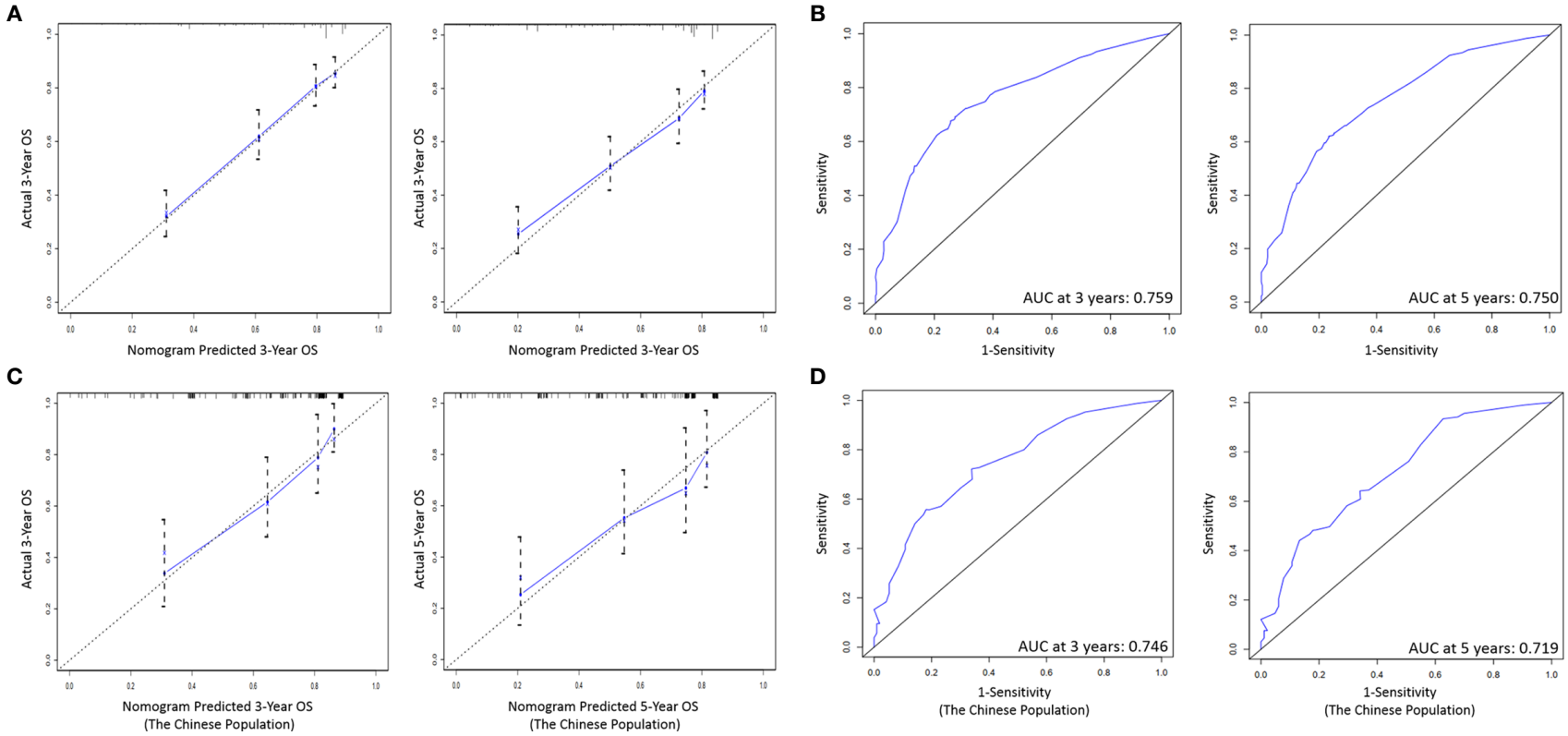

FIGURE 6 | **(A)** Calibration curves of the prognostic nomogram for 3-, and 5-year OS in the training set. **(B)** The ROC curve of the prognostic nomogram in the training set. **(C)** Calibration curves of the prognostic nomogram for 3-, and 5-year OS in the validation set. **(D)** The ROC curve of the prognostic nomogram in the validation set. ROC, Receiver operating characteristic; OS, Overall survival.

develop a nomogram to predict its impact on the OS. What is important is that we use real-world data sets with reliable statistics for verification. We sought to emphasize two major points: (I) ELN and LND are independent predictors for survival of PeCa. (II) A greater number of ELNs and lower LND are associated with better long-term survival of PeCa. We recommended 13 ELNs and LND >9.3% as the cutoff value for evaluating the prognosis of PeCa patients. Therefore, surgeons and pathologists should try their best to explore the LNs and the minimum recommended number for assessing the integrity of LN sampling is 13 and LND needs to be at least 9.3%. Based on real patient data, our research emphasize that surgeons should fully sample and dissect LNs in clinical practice, and carefully explore LNs.

Due to the limitation of retrospective and small-scale real data, the prognostic significance of our results may be discounted a little. First, the main limitation is that the universality of our study may be limited by the fact that it is conducted in a single cultural/social context. Our research is carried out in one country, which is probably a relatively homogeneous population. Due to the lack of sample size and stratified sampling, it cannot represent the true situation of all PeCa patients, and the results will inevitably be influenced by local culture. Therefore, this research needs to be carried out in more countries and regions. Second, the results may still be affected by the selection bias inherent in the design of this study, because adjuvant therapy (including adjuvant chemotherapy and/or radiotherapy) and pelvic lymphadenectomy may affect other parameters. Third, we were unable to investigate other important issues, such as the influence of the number of LNs at stations N1 and N2. As the treatment of PeCa progresses, the prognostic significance of our ELN and LND cut-off values may be changed, so this finding needs to be verified in other cohorts. Fourth, SEER databases may include inhomogeneous data about data collection deriving also from different intern protocols adopted by each center enrolled patients coming from.

Despite these limitations, our analysis demonstrates that the greater the number of LNs examined, the smaller the LND value, and the higher the long-term OS of patients with PeCa. We recommend checking at least 13 LNs and LND >9.3% as a cut-off point for assessing the prognostic stratification of patients with PeCa. This further proves that ELN and LND are tools for predicting PeCa. More institutional research is needed to further determine the clinically relevant prognosis data of the disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PG and XZ designed the study. JG provided the databases. PG, TZ, HL, and XL assembled and analyzed the data. PG wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Akers C, Holden F. An Overview of the Diagnoses and Treatments for Penile Cancer. *Br J Nurs* (2020) 29:S6–14. doi: 10.12968/bjon.2020.29.9.S6
2. Ball MW, Schwen ZR, Ko JS, Meyer A, Netto GJ, Burnett AL, et al. Lymph Node Density Predicts Recurrence and Death After Inguinal Lymph Node Dissection for Penile Cancer. *Investig Clin Urol* (2017) 58:20–6. doi: 10.4111/icu.2017.58.1.20
3. O'Brien JS, Perera M, Manning T, Bozin M, Cabarkapa S, Chen E, et al. Penile Cancer: Contemporary Lymph Node Managemen. *J Urol* (2017) 197:1387–95. doi: 10.1016/j.juro.2017.01.059
4. Maciel CVM, Machado RD, Morini MA, Mattos PAL, Dos Reis R, Dos Reis RB, et al. External Validation of Nomogram to Predict Inguinal Lymph Node Metastasis in Patients With Penile Cancer and Clinically Negative Lymph Nodes. *Int Braz J Urol* (2019) 45:671–8. doi: 10.1590/S1677-5538.IBJU.2018.0756.
5. Resch I, Abufaraj M, Hübner NA, Shariat SF. An Update on Systemic Therapy for Penile Cancer. *Curr Opin Urol* (2020) 30:229–33. doi: 10.1097/MOU.0000000000000733
6. Li ZS, Yao K, Chen P, Zou ZJ, Qin ZK, Liu ZW, et al. Disease-Specific Survival After Radical Lymphadenectomy for Penile Cancer: Prediction by Lymph Node Count and Density. *Urol Oncol* (2014) 32:893–900. doi: 10.1016/j.urolonc.2013.11.008
7. Fang AC, Ahmad AE, Whitson JM, Ferrell LD, Carroll PR, Konety BR. Effect of a Minimum Lymph Node Policy in Radical Cystectomy and Pelvic Lymphadenectomy on Lymph Node Yields, Lymph Node Positivity Rates, Lymph Node Density, and Survivorship in Patients With Bladder Cancer. *Cancer* (2010) 116:1901–8. doi: 10.1002/cncr.25011
8. Biffi R, Botteri E, Cenciarelli S, Luca F, Pozzi S, Valvo M, et al. Impact on Survival of the Number of Lymph Nodes Removed in Patients With Node-Negative Gastric Cancer Submitted to Extended Lymph Node Dissection. *Eur J Surg Oncol* (2011) 37:305–11. doi: 10.1016/j.ejso.2011.01.013
9. Li ZS, Yao K, Chen P, Wang B, Mi QW, Chen JP, et al. Development of a New Classification Method for Penile Squamous Cell Carcinoma Based on Lymph Node Density and Standard Pathological Risk Factors: The ND Staging Syste. *J Cancer* (2016) 2016:262–7. doi: 10.7150/jca.13171
10. Lughezzani G, Catanzaro M, Torelli T, Piva L, Biasoni D, Stagni S, et al. Relationship Between Lymph Node Ratio and Cancer-Specific Survival in a Contemporary Series of Patients With Penile Cancer and Lymph Node Metastases. *BJU Int* (2015) 116:727–33. doi: 10.1111/bju.12510
11. Ooki A, Yamashita K, Kobayashi N, Katada N, Sakuramoto S, Kikuchi S, et al. Lymph Node Metastasis Density and Growth Pattern as Independent

Prognostic Factors in Advanced Esophageal Squamous Cell Carcinoma. *World J Surg* (2007) 31:2184–91. doi: 10.1007/s00268-007-9198-9
12. Herr HW. The Concept of Lymph Node Density–is it Ready for Clinical Practice? *J Urol* (2007) 177:1273–6. doi: 10.1016/j.juro.2006.11.094
13. Kassouf W, Leibovici D, Munsell MF, Dinney CP, Grossman HB, Kamat AM. Evaluation of the Relevance of Lymph Node Density in a Contemporary Series of Patients Undergoing Radical Cystectomy. *J Urol* (2006) 176:53–7. doi: 10.1016/S0022-5347(06)00510-6
14. Liang W, He J, Shen Y, Shen J, He Q, Zhang J, et al. Impact of Examined Lymph Node Count on Precise Staging and Long-Term Survival of Resected Non-Small-Cell Lung Cancer: A Population Study of the US SEER Database and a Chinese Multi-Institutional Registr. *J Clin Oncol* (2017) 35:1162–70. doi: 10.1200/JCO.2016.67.5140
15. Svatek RS, Munsell M, Kincaid JM, Hegarty P, Slaton JW, Busby JE, et al. Association Between Lymph Node Density and Disease Specific Survival in Patients With Penile Cance. *J Urol* (2009) 182:2721–7. doi: 10.1016/j.juro.2009.08.029
16. Hakenberg OW, Compérat EM, Minhas S, Necchi A, Protzel C, Watkin N. EAU Guidelines on Penile Cancer: 2014 Update. *Eur Urol* (2015) 67:142–50. doi: 10.1016/j.eururo.2014.10.017
17. Peak TC, Russell GB, Dutta R, Rothberg MB, Chapple AG, Hemal AK. A National Cancer Database-Based Nomogram to Predict Lymph Node Metastasis in Penile Cancer. *BJU Int* (2019) 123:1005–10. doi: 10.1111/bju.14652
18. Zheng W, Li K, Zhu W, Ding Y, Wu Q, Tang Q, et al. Nomogram Prediction of Overall Survival Based on Log Odds of Positive Lymph Nodes for Patients With Penile Squamous Cell Carcinoma. *Cancer Med* (2020) 9:5425–35. doi: 10.1002/cam4.3232
19. Doll KM, Rademaker A, Sosa JA. Practical Guide to Surgical Data Sets: Surveillance, Epidemiology, and End Results (SEER) Databas. *JAMA Surg* (2018) 153:588–9. doi: 10.1001/jamasurg.2018.0501
20. Leone A, Diorio GJ, Pettaway C, Master V, Spiess PE. Contemporary Management of Patients With Penile Cancer and Lymph Node Metastasis. *Nat Rev Urol* (2017) 14:335–47. doi: 10.1038/nrurol.2017.47
21. Salazar A, Júnior EP, Salles PGO, Silva-Filho R, Reis EA, Mamede M. 18f-FDG PET/CT as a Prognostic Factor in Penile Cancer. *Eur J Nucl Med Mol Imaging* (2019) 46:855–63. doi: 10.1007/s00259-018-4128-7
22. Bandini M, Spiess PE, Pederzoli F, Marandino L, Brouwer OR, Albersen M, et al. A Risk Calculator Predicting Recurrence in Lymph Node Metastatic Penile Cancer. *BJU Int* (2020) 126:577–85. doi: 10.1111/bju.15177
23. Pagliaro LC, Williams DL, Daliani D, Williams MB, Osai W, Kincaid M, et al. Neoadjuvant Paclitaxel, Ifosfamide, and Cisplatin Chemotherapy for Metastatic Penile Cancer: A Phase II Study. *J Clin Oncol* (2010) 28:3851–7. doi: 10.1200/JCO.2010.29.5477
24. Chiramel J, Almond R, Slagter A, Khan A, Wang X, Lim KHJ, et al. Prognostic Importance of Lymph Node Yield After Curative Resection of Gastroenteropancreatic Neuroendocrine Tumours. *World J Clin Oncol* (2020) 11:205–16. doi: 10.5306/wjco.v11.i4.205
25. Campbell RA, Slopnick EA, Ferry EK, Zhu H, Kim SP. Disparity Between Pre-Existing Management of Penile Cancer and NCCN Guidelines. *Urol Oncol* (2017) 35:e9–531.e14:531. doi: 10.1016/j.urolonc.2017.03.002
26. Hakenberg OW, Dräger DL, Erbersdobler A, Naumann CM, Jünemann KP. Protzel C. *Diagnosis Treat Penile Cancer Dtsch Arztebl Int* (2018) 115:646–52. doi: 10.1016/j.urolonc.2017.03.002
27. Bada M, Berardinelli F, Nyirády P, Varga J, Ditonno P, Battaglia M, et al. Adherence to the EAU Guidelines on Penile Cancer Treatment: European, Multicentre, Retrospective Study. *J Cancer Res Clin Oncol* (2019) 145:921–6. doi: 10.1007/s00432-019-02864-9
28. Mao W, Huang X, Kong M, Fan J, Geng J. More Lymph Node Dissection Improves Survival in Patients With Newly Diagnosed Lymph Node-Positive Penile Cancer. *Int Urol Nephrol* (2019) 51:641–54. doi: 10.1007/s11255-019-02084-7
29. Zhu Y, Gu CY, Ye DW. Population-Based Assessment of the Number of Lymph Nodes Removed in the Treatment of Penile Squamous Cell Carcinoma. *Urol Int* (2014) 92:186–93. doi: 10.1159/000354401
30. Zhu Y, Ye DW, Yao XD, Zhang SL, Dai B, Zhang HL. New N Staging System of Penile Cancer Provides a Better Reflection of Prognosis. *J Urol* (2011) 186:518–23. doi: 10.1016/j.juro.2011.03.104
31. Zhu Y, Gu CY, Ye DW. Validation of the Prognostic Value of Lymph Node Ratio in Patients With Penile Squamous Cell Carcinoma: A Population-Based Study. *Int Urol Nephrol* (2013) 45:1263–71. doi: 10.1007/s11255-013-0502-3
32. Novara G, Galfano A, De Marco V, Artibani W, Ficarra V. Prognostic Factors in Squamous Cell Carcinoma of the Penis. *Nat Clin Pract Urol* (2007) 4:140–6. doi: 10.1038/ncpuro0751

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Deep Inguinal Lymph Node Metastases Can Predict Pelvic Lymph Node Metastases and Prognosis in Penile Squamous Cell Carcinoma

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Objectives: To evaluate the relationship between deep inguinal lymph node metastasis (ILNM) and pelvic lymph node metastasis (PLNM) and explore the prognostic value of deep ILNM in penile squamous cell carcinoma (PSCC).

Materials and Methods: The records of 189 patients with ILNM treated for PSCC were analysed retrospectively. Logistic regression models were used to test for predictors of PLNM. Cox regression was performed in univariable and multivariable analyses of cancer-specific survival (CSS). CSS was compared using Kaplan-Meier analyses and log rank tests.

Results: PLNM were observed in 53 cases (28.0%). According to logistic regression models, only deep ILNM (OR 9.72, p<0.001) and number (≥3) of metastatic inguinal lymph nodes (ILNs) (OR 2.36, p=0.03) were independent predictors of PLNM. The incidences of PLNM were 18% and 19% with negative deep ILNM and extranodal extension (ENE); and 76% and 42% with positive deep ILNM and ENE, respectively. The accuracy of deep ILNM, ENE, bilateral involvement and number (≥3) of ILNMs for predicting PLNM were 81.0%, 65.6%, 63.5% and 67.2%, respectively. The CSS was significantly different in patients with positive and negative deep ILNM (median 1.7 years *vs* not reached, p<0.01). Patients who presented with deep ILNM had worse CSS (median 3.8 years *vs* not reached, p<0.01) in those with negative PLNs.

Conclusions: Deep ILNM is the most accurate factor for predicting PLNM in PSCC according to our data. We recommend that patients with deep ILNM should be referred for pelvic lymph node dissection. Involvement of deep ILNs indicates poor prognosis. We propose that patients with metastases of deep ILNs may be staged as pN3.

Keywords: lymph node dissection, neoplasm metastasis, penile neoplasms, prognosis, staging

INTRODUCTION

Lymph node metastasis (LNM) is the major prognostic factor for survival of penile squamous cell carcinoma (PSCC) (1). Regional lymph nodes (LNs) of the penis include inguinal and pelvic nodes. Therapeutic radical inguinal lymph node dissection (ILND) and pelvic lymph node dissection (PLND) are important treatments for PSCC (2). Inguinal lymph nodes (ILNs) consist of superficial and deep nodes, and both superficial and deep ILNs should be removed in complete ILND (3). Lymphatic drainage of the penis is to the superficial and deep ILNs and to the pelvic lymph nodes (PLNs) (4). Thus, PSCC metastasizes in a stepwise fashion from the primary tumor to the ILNs and PLNs (4, 5).

PLND is not recommended when metastasis of deep ILNM is observed according to latest guidelines on penile cancer, although it was recommended before 2014 (6, 7). Additionally, superficial and deep ILNs were not distinguished according to guidelines. The recommendation is mainly based on a study by Leijte et al. (8). However, the relationship between deep inguinal LNM (ILNM) and pelvic LNM (PLNM) and the prognosis of deep ILNM were not evaluated in that study. Interestingly, ILND is routinely performed in patients with groin LNM from melanoma. PLND should be performed if deep ILNs are positive according to NCCN guidelines for cutaneous melanoma (9). The tumor status of deep ILNs is associated with PLNM and survival in melanoma and vulvar cancer (10, 11).

Few studies with small series of cases have evaluated the relationship between the tumor status of deep ILNs and PLNs in penile cancer (12, 13), which showed that deep ILNM may be associated with PLNM. Unfortunately, data on the clinical characteristics of deep ILNM in penile cancer are still scarce. Thus, the tumor status of deep ILNs is ignored by the latest guidelines on penile cancer.

In this retrospective study, we aimed to assess whether deep ILNM is associated with PLNM and explore the prognostic value of deep ILNM in PSCC.

MATERIALS AND METHODS

Study Population

After institutional review board and ethics committee approval was obtained, data were collected on patients in our institution with PSCC treated between January 2000 and June 2020. The informed consent was waived since the retrospective nature of this study. Patients were screened according to following inclusion criterions: 1) Pathologically confirmed PSCC; 2) Bilateral ILND were performed and pathologically confirmed with nodes metastases; 3) Deep inguinal lymphatic tissues were dissected separately; 4) Bilateral PLND were performed, or not performed but followed up with more than two years without evidence of PLNM. Patients who did not receive bilateral PLND were grouped with those without PLNM at histopathological evaluation (8, 14, 15). Patients who received neoadjuvant therapy or had less than 10 total ILNs removed without a fixed nodal mass were excluded.

Indications and Surgical Technique

All the surgeries were performed by experienced surgeons. ILND was indicated according to established guidelines, which have been discussed previously (16, 17). After superficial nodes were removed, the cribriform fascia near the femoral canal was divided. Deep ILNs lying in the femoral canal medial to the femoral vein were dissected (**Figure 1A**). The femoral canal and obturator foramen can be communicated after removal of deep inguinal lymphatic tissue and PLNs (**Figures 1B, C**). The femoral canal should be closed after dissection of deep ILNs (**Figure 1D**) in cases of hernia.

It was controversial to perform PLND in penile cancer before 2009. The decision to perform PLND varied over time and at each institution (14, 18, 19). Thus, only some patients at our institution received PLND before 2009. After that time, synchronous or secondary PLND was indicated for patients if two or more positive ILNs, ENE, or suspicious pelvic imaging were found following radical ILND. PLND consisted of the removal of common iliac, external iliac, internal iliac, obturator and presacral LNs, which was described previously (15, 20).

Staging, Node Count and Follow-Up

All LN specimens were reviewed by two dedicated uropathologists at our institution. After pathological review, clinical and pathological nodal categories were determined according to the 8th edition AJCC staging system for penile cancer. A fixed or gross nodal mass was counted as one LN regardless of size and ENE (14, 21). Deep inguinal LNs were counted as zero and categorized as negative if no LNs were identified in deep inguinal lymphatic tissue. Previous studies revealed that having more than 2 positive ILNs was an independent predictive factor for PLNM (14, 19). Thus, a positive ILNs cutoff of 3 or greater was set in this study for the logistic regression analyses. Follow-up, including physical examination, ultrasound, CT scan or MRI, was performed every 3 months for the first 2 years, every 6 months for the next 3 years, and annually thereafter, for all patients enrolled.

Statistical Analysis

Mann-Whitney *U* and chi-square tests were used to compare continuous and categorical variables, respectively. Univariate and multivariable logistic regression models were used to determine independent predictors for PLNM. Univariable and multivariable Cox regression models were used to test factors of cancer-specific survival (CSS). The Kaplan-Meier method was used to explore CSS rates, and differences were assessed using the log rank test. All reported *p* values are two-sided, with statistical significance considered at $p<0.05$. All statistical analyses were

Abbreviations: AUC, area under the curve; CSS, cancer-specific survival; ENE, extranodal extension; ILND, inguinal lymph node dissection; ILNM, inguinal lymph node metastasis; ILN, Inguinal lymph node; LNM, lymph node metastasis; LN, lymph node; NPV, negative predictive value; PLND, pelvic lymph node dissection; PLNM, pelvic lymph node metastasis; PLN, pelvic lymph node; PPV, positive predictive value; PSCC, penile squamous cell carcinoma.

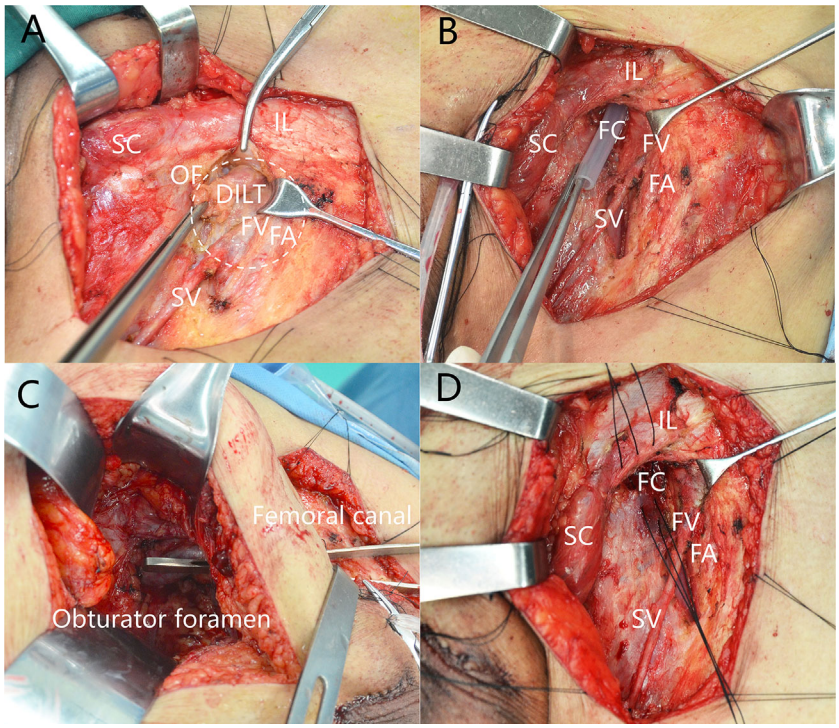

FIGURE 1 | Deep inguinal lymph nodes dissection. **(A)** Position of deep inguinal lymph nodes. **(B)** The femoral canal is empty after removal of DILT. **(C)** Femoral canal communicates with obturator after removal of DILT and pelvic LNs. **(D)** Closing the femoral canal. DILT, deep inguinal lymphatic tissue; FA, femoral artery; FC, femoral canal; FV, femoral vein; IL, inguinal ligament; OF, oval fossa; SC, spermatic cord; SV, saphenous vein.

performed with SPSS version 22 (SPSS Inc., Chicago, IL, USA) and R statistical package version 3.6.3 (R Project for Statistical Computing, www.r-project.org).

RESULTS

Patient Characteristics

A total of 632 patients received penectomies during the period we analysed, and 189 of them were eligible and included in this study. PLNM was confirmed histopathologically based on PLND in 53 (28.0%) patients. A total of 128 (67.7%) patients received bilateral PLND. 61 patients (32.3%) who did not receive bilateral PLND with negative follow-up were grouped with negative PLNM. Deep ILNs were not identified in 95 (25.1%) groins. Clinical and pathological characteristics are summarized in **Table 1**. Mean follow-up was 4.1 (IQR 1.5-5.9) years.

Predicting PLNM by Pathological Characteristics of ILNs

Patients who presented with PLNM had a significantly higher incidence of deep ILNM (47.2% *vs* 5.9%, $p<0.001$), ENE (58.5% *vs* 31.6%, $p=0.001$), bilateral involvement of ILNs (60.4% *vs* 35.3%, $p=0.002$), and a greater number of positive ILNs (median 4 *vs* 2, $p<0.001$) than those with negative PLNs (**Table 1**). On univariable logistic regression analyses, deep ILNM (OR 14.29, $p<0.001$), ENE (OR 3.05, $p=0.001$), 3 or more positive ILNs (OR 4.52, $p<0.001$) and bilateral involvement (OR 2.79, $p=0.002$) were significant predictors of PLNM (**Table 2**). Only 2 factors (deep ILNM and 3 or more positive ILNs) emerged as independent predictors of PLNM in the multivariable logistic regression models (**Table 2**). When patients were classified based on the number of positive ILNs, the incidence of PLNM increased in parallel with the number of positive ILNs for patients with positive and negative ENE (**Supplementary Table 1**). This was also observed in patients with bilateral and unilateral involvement. However, the incidence of PLNM was relatively lower in patients with negative deep ILNs than in those with positive deep ILNs. PLNM incidences were consistently high in patients with positive deep ILNs (**Supplementary Table 1**).

The predictive values of ILNs characteristics for predicting PLNM are shown in **Figure 2**. The specificity (94.1%) and positive predictive value (PPV) (75.8%) of deep ILNM were higher than those of any other predictor, although the sensitivity (47.2%) was relatively low. The negative predictive value (NPV) was comparable for all predictors. ENE, ≥3 positive ILNs and bilateral involvement had similar predictive values. The accuracy (81.0% *vs* 65.6% *vs* 57.2% *vs* 63.5%) (true positive and true negative) and the area under the curve (AUC) (0.71) of deep ILNM were better than those of any other factors.

TABLE 1 | Clinical and pathological characteristics of the 189 patients with penile SCC.

Characteristics	Overall	ILNM only	ILNM and PLNM	*p* value
Number of patients	189	136	53	–
Age, Median (IQR)	52 (44–62)	51 (43-59)	55 (47-67)	0.423^
Treatment of primary tumor, n (%)				0.714*
Circumcision	16 (8.5%)	10 (7.4%)	6 (11.3%)	
Partial penectomy	137 (72.5%)	100 (73.5%)	37 (69.8%)	
Total penectomy	25 (13.2%)	19 (14.0%)	6 (11.3%)	
Unknow	11 (5.8%)	7 (5.1%)	4 (7.5%)	
pT stage, n (%)				0.428*
≤pT1	79 (41.8%)	62 (45.6%)	17 (32.1%)	
pT2	56 (29.6%)	37 (27.2%)	19 (35.8%)	
pT3	39 (20.6%)	26 (19.1%)	13 (24.5%)	
pT4	5 (2.6%)	3 (2.2%)	2 (3.8%)	
pTx	10 (5.3%)	8 (5.9%)	2 (3.8%)	
Tumor grade, n (%)				0.229*
G1	73 (38.6%)	58 (42.6%)	15 (28.3%)	
G2	88 (46.6%)	61 (44.9%)	27 (50.9%)	
≥G3	19 (10.1%)	11 (8.1%)	8 (15.1%)	
Gx	9 (4.8%)	6 (4.4%)	3 (5.7%)	
No. of ILNs removed, Median (IQR)	24 (17-29)	24 (18-30)	20 (16-27)	0.058^
No. of deep ILNs removed, Median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	0.641^
No. of PLNs removed, Median (IQR)	20 (14-28)	20 (14-30)	21 (14-27)	0.827^
No. of positive ILNs, Median (IQR)	2 (1-4)	2 (1-3)	4 (2-6)	<0.001^
Deep ILNs, n (%)				<0.001*
Positive	33 (17.5%)	8 (5.9%)	25 (47.2%)	
Negative	156 (82.5%)	128 (94.1%)	28 (52.8%)	
Extranodal extension of ILNs, n (%)				0.001*
Present	74 (39.2%)	43 (31.6%)	31 (58.5%)	
Absent	115 (60.8%)	93 (68.4%)	22 (41.5%)	
Diameter of ILN, n (%)				0.101*
<30 mm	88 (46.6%)	68 (50.0%)	20 (37.7%)	
≥30 mm	92 (48.7%)	60 (44.1%)	32 (60.4%)	
Unknow	9 (4.8%)	8 (5.9%)	1 (1.9%)	
Side involvement of ILNs, n (%)				0.002*
Bilateral	80 (42.3%)	48 (35.3%)	32 (60.4%)	
Unilateral	109 (57.7%)	88 (64.7%)	21 (40.6%)	
Lymphovascular invasion, n (%)				0.087*
Present	66 (34.9%)	41 (30.1%)	25 (47.2%)	
Absent	118 (62.4%)	91 (66.9%)	27 (50.9%)	
Unknow	5 (2.6%)	4 (2.9%)	1 (1.9%)	
Adjuvant therapy, n (%)				0.01*
Positive	98 (51.9%)	62 (45.6%)	32 (61.4%)	
Negative	75 (39.7%)	66 (48.5%)	13 (24.5%)	
Unknow	16 (8.5%)	8 (5.9%)	8 (15.1%)	

*SCC, squamous cell carcinoma; IQR, interquartile range; ILN, inguinal lymph nodes; ILNM, inguinal lymph node metastases; PLN, pelvic lymph nodes; PLNM, pelvic lymph node metastases. *Chi-square test; ^Mann-Whitney's test.*

TABLE 2 | Univariable and multivariable logistic regression analysis predicting PLNM by inguinal lymph node characteristics.

Predictors	Univariable		Multivariable	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
+ Deep ILN (no *vs* yes)	14.29 (5.84-34.96)	<0.001	9.72 (3.77-25.08)	<0.001
+ ENE (no *vs* yes)	3.05 (1.58-5.87)	0.001	–	–
>2 Positive ILNs (no *vs* yes)	4.52 (2.28-8.98)	<0.001	2.36 (1.09-5.13)	0.03
Bilateral involvement (no *vs* yes)	2.79 (1.45-5.37)	0.002	–	–
>30mm diameter of metastatic (no *vs* yes)	1.81 (0.94-3.50)	0.076	–	–

PLNM, pelvic lymph node metastasis; ILN, inguinal lymph node; ENE, extranodal extension.

Survival Analysis

The median CSS was 2.9 (IQR 1.5-5.9) years. Univariable Cox regression analyses showed that deep ILNM, ENE, bilateral involvement, 3 or more positive inguinal LNs and diameter of metastatic ILNs were significant prognostic factors of CSS (**Table 3**). In multivariable Cox regression analyses, deep ILNM

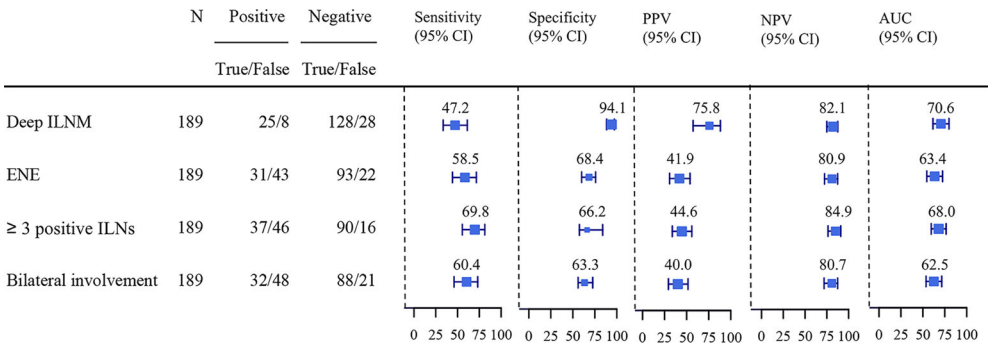

FIGURE 2 | Sensitivity, specificity, PPV, NPV and AUC of ILNs characteristics predicting PLNM. PLNM, pelvic lymph node metastasis; ILNM, inguinal lymph node metastasis; ENE, extranodal extension; AUC, Area under the curve; PPV, positive predictive value; NPV, negative predictive value.

(HR 2.07, p =0.007), ENE (HR 2.72, p <0.001) and bilateral involvement (HR 2.37, p <0.001) remained independent prognostic factors for CSS (**Table 3**). The CSS was significantly different in patients with positive and negative deep ILNM (median 1.7 years *vs* not reached) and in those with positive and negative ENE (median 2.3 years *vs* not reached) (**Figures 3A, B**). Patients who presented with deep ILNM still had worse CSS (median 3.8 years *vs* not reached) in those with negative PLNs. (**Figure 3C**). CSS was similar between those with deep ILNM and ENE in patients with PLNM (median 1.6 *vs* 1.6 years) (**Supplementary Figure 1**). However, considering patients with negative PLNs, there was still no significant difference in CSS between patients with deep ILNM and ENE (median 3.8 *vs* 2.9 years) (**Figure 3D**).

DISCUSSION

PLNM is a major prognostic factor in PSCC patients, which results in a 5-year survival of 12%~33% (14, 19, 22, 23). Approximately one-third of patients with ILNM from PSCC have PLNM (14). Thus, it is important to identify patients with PLNM early. Conventional images, including CT and MRI, are limited in identifying patients with PLNM and result in low sensitivity. Thus, assessment of the pathological characteristics of ILNs is indicated for PLND (6). In this retrospective study, we analyzed whether metastases of deep ILNs can indicate involvement of PLNs.

Previous studies have shown that the histopathological characteristics of ILNs, including the number of positive nodes, tumor grade of the involved nodes, lymph node density, diameter, and ENE, are predictive factors associated with PLNM (14, 18, 19). However, these studies ignored the tumor status of deep ILNs. Although lymphatic drainage of the penis occurs to the superficial and deep ILNs and to the PLNs sequentially; PSCC metastasizes along a similar stepwise pathway (4, 5). Thus, it is important to evaluate the status of deep ILNs in PSCC. We usually dissected superficial and deep ILNs separately in our center in the last 20 years, and we observed that metastases of PLNs usually occurred when deep ILNs were involved. Therefore, the significance of deep ILNM needs to be evaluated due to the metastatic pathway of PSCC.

Leijte et al. (8) previously proposed that deep ILNM should be removed from pN3 cases and the distinction between superficial and deep ILNs should be eliminated, because they found that superficial and deep ILNs cannot be easily distinguished (8). These recommendations were later adopted by the AJCC staging system and guidelines on penile cancer. However, the relationship between deep ILNM and PLNM, and the prognostic value of deep ILNM, were not analysed in that study. Furthermore, superficial and deep ILNs can be distinguished during surgery according to our experience, as their anatomical positions are totally different (**Figure 1A**) (3). The indications for PLND in the latest guidelines on penile cancer were mainly based on a study by Lughezzani et al. (14). However, the relationship between metastases of deep ILNs and metastases of PLNs was not analyzed in that study.

Although a few studies have evaluated the relationship between the tumor status of deep ILNs and PLNs, the number of cases in

TABLE 3 | Univariable and multivariable Cox regression analyses of variables on CSS.

Prognostic variables	Univariable		Multivariable	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
+ Deep ILN (no *vs* yes)	4.08 (2.51-6.64)	<0.001	2.07 (1.22-3.50)	0.007
+ ENE (no *vs* yes)	3.76 (2.38-5.99)	<0.001	2.72 (1.66-4.45)	<0.001
>2 Positive ILNs (no *vs* yes)	3.11 (1.98-4.89)	<0.001	2.37 (1.49-3.78)	<0.001
Bilateral involvement (no *vs* yes)	3.38 (2.14-5.34)	<0.001	–	–
>30mm diameter of metastatic (no *vs* yes)	1.63 (1.04-2.57)	0.035	–	–

CSS, cancer-specific survival; ILNM, inguinal lymph node; ENE, extranodal extension.

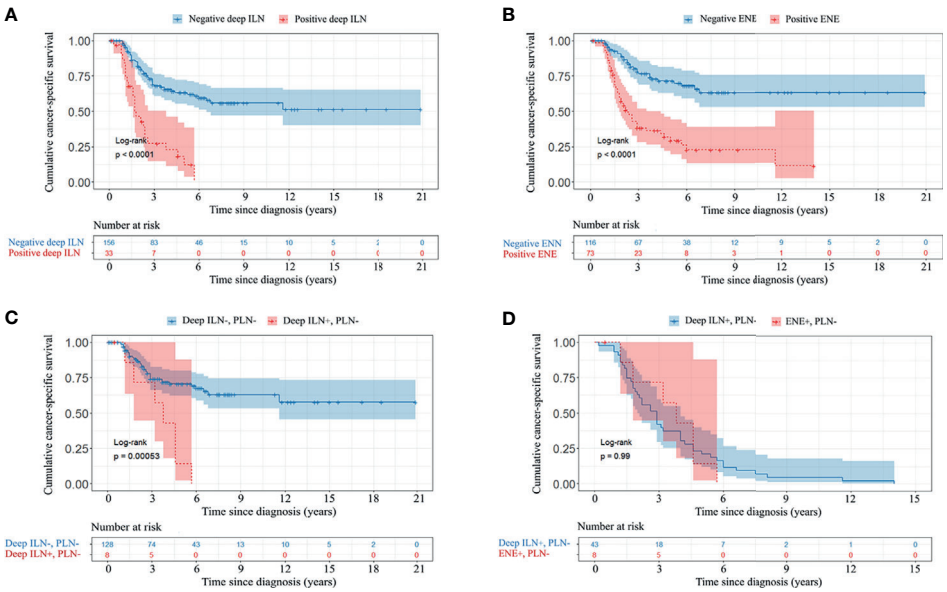

FIGURE 3 | Kaplan-Meier CSS curve of patients with different ILN and PLN characteristics. **(A)**, deep ILN. **(B)**, ENE. **(C)**, deep ILNM without PLNM. **(D)**, deep ILNM and ENE without PLNM. CSS, cancer-specific survival; PLN, pelvic lymph node; ILN, inguinal lymph node; ENE, extranodal extension.

these studies is very small (24 and 30 patients with positive inguinal nodes, respectively) (12, 13). Our study shows that deep ILNM emerged as an independent predictor of PLNM. The specificity, PPV, accuracy and AUC of deep ILNM were higher than those of any other factors evaluated in this study. PLNM presented in 75% of patients with deep ILNM, which only occurred in 46%, 42%, and 40% of patients with ≥3 positive inguinal LNs, ENE, and bilateral involvement, respectively. Moreover, PLNM incidence was high in all patients with deep ILNM, regardless of the number of positive ILNs in our study, even in those with only 2 positive ILNs (75%). This may endorse the recommendation that patients with deep ILNM should receive PLND. Deep ILNM was not observed in patients with 1 positive inguinal LN. This may indicate that deep ILNs are not sentinel nodes. The sensitivity (47.2%) of deep ILNM for predicting PLNM is relatively low. This result suggests that negative deep ILNs cannot rule out PLNM. One possible explanation is that occult metastases of deep ILNs were not identified histopathologically; and another is that there may be other direct pathways of lymphatic drainage from superficial ILNs to PLNs, bypassing deep ILNs.

The prognostic value of deep ILNs in penile cancer was not evaluated previously. Notably, when all patients were considered, patients with positive deep ILNM had a significantly worse prognosis than those with negative ILNM in our study. This was also observed in patients with negative PLNM. Furthermore, the prognosis was similar between patients with positive deep ILNM and ENE in negative PLN patients. All these results indicate that deep ILNM is a prognostic factor associated with poor survival, which is similar to inguinal ENE (24). ENE is categorized as pN3 according to AJCC staging system, thus, metastases of deep ILNs may be categorized as pN3 according to our data. However, this proposal is not validated yet, and more data are required to verify it.

The retrospective nature and long interval of our series represents a potential limitation. However, All the surgeries were performed by experienced surgeons. Additionally, to the best of our knowledge, this study has the largest sample size evaluating the relationship between the tumor status of ILNs and PLNs. Therefore, large sample size, standardized and similar treatment strategy minimized the shortcomings of retrospective design. Penile cancer is rare, so long duration is required to collect enough cases. Previous studies focus on penile cancer even across longer period of time (14, 19). Also, prospective studies evaluating relationship of inguinal and pelvic metastases are unlikely, due to the low incidence of penile cancer.

There were 95 groins (25.1%) with no LNs identified in deep inguinal lymphatic tissue, which could be considered a limitation of our study. However, the average number of deep inguinal LNs dissected was 1.4 per groin, which is consistent with previous studies (11, 25). Inguinal regions of 19 male cadavers were dissected by de Carvalho et al. (25). Deep ILNs were not encountered in all cases, even though all cadavers were dissected carefully. Absence of deep inguinal LNs was also observed in a study by Zhu et al. (13). Several factors contribute to the lack of confirmation of deep ILNs. First, deep ILNs may be absent in some groins. In addition, deep ILNs may be missed during histopathological analyses due to their small size and number. Patients with deep ILNs not identified were categorized as negative in our study. This may lower the sensitivity of deep ILNs predicting PLNM. However, this does not change our opinion that patients with deep ILNM should receive PLND.

The inclusion of patients (32.3%, 61) who did not receive PLND may also be considered a limitation. However, these patients were followed up more than two years, and no pelvic metastases occurred. Virtually all metastases manifest within this period (8, 14, 15). The follow-up of some patients with negative PLNs was relatively short. However, all patients received standardized surgery. The recurrence rate was relatively low in our institution, which was reported previously (16, 17). Furthermore, bilateral, rather than unilateral, PLND was performed in our study, although the guidelines recommend that bilateral PLND is not necessary for all patients. Zargar-Shoshtari et al. (26) found that metastases can spread from ILNs on one side to the PLNs on the other side. This was also found in our previous study (20). As such, metastatic PLNs can be removed more completely by bilateral PLND.

There are other limitations to our data and findings. Patients who received neoadjuvant chemotherapy were excluded from our study. Though this avoided the impact of a therapy response from neoadjuvant chemotherapy, bias may have occurred as some advanced patients were not included. However, approximately 16% of patients can achieve a pathological complete response with neoadjuvant chemotherapy (27). It is difficult to distinguish patients with pathological complete response and absent pelvic metastases. Thus, patients who received neoadjuvant chemotherapy had to be excluded. Additionally, a bulky nodal mass was counted as one lymph node in our study. The true number of involved LNs is unknown in such cases. The number of positive ILNs remained statistically significant in the multivariable analysis, even though a bulky nodal mass was counted as one.

In conclusion, metastases of deep ILNs is the most accurate nodal feature predicting PLNM in PSCC according to our data. We recommend that patients with deep ILNM should receive PLND according to our findings, regardless of the number of positive ILNs and other histopathological characteristics of ILNs. Additionally, metastases of deep ILNs affect prognosis. We propose that patients with involvement of deep ILNs may be staged as pN3, but this proposal need to be verified in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional review board and ethical committee of Sun Yat-sen University Cancer Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZY, HH, FZ, and KY designed this study. ZY drafted the manuscript. ZY, XT, YW, and YZ collected the data. ZY, DC, ZW, ZL YL, and ZQ analysed and interpreted the data, prepared the figures and tables for the manuscript. HH, FZ, and KY surprised this study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.715799/full#supplementary-material

Supplementary Table 1 | Pelvic lymph node metastasis based on inguinal lymph node characteristics. ILN, inguinal lymph node; ENE, extranodal extension; PLNM, pelvic lymph node metastasis.

Supplementary Figure 1 | Kaplan-Meier CSS curve of patients with deep ILNM, ENE and PLNM. CSS, cancer-specific survival; PLN, pelvic lymph node; ILN, inguinal lymph node; ENE, extranodal extension.

REFERENCES

1. Ficarra V, Akduman B, Bouchot O, Palou J, Tobias-Machado M. Prognostic Factors in Penile Cancer. *Urology* (2010) 76(2 Suppl 1):S66–73. doi: 10.1016/j.urology.2010.04.008
2. Protzel C, Alcaraz A, Horenblas S, Pizzocaro G, Zlotta A, Hakenberg OW. Lymphadenectomy in the Surgical Management of Penile Cancer. *Eur Urol* (2009) 55(5):1075–88. doi: 10.1016/j.eururo.2009.02.021
3. Daseler EH, Anson BJ, Reimann AF. Radical Excision of the Inguinal and Iliac Lymph Glands; a Study Based Upon 450 Anatomical Dissections and Upon Supportive Clinical Observations. *Surg Gynecol Obstet* (1948) 87(6):679–94.
4. Cabanas RM. An Approach for the Treatment of Penile Carcinoma. *Cancer* (1977) 39(2):456–66. doi: 10.1002/1097-0142(197702)39:2<456::aid-cncr2820390214>3.0.co;2-i
5. Leijte JA, Valdes Olmos RA, Nieweg OE, Horenblas S. Anatomical Mapping of Lymphatic Drainage in Penile Carcinoma With SPECT-CT: Implications for the Extent of Inguinal Lymph Node Dissection. *Eur Urol* (2008) 54 (4):885–90. doi: 10.1016/j.eururo.2008.04.094
6. Hakenberg OW, Minhas ES, Necchi A, Protzel C, Watkin N, Compérat E. *EAU Guidelines on Penile Cancer 2020. European Association of Urology Guidelines 2020 Edition. Presented at the EAU Annual Congress Amsterdam 2020.* Arnhem, The Netherlands: European Association of Urology Guidelines Office (2020).
7. National Comprehensive Cancer Network. *Penile Cancer (Version 1, 2021).* (2021). Available at: https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf.
8. Leijte JA, Gallee M, Antonini N, Horenblas S. Evaluation of Current TNM Classification of Penile Carcinoma. *J Urol* (2008) 180(3):933–8. doi: 10.1016/j.juro.2008.05.011

9. National Comprehensive Cancer Network. *Cutaneous Melanoma (Version 3, 2020)*. (2020). Available at: https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf.
10. Essner R, Scheri R, Kavanagh M, Torisu-Itakura H, Wanek LA, Morton DL. Surgical Management of the Groin Lymph Nodes in Melanoma in the Era of Sentinel Lymph Node Dissection. *Arch Surg* (2006) 141(9):877–82. doi: 10.1001/archsurg.141.9.877
11. Tu H, Sun P, Gu HF, Zhang XK, Huang H, Wan T, et al. Clinical Significance and Prognostic Value of Femoral Lymph Node Metastasis in FIGO Stage III Vulvar Carcinoma. *Eur J Surg Oncol* (2017) 43(9):1768–75. doi: 10.1016/j.ejso.2017.05.019
12. Zhu Y, Zhang SL, Ye DW, Yao XD, Jiang ZX, Zhou XY. Predicting Pelvic Lymph Node Metastases in Penile Cancer Patients: A Comparison of Computed Tomography, Cloquet's Node, and Disease Burden of Inguinal Lymph Nodes. *Onkologie* (2008) 31(1-2):37–41. doi: 10.1159/0000112462
13. Zhu Y, Zhang SL, Ye DW, Yao XD, Dai B, Zhang HL, et al. Prospectively Packaged Ilioinguinal Lymphadenectomy for Penile Cancer: The Disseminative Pattern of Lymph Node Metastasis. *J Urol* (2009) 181 (5):2103–8. doi: 10.1016/j.juro.2009.01.041
14. Lughezzani G, Catanzaro M, Torelli T, Piva L, Biasoni D, Stagni S, et al. The Relationship Between Characteristics of Inguinal Lymph Nodes and Pelvic Lymph Node Involvement in Penile Squamous Cell Carcinoma: A Single Institution Experience. *J Urol* (2014) 191(4):977–82. doi: 10.1016/j.juro.2013.10.140
15. Li Z, Guo S, Wu Z, Han H, Li Z, Wang Y, et al. Subclassification of Pn3 Staging Systems for Penile Cancer: Proposal for Modification of the Current TNM Classification. *Urol Oncol* (2017) 35(9):543.e1–.e6. doi: 10.1016/j.urolonc.2017.04.009
16. Yao K, Tu H, Li YH, Qin ZK, Liu ZW, Zhou FJ, et al. Modified Technique of Radical Inguinal Lymphadenectomy for Penile Carcinoma: Morbidity and Outcome. *J Urol* (2010) 184(2):546–52. doi: 10.1016/j.juro.2010.03.140
17. Yao K, Zou ZJ, Li ZS, Zhou FJ, Qin ZK, Liu ZW, et al. Fascia Lata Preservation During Inguinal Lymphadenectomy for Penile Cancer: Rationale and Outcome. *Urology* (2013) 82(3):642–7. doi: 10.1016/j.urology.2013.05.021
18. Liu JY, Li YH, Zhang ZL, Yao K, Ye YL, Xie D, et al. The Risk Factors for the Presence of Pelvic Lymph Node Metastasis in Penile Squamous Cell Carcinoma Patients With Inguinal Lymph Node Dissection. *World J Urol* (2013) 31(6):1519–24. doi: 10.1007/s00345-013-1024-4
19. Lont AP, Kroon BK, Gallee MP, van Tinteren H, Moonen LM, Horenblas S. Pelvic Lymph Node Dissection for Penile Carcinoma: Extent of Inguinal Lymph Node Involvement as an Indicator for Pelvic Lymph Node Involvement and Survival. *J Urol* (2007) 177(3):947–52. doi: 10.1016/j.juro.2006.10.060
20. Yao K, Chen Y, Ye Y, Wu Z, Chen D, Han H, et al. Lymph Node Mapping in Penile Cancer Patients Undergoing Pelvic Lymph Node Dissection. *J Urol* (2020) 205(1):145–51. doi: 10.1097/JU.0000000000001322
21. Carver BS, Cronin AM, Eggener S, Savage CJ, Motzer RJ, Bajorin D, et al. The Total Number of Retroperitoneal Lymph Nodes Resected Impacts Clinical Outcome After Chemotherapy for Metastatic Testicular Cancer. *Urology* (2010) 75(6):1431–5. doi: 10.1016/j.urology.2009.11.076
22. Graafland NM, van Boven HH, van Werkhoven E, Moonen LM, Horenblas S. Prognostic Significance of Extranodal Extension in Patients With Pathological Node Positive Penile Carcinoma. *J Urol* (2010) 184(4):1347–53. doi: 10.1016/j.juro.2010.06.016
23. Zargar-Shoshtari K, Djajadiningrat R, Sharma P, Catanzaro M, Zhu Y, Nicolai N, et al. Establishing Criteria for Bilateral Pelvic Lymph Node Dissection in the Management of Penile Cancer: Lessons Learned From an International Multicenter Collaboration. *J Urol* (2015) 194(3):696–701. doi: 10.1016/j.juro.2015.03.090
24. Zhang ZL, Yu CP, Liu ZW, Velet L, Li YH, Jiang LJ, et al. The Importance of Extranodal Extension in Penile Cancer: A Meta-Analysis. *BMC Cancer* (2015) 15:815. doi: 10.1186/s12885-015-1834-4
25. de Carvalho JP, Patricio BF, Medeiros J, Sampaio FJ, Favorito LA. Anatomic Aspects of Inguinal Lymph Nodes Applied to Lymphadenectomy in Penile Cancer. *Adv Urol* (2011) 2011:952532. doi: 10.1155/2011/952532
26. Zargar-Shoshtari K, Sharma P, Djajadiningrat R, Catanzaro M, Ye DW, Zhu Y, et al. Extent of Pelvic Lymph Node Dissection in Penile Cancer may Impact Survival. *World J Urol* (2016) 34(3):353–9. doi: 10.1007/s00345-015-1593-5
27. Azizi M, Aydin AM, Hajiran A, Lai A, Kumar A, Peyton CC, 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):1147–55. doi: 10.1097/JU.0000000000000746

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CASE REPORT
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Warthy-Basaloid Squamous Cell Carcinoma of Penile – Case Report

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Objective: The aim of the study was to present a case of penile squamous cell carcinoma and immunohistochemical identification and evaluation of E-cadherin and β-catenin expression.

Methods: We are presenting a 70-year old man with a variant of penile squamous cell carcinoma with mixed warty and basaloid features. After diagnosis, the patient underwent partial penectomy. Samples taken from the material after surgery were subjected to basic histological staining and immunohistochemical identification of E-cadherin and β-catenin. A Real-time PCR study was conducted to investigate the expression of E-cadherin and β-catenin.

Results: Routine histopathological examinations revealed the characteristic features of warty-basaloid squamous cell carcinoma. In the case studied, a positive immunohistochemical reaction was observed for E-cadherin and β-catenin. QRT-PCR analysis showed a statistically significant decrease in E-cadherin expression in tumor samples compared to healthy tissue. In contrast, expression of the gene encoding β-catenin was slightly higher in tumor samples compared to normal tissue.

Conclusions: The reduced level of the complex of adhesive elements, E-cadherin-β-catenin, disturbs cell differentiation, promotes a more invasive phenotype-stromal infiltration and the formation of distant metastases. In the described case of the penile tumor, a decrease in E-cadherin expression was noted, which could be related to the occurrence of neoplastic infiltration of the spongy body space. In summary, E-cadherin and β-catenin expression and the immunoreactivity of these proteins are expressed at different levels in tumor cells and in penile interstitial cells. Regulation of expression during various physiological and pathophysiological processes indicates a potentially important role of E-cadherin and β-catenin in cell proliferation and adhesion.

Keywords: penile squamous cell carcinoma, warty carcinoma, basaloid carcinoma, human papillomavirus, adhesion molecules

INTRODUCTION

The human papillomavirus (HPV) is the most common viral infection of the reproductive tract. Wherein, it does not cause systemic infection, but a local one and most of them are asymptomatic and resolves spontaneously. Persistent infections cause changes to the skin (warts) and can lead to the development of cancer. HPV infects epithelial cells of the skin and mucous membranes, and their development cycle is related to the differentiation of infected cells. HPV infection is closely related to penile cancer, but the relationship between HPV infection and cancer formation is not fully understood (1, 2).

Penile cancer is a fairly rare cancer with approximately 26 000 cases diagnosed worldwide each year. Most penis cancers are squamous cell carcinomas, but there is a wide spectrum of histological subtypes. Numerous observations indicate the participation of HPV in the incidence of penile cancer. The role of HPV has been confirmed in the etiology of squamous cell carcinoma of the penis in men, but it has been established that other factors are also involved in this process (3, 4).

There are three types of penile squamous cell carcinoma, usually associated with the HPV: warty-basaloid, warty carcinomas and basaloid. In invasive penile tumors, papillary-basal carcinomas were most often associated with HPV. It is supposed that these viruses are present only in the initial stage of the precancerous state, and later, along with the development of neoplastic changes, their genes cannot be found. So they are undetectable at this stage (5).

HPV infection is not able to induce complete neoplastic transformation. This process requires the participation of other factors, such as the escape of cells from the control of the immune system, changes in the expression of viral genes, and subsequent genetic changes. Studies have shown that an interaction can occur between the HPV oncoprotein and p53, which leads to the inactivation of the p53 gene, which is a negative regulator of tumor cell growth. In response to DNA damage, it may arrest the G1 cell cycle and/or apoptosis. Research is constantly being carried out to identify successive genetic changes related to the oncogenesis process stimulated by HPV, or involving HPV (6, 7).

In the course of tumor progression, epithelial cells, in the course of oncogenesis, begin to acquire the features of mesenchymal cells. It is associated with the loss or reduction of intercellular connections, and weakened interaction with the basement membrane. Intercellular junctions maintain apical-basal polarization, maintain tissue integrity, and enable interaction and signal transmission between cells and between the extracellular matrix. Weak cell adherence may lead to impaired control of the cell cycle, separation of single cells from the primary site, which creates conditions for the formation of neoplastic metastases (8).

One of the basic adhesion molecules responsible for the formation of intercellular connections and the mutual recognition of cells is E-cadherin. As a result of its loss, the accumulation of β-catenin in the cytoplasm of the cell occurs and its translocation to the cell nucleus, where it regulates the transcription of many genes involved in the proliferation and differentiation of cells.

E-cadherin expression reduction or function shutdown is associated with the loss of intercellular connections, proper polarity and the acquisition of the ability to migrate and invade, which are key phenomena responsible for the progression of neoplastic disease (8, 9).

Warthy-basal cell carcinoma of the penis is a rare disease that is clinically and pathologically diverse. New factors involved in the cancer progression are still being searched for. E-cadherin and B-catenin could prove to be important biomarkers that have not yet been assessed together in this type of cancer.

The aim of the study was to present the case of penile squamous cell carcinoma and immunohistochemical identification and evaluation of E-cadherin and B-catenin expression. This study is rare and contains new data.

MATERIAL AND METHODS

Clinical Presentation

We are presenting a 70-year old male patient who noticed a change in the area of the glans penis in 2017. In July 2019, a biopsy was performed. The histopathological analysis of this biopsy was found (hist-pat. *Planocellulare invasivum*). The patient was proposed a partial penile amputation, for which the patient did not consent.

He was hospitalized only at the beginning of 2021 at the Clinical Hospital in Białystok, the lesion has increased by about 10-15 mm. Before the surgery color Doppler ultrasound of the penis was performed. There was a suspicion of cavernous infiltration. This was confirmed in histopathomorphology. Patient underwent a partial penectomy procedure.

The study protocol was approved by the Bioethics Committee, Medical University of Bialystok (R-I-002/282/2019) and prior written informed consent was obtained from patient.

Clinicopathologic Features

The distal part of the penis measures 5 x 3.2 x 3.5 cm. In the area of the glans and foreskin, there is an exophytic ulcerative tumor 3.7x2x1.5 cm. On the glans cross-sections, a whitish infiltrate is present, covering the distal part of the corpora cavernosa and penetrating superficially into the left corpora cavernosa.

Pathomorphological Diagnosis

Histological type: warthy-basaloid squamous cell carcinoma. Histological maturity grade: G2 - moderately differentiated. The tumor invades the spongy body, the infiltration is at least 8 mm thick. There was no invasion of the perineural spaces. Pathomorphological stage (pTNM): pT2 pNx pMx. Inguinal nodes were not palpable.

The diagnosed variant of squamous cell carcinoma is typically associated with an HPV infection. P16 test - diffuse positive.

Immunohistochemistry

Material were embedded in paraffin in a routine manner. The paraffin blocks were cut into 4 μm sections and attached to positively charged glass slides and stained in hematoxylin and eosin for general histological evaluation. Immunostaining was performed by the following protocol: sections were deparaffined and hydrated in pure alcohols. For antigen retrieval, the sections were subjected to pretreatment in pressure chamber and heated for 1 min at 21 psi at 125°C, using Target Retrieval Solution Citrate pH=6.0 (S 2369 Agilent Technologies, Inc. 5301 Stevens Creek Blvd Santa Clara, CA 95051, USA). After cooling down to room temperature, the sections were incubated with Dako REAL Peroxidase-Blocking Solution (S 2023 Agilent Technologies, Inc.) for 10 minutes to block endogenous peroxidase activity. The sections with the primary antibodies: β-catenin, (ab32572 Abcam, UK) and E-cadherin (ab76055 Abcam, UK) were incubated 24 hours at +4°C in a humidified chamber. The antibodies were previously diluted in Antibody Diluent Background Reducing (S 3022 Agilent Technologies, Inc.) in relation 1: 2 000 for β-catenin and 1: 500 for E-cadherin. Procedure was followed by incubation (1 hour) with secondary antibody (EnVision FLEX, High pH (Link), HRP. Rabbit/Mouse. (K800021-2 Agilent Technologies, Inc.). The bound antibodies were visualized by 1-min incubation with DAB Flex chromogen. The sections were finally counterstained in hematoxylin QS (H-3404, Vector Laboratories; Burlingame, CA), mounted and evaluated under light microscope. Appropriate washing with Wash Buffer (S 3006 Agilent Technologies, Inc.) was performed between each step (3 times for 2 minutes). Sections were dehydrated with absolute alcohol followed by xylene, and coverslipped with Entellan (Merck). The specificity of the antibodies was confirmed using a negative control, which involved replacing the antibodies with the Antibody Diluent (no staining).

Real-Time PCR

Tumor and normal tissue samples taken from the material after partial penectomy were placed in an RNA-later solution. Total RNA was isolated using NucleoSpin® RNA Isolation Kit (Machery-Nagel). Quantification and quality control of total RNA was determined using a spectrophotometer - NanoDrop 2000 (ThermoScientific). An aliquot of 1 μg of total RNA was reverse transcribed into cDNA using iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (BIO-RAD). Synthesis of cDNA was performed in a final volume of 20 μl using an Thermal Cycler (Model SureCycler 8800, Aligent Technologies). For reverse transcription, the mixtures were incubated at 46°C for 20 min, then heated to 95°C for 1 min and finally cooled quickly at 4°C. Quantitative real-time PCR reactions were performed using Stratagene Mx3005P (Aligent Technologies) with the SsoAdvanced™ Universal SYBER® Green Supermix (BIO-RAD). Specific primers for E-cadherin, β-catenin and GAPDH (GAPDH) were designed by BIO-RAD Company. The housekeeping gene GAPDH (GAPDH) was used as a reference gene for quantification. To determine the amounts of levels of test genes expression, standard curves were constructed for each gene separately with serially diluted PCR products. PCR products were obtained by cDNA amplification using specific primers as follows: E-cadherin (qHsaCEP0049339, BIO-RAD), β-catenin (qHsaCID0010363, BIO-RAD), and GAPDH (qHsaCED0038674, BIO-RAD). QRT-PCR was carried out in a doublet in a final volume of 20 μl under the following conditions: 2 min polymerase activation at 95°C, 5 s denaturation at 95°C, 30 s annealing at 60°C for 35 cycles. PCR reactions were checked, including no-RT-controls, omitting of templates, and melting curve to ensure only one product was amplified. The relative quantification of gene expression was determined by comparing Ct values using the ΔΔCt method. All results were normalized to GAPDH.

Statistical Analysis

All data were analyzed for statistical significance using the Statistica version 12.0 computer software package. The mean values were computed automatically; significant differences were determined by one-way ANOVA test; $p < 0.05$ was considered significant.

RESULTS

Routine histopathological examinations showed the characteristic features of warty-basaloid squamous cell carcinoma (**Figure 1**). Invasive tumor nest with central atypical parakeratosis, pleomorphic koilocytosis and peripheral small and uniform cells with basaloid features. The papillae had conspicuous fibrovascular cores. Cells with basaloid features predominated, although rounded and spindle cells were also noted. An evident clear cell koilocytosis on surface was present in this case (**Figure 1**).

A positive immunohistochemical reaction for E-cadherin and β-catenin was observed in the studied warty-basaloid squamous cell carcinoma case (**Figures 2**, **3**).

The immunoreactivity of E-cadherin in penile cancer neoplastic cells is mainly located in the cell membrane (**Figure 2**). Cells with a highly stained cell membrane were adjacent to weakly stained or negative cells (**Figure 2**).

In the attached photos (**Figure 3**) we observe the membrane immunoexpression of β-catenin, as well as the presence of β-catenin in the cytoplasmic compartment and translocation to the cell nucleus.

QRT-PCR analysis showed a statistically significant decrease in E-cadherin expression in tumor samples compared to healthy tissue. In contrast, expression of the gene encoding β-catenin was slightly higher in tumor samples compared to normal tissue (**Figure 4**).

DISCUSSION

Penile cancer can follow various etiological pathways, one of them is associated with HPV infection, and the others are associated

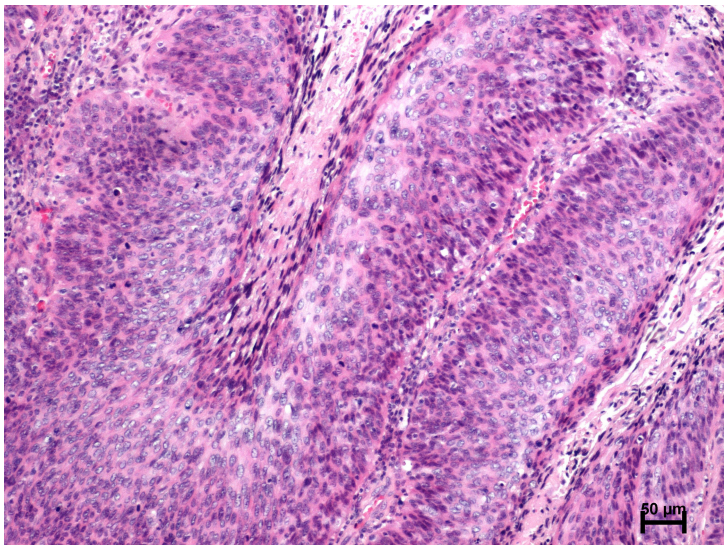

FIGURE 1 | Microscopic features of warty–basaloid carcinoma (H+E).

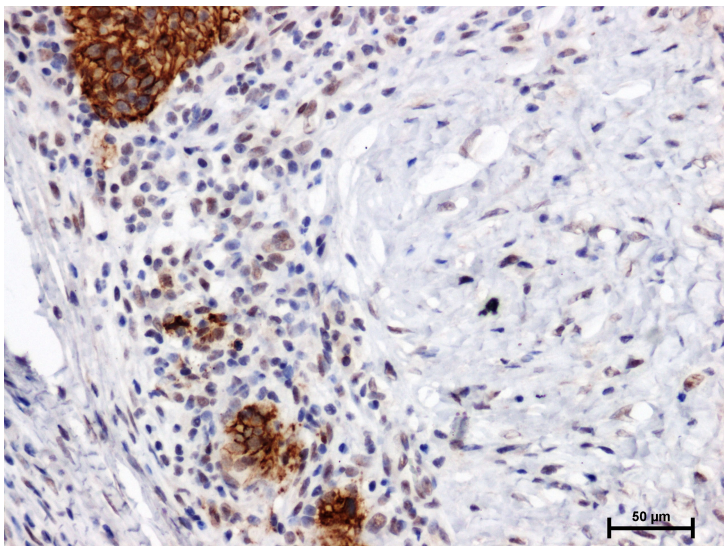

FIGURE 2 | Immunoidentification of E-cadherin in warty–basaloid carcinoma of penile.

with chronic inflammation, phimosis, etc. The prevalence of different histological types of penile cancer varies, the most common type is papillary and/or basal cell carcinoma, less frequently keratinizing variants. The presence of cancer cells with basaloid features is strongly associated with the presence of HPV (5, 10, 11).

We describe a mixed neoplasm exhibiting warty and basaloid features. Considering the pathogenesis associated with the human papilloma virus, as well as mixed morphological forms, warty and basaloid carcinomas would represent the low- and high-grade ends of a clinicopathological spectrum (4, 12, 13).

E-cadherin, as a component of the E-cadherin-catenin adhesive complex, acts as an "invasion suppressor". Reducing its level leads to a weakening of intercellular adhesive interactions, disrupts the integrity and structure of the tissue, and promotes the aggressive features of neoplastic cells - infiltration of the stroma, invasion of blood vessel and formation of metastasis (14, 15).

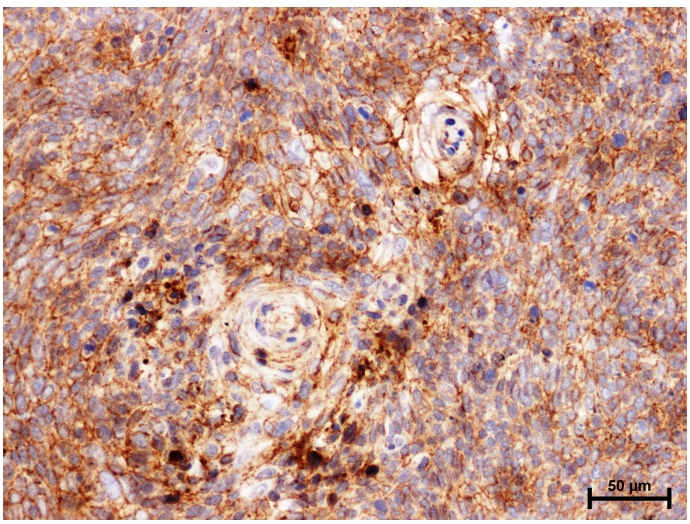

FIGURE 3 | Immunoidentification of β-catenin in warty–basaloid carcinoma of penile.

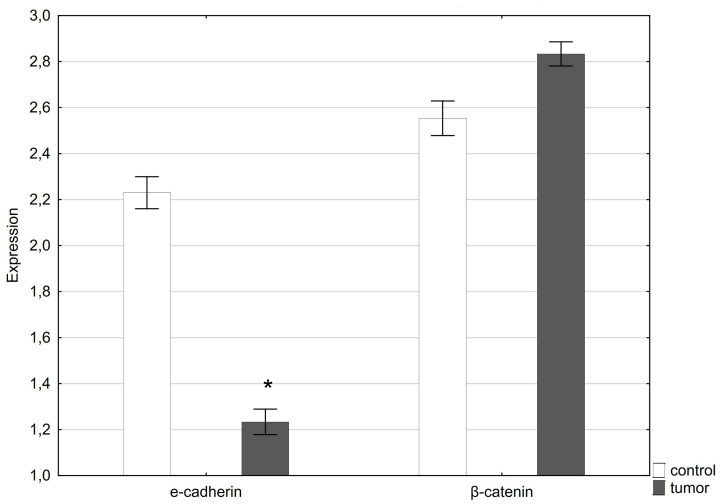

FIGURE 4 | Expression of E-cadherin and β-catenin in normal penile tissue (control) and penile tumor. *p < 0.05 was considered significant.

The decreased level of E-cadherin stimulates the proliferative activity of cells, as well as the process of epithelial-mesenchymal transformation, during which neoplastic cells lose the ability to form epithelial structures and develop a mesenchymal phenotype, which allows for cell dissociation, invasion of the environment and the formation of metastases (16).

In the described case of a penile tumor, a decrease in the expression of E-cadherin was noted, which could be related to the occurrence of a neoplastic infiltration of the spongy body space.

In normal epithelium, β-catenin is located in the cell membrane. Reduced membrane immunoexpression, as well as the presence of β-catenin in the cytoplasmic compartment and translocation to the cell nucleus are interpreted as indicators of decreased immunoexpression of this protein (17).

In the performed immunohistochemical reaction showing β-catenin in the examined penile tumor, positive membrane, cytoplasmic and nuclear reactions were observed. Furthermore, we found a slight increase in the expression of the gene encoding beta-catenin in the tumor tissue as compared to the control.

In over half of all cancer cases, such as breast cancer, leukemia, melanoma, colorectal cancer and liver cancer, β-catenin accumulates in the nucleus or the cytoplasm. Research

has shown that β-catenin promotes the progression of tumors *via* suppressing the T-cell responses (18, 19).

Reduced level of E-cadherin-β-catenin adhesive complex elements disturbs cell differentiation, promotes a more invasive phenotype-stromal infiltration and the formation of distant metastases (8).

In conclusion, E-cadherin and β-catenin expression and the immunoreactivity of these proteins are expressed at different levels in tumor cells and in penile interstitial cells. Regulation of expression during various physiological and pathophysiological processes indicates a potentially important role of E-cadherin and β-catenin in cell proliferation and adhesion. The reduced level of E-cadherin-β-catenin, disturbs cell differentiation, promotes a more invasive phenotype-stromal infiltration and the formation of distant metastases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Bioethics Committee, Medical University of Bialystok (R-I-002/282/2019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IK and ND conceived of and designed the experiments. ND and GM analyzed the data. IK, ND, and GM contributed reagents/materials/analysis tools. Writing – original draft preparation: ND. Writing – review and editing: IK. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Kidd LC, Chaing S, Chipollini J, Giuliano AR, Spiess PE. Sharma P Relationship Between Human Papillomavirus and Penile Cancer-Implications for Prevention and Treatment. *Transl Androl Urol* (2017) 6:791–802. doi: 10.21037/tau.2017.06.27
2. Sarier M, Ceyhan AM, Sepin N, Ozel E, Inal MM, Kukul E, et al. HPV Infection in Urology Practice. *Int Urol Nephrol* (2020) 52:1–8. doi: 10.1007/s11255-019-02302-2
3. De Martel C, Plummer M, Vignat J, Franceschi S. Worldwide Burden of Cancer Attributable to HPV by Site, Country and HPV Type. *Int J Cancer* (2017) 141:664–70. doi: 10.1002/ijc.30716
4. Guimaraes GC, Cunha IW, Soares FA, Lopes A, Torres J, Chaux A, et al. Penile Squamous Cell Carcinoma Clinicopathological Features, Nodal Metastasis and Outcome in 333 Cases. *J Urol* (2009) 182:528–34. doi: 10.1016/j.juro.2009.04.028
5. Cubilla AL, Lloveras B, Alejo M, Clavero O, Chaux A, Kasamatsu E, et al. The Basaloid Cell Is the Best Tissue Marker for Human Papillomavirus in Invasive Penile Squamous Cell Carcinoma: A Study of 202 Cases From Paraguay. *Am J Surg Pathol* (2010) 34:104–14. doi: 10.1097/PAS.0b013e3181c76a49
6. Fischer M, Uxa S, Stanko C, Magin TM, Engeland K. Human Papilloma Virus E7 Oncoprotein Abrogates the P53-P21-DREAM Pathway. *Sci Rep* (2017) 7:2603. doi: 10.1038/s41598-017-02831-9
7. Buitrago-Pérez A, Garaulet G, Vázquez-Carballo A, Paramio JM, García-Escudero R. Molecular Signature of HPV-Induced Carcinogenesis: pRb, P53 and Gene Expression Profiling. *Curr Genomics* (2009) 10:26–34. doi: 10.2174/138920209787581235
8. Tian X, Liu Z, Niu B, Zhang J, Tan TK, Lee SR, et al. E-Cadherin/β-Catenin Complex and the Epithelial Barrier. *J BioMed Biotechnol* (2011) 2011:567305. doi: 10.1155/2011/567305
9. Ripoli M, Barbano R, Balsamo T, Piccoli C, Brunetti V, Coco M, et al. Hypermethylated Levels of E-Cadherin Promoter in Huh-7 Cells Expressing the HCV Core Protein. *Virus Res* (2011) 160:74–81. doi: 10.1016/j.virusres.2011.05.014
10. Chaux A, Reuter V, Lezcano C, Velazquez EF, Torres J, Cubilla AL. Comparison of Morphologic Features and Outcome of Resected Recurrent and Nonrecurrent Squamous Cell Carcinoma of the Penis: A Study of 81 Cases. *Am J Surg Pathol* (2009) 33:1299–306. doi: 10.1097/PAS.0b013e3181a418ae
11. Chaux A, Pfannl R, Lloveras B, Alejo M, Clavero O, Lezcano C, et al. Distinctive Association of P16ink4a Overexpression With Penile Intraepithelial Neoplasia Depicting Warty and/or Basaloid Features: A Study of 141 Cases Evaluating a New Nomenclature. *Am J Surg Pathol* (2010) 34:385–92. doi: 10.1097/PAS.0b013e3181cdad23
12. Cubilla AL, Reuter VE, Velazquez E, Piris A, Saito S, Young RH. Histologic Classification of Penile Carcinoma and Its Relation to Outcome in 61 Patients With Primary Resection. *Int J Surg Pathol* (2001) 9:111–20. doi: 10.1177/106689690100900204
13. Cubilla AL, Velazques EF, Reuter VE, Oliva E, Mihm MCJr, Young RH. Warty (Condylomatous) Squamous Cell Carcinoma of the Penis: A Report of 11 Cases and Proposed Classification of 'Verruciform' Penile Tumors. *Am J Surg Pathol* (2000) 24:505–12. doi: 10.1097/00000478-200004000-00004
14. Christofori G. New Signals From the Invasive Front. *Nature* (2006) 441:444–50. doi: 10.1038/nature04872
15. Vincan E, Barker N. The Upstream Components of the Wnt Signalling Pathway in the Dynamic EMT and MET Associated With Colorectal Cancer Progression. *Clin Exp Metastasis* (2008) 25:657–63. doi: 10.1007/s10585-008-9156-4
16. Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S, et al. Epithelial-Mesenchymal Transition in Cancer Development and Its Clinical Significance. *Cancer Sci* (2010) 101:293–9. doi: 10.1111/j.1349-7006.2009.01419.x
17. Nguyen VHL, Hough R, Bernaudo S, Peng C. Wnt/β-Catenin Signalling in Ovarian Cancer: Insights Into Its Hyperactivation and Function in Tumorigenesis. *J Ovarian Res* (2019) 12:122. doi: 10.1186/s13048-019-0596-z
18. Kotsinas A, Evangelou K, Zacharatos P, Kittas C, Gorgoulis VG. Proliferation, But Not Apoptosis, Is Associated With Distinct Beta-Catenin Expression Patterns in Non-Small-Cell Lung Carcinomas: Relationship With Adenomatous Polyposis Coli and G(1)-To S-Phase Cell-Cycle Regulators. *Am J Pathol* (2002) 161:1619–34. doi: 10.1016/s0002-9440(10)64440-9
19. Tao J, Calvisi DF, Ranganathan S, Cigliano A, Zhou L, Singh S, et al. Activation of Beta-Catenin and Yap1 in Human Hepatoblastoma and

Induction of Hepatocarcinogenesis in Mice. *Gastroenterology* (2014) 147:690–701. doi: 10.1053/j.gastro.2014.05.004

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Does the Identification of a Minimum Number of Cases Correlate With Better Adherence to International Guidelines Regarding the Treatment of Penile Cancer? Survey Results of the European PROspective Penile Cancer Study (E-PROPS)

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Background: Penile cancer represents a rare malignant disease, whereby a small caseload is associated with the risk of inadequate treatment expertise. Thus, we hypothesized that strict guideline adherence might be considered a potential surrogate for treatment quality. This study investigated the influence of the annual hospital caseload on guideline adherence regarding treatment recommendations for penile cancer.

Methods: In a 2018 survey study, 681 urologists from 45 hospitals in four European countries were queried about six hypothetical case scenarios (CS): local treatment of the primary tumor pTis (CS1) and pT1b (CS2); lymph node surgery inguinal (CS3) and pelvic (CS4); and chemotherapy neoadjuvant (CS5) and adjuvant (CS6). Only the responses from 206 head and senior physicians, as decision makers, were evaluated. The answers were assessed based on the applicable European Association of Urology (EAU) guidelines regarding their correctness. The real hospital caseload was analyzed based on multivariate logistic regression models regarding its effect on guideline adherence.

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Results: The median annual hospital caseload was 6 (interquartile range (IQR) 3–9). Recommendations for CS1–6 were correct in 79%, 66%, 39%, 27%, 28%, and 28%, respectively. The probability of a guideline-adherent recommendation increased with each patient treated per year in a clinic for CS1, CS2, CS3, and CS6 by 16%, 7.8%, 7.2%, and 9.5%, respectively (each $p < 0.05$); CS4 and CS5 were not influenced by caseload. A caseload threshold with a higher guideline adherence for all endpoints could not be perceived. The type of hospital care (academic vs. non-academic) did not affect guideline adherence in any scenario.

Conclusions: Guideline adherence for most treatment recommendations increases with growing annual penile cancer caseload. Thus, the results of our study call for a stronger centralization of diagnosis and treatment strategies regarding penile cancer.

Keywords: penile neoplasms, guideline adherence, organ-sparing treatment, lymph node dissection, chemotherapy

INTRODUCTION

In Europe, the age-standardized incidence rate for penile cancer in 2018 was 0.9/100,000 (1). Penile cancer represents a rare malignant disease, whereby due to a low annual caseload, most centers have limited experience in the accurate management of such patients (2). Hence, it is difficult to conduct prospective randomized studies on penile cancer since the evidence supporting guideline recommendations and the research interest are scarce (3). However, an evaluation of guideline recommendations may help pool the currently reported expertise.

Guideline adherence in the treatment of penile cancer is low (4, 5). In an evaluation of the Swedish National penile cancer register from 2000 to 2012, Kirrander et al. found 71% guideline adherence for organ-preserving surgery and 50% for lymph node dissection (4). Moreover, according to the *Surveillance, Epidemiology, and End Results Program* from 1998 to 2015, guideline adherence for inguinal lymphadenectomy (ILND) did not reach 25% (5). Concurrently, several studies support the association between annual caseload and outcomes, suggesting that improved guideline adherence has a beneficial impact on the prognosis of penile cancer patients (6–11).

Regarding guideline-adherent ILND, Mistretta and colleagues demonstrated a 75% reduction in cancer-specific mortality and 58% in N1–3 stages (5). Furthermore, in a retrospective study involving 425 patients from 12 European and American centers, Cindolo et al. showed a 41% reduction in overall mortality and 49% when guidelines on primary tumor and lymph node management were strictly followed (12).

There has been an urge to centralize treatment of penile cancer in certified cancer centers despite the largely inconsistent results (13, 14). Kilsdonk et al. reported that centralized treatment in Great Britain since 2002 has resulted in a much higher uptake of organ-preserving surgery but not in an improvement of 1- and 5-year survival rates (15). Other studies have shown a favorable influence of treatment centralization on overall survival, though these effects are largely attributed to an adequate use of ILND and indication-specific perioperative chemotherapy (14, 16–18).

Reaching an established minimum caseload for specific tumor entities is an important criterion for certified cancer centers. To the best of our knowledge, no reliable data are available to date on the impact of caseload and treatment setting (academic vs. non-academic) on penile cancer guideline adherence. The purpose of this study was to examine the impact of hospitals' annual caseload of penile cancer patients on adherence to key clinical aspects of current guideline recommendations. A questionnaire-based compilation of fictitious treatment decisions was distributed among urological chief physicians and senior staff members to determine a potential minimum caseload in specialized penile cancer centers.

MATERIALS AND METHODS

Study Design, Participants, and Endpoints

The E-PROPS working group (European PROspective Penile Cancer Study) intends to collate the therapeutic procedures in penile cancer patients using three sequential modules. Module 1 involves data collection/evaluation of a questionnaire addressed to 681 urologists from 45 hospitals in Germany (n = 34), Austria (n = 8), Switzerland (n = 2), and Italy/South Tyrol (n = 1) in 2018. It contained 14 questions evaluating the position of respondents in their respective hospitals, their responsibility in treatment decisions, and their theoretical knowledge on surgery on primary tumors, inguinal/pelvic lymph node dissection, and perioperative chemotherapy in penile cancer patients. The following parameters of participating centers were additionally recorded: level of care (university hospital, maximum care hospital, specialized hospital, and primary care hospital), responsibility for penile cancer chemotherapy at the hospital (urology only, oncology only, or both), number of beds and staff, and the number of penile cancer patients treated in 2017.

The survey was established and analyzed in accordance with the STROBE criteria (19–22) and granted approval by the Institutional Review Board of the University of Regensburg.

Of 557 evaluable questionnaires, only those completed by chief physicians and senior staff members (n = 206) were selected

for analysis, assuming that this occupational group is largely responsible for all treatment decisions in penile cancer patients. Specifically, this involved decisions in six case scenarios (CS): local treatment of primary tumor stages pTis (CS1) and pT1b (CS2), indication for inguinal (CS3) and pelvic (CS4) lymphadenectomy, and neoadjuvant (CS5) and adjuvant (CS6) chemotherapy.

The endpoint in all statistical tests was a guideline-adherent recommendation for the prespecified CS, whereby the accuracy of the answers was assessed according to European Association of Urology (EAU) guidelines on penile cancer applicable at the time (23).

The real annual caseload of penile cancer patients treated in all participating institutions was reviewed in unadjusted and multivariate analyses to assess the potential influence on guideline adherence. The inclusion of penile cancer caseload reported per center for 2017 was continuous and dichotomized into 1–5 vs. >5, 1–7 vs. >7, 1–8 vs. >8, and 1–9 vs. >9 patients. Dichotomization was based on the number of cases reported and introduced to potentially obtain an idea of a threshold for an annual caseload.

Statistical Analysis

Metric variables are presented as medians and interquartile ranges (IQRs). The relationship between metric and categorical variables was investigated using Spearman's rank correlation, between categorical variables using the chi^2 test. The effect size of significant results is indicated by the rank correlation coefficient Rho, using the chi^2 test with Phi. In both cases, a value of 0.1 corresponds to a weak effect, 0.3 to a medium effect, and ≥0.5 to a strong effect (24).

The independent influence of the annual caseload on guideline adherence was analyzed using multivariate binary logistic regression models with stepwise backward variable selection, whereby the following independent variables were included into the regression models: level of care (university hospital yes/no), responsibility for chemotherapy (oncology alone vs. urology alone or in cooperation with oncology), number of staff and beds in the hospital (continuously coded), and providing the following relevant treatment options regarding penile cancer in the hospital (yes/no): radiotherapy, organ preservation, laser therapy, and annual penile cancer caseload of the hospital (continuously coded, as well as according to the abovementioned dichotomies).

A probability of error of <5% was accepted as a significant result in all tests ($p < 0.05$). All statistical analyses were performed using SPSS®V26 (IBM, Armonk, USA).

RESULTS

Descriptive Results

The overall response rate was 81.8%, with 557/681 questionnaires answered. In the study group considered here (n = 206), four (1.9%), 15 (7.3%), 40 (19%), and 147 (71%) of completed questionnaires came from hospitals in Italy/South Tyrol, Switzerland, Austria, and Germany, respectively. Answers from university (n = 99; 48%) and non-university hospitals (n = 107; 52%) were almost equally distributed. The participating clinics had 40 (IQR 33–53) beds with 16 (IQR 11–20) medical staff and treated six (IQR 3–9) penile cancer patients in 2017. Of the respondents, 182 (88%) performed penile cancer operations on their own. Radiation therapy, organ-preserving procedures, and laser therapy for penile cancer patients were available in 46%, 91%, and 82% of cases, respectively. Chemotherapy in penile cancer was performed by urology alone, urology and oncology together, or oncology alone in 35%, 21%, and 44% of cases, respectively.

University hospitals treated significantly more penile cancer patients per year than non-university hospitals, with seven (IQR 5–8) and five cases (IQR 2–9; $p < 0.0001$; Rho 0.269), respectively.

Figure 1 shows an overview of the proportion of guideline-adherent recommendations with regard to scenarios CS1–CS6.

Unadjusted Relationship Analysis Between Annual Penile Cancer Caseload and Guideline-Adherent Recommendation

With continuous inclusion of the caseload, recommended treatment only complies with guidelines for treatment of primary tumors in stages pTis (CS1) and pT1b (CS2), while the effect size (Spearman's Rho) tends to be low (**Table 1**).

With dichotomous inclusion of the annual caseload, a dichotomization of ≤8 vs. >8 cases seems to discriminate best between the chance of guideline-adherent and non-guideline-adherent recommendations, at least with regard to the therapy of the primary tumor. A higher caseload had a significant effect on CS1 and CS2, although the effect size (Phi) of <0.2 must be equally considered as low (**Table 1**).

Multivariate Relationship Analysis Between Annual Penile Cancer Caseload and Guideline-Adherent Recommendation

Table 2 shows the results of the multivariate analysis. Regarding the different CS, most often, an independent positive influence of the annual penile cancer caseload on the probability of a guideline-adherent recommendation for the treatment of the primary tumor in the pTis-stage (CS1) was observed.

Essentially, for four out of the six endpoints (CS1–3 and CS6), a significant influence of the annual caseload continuously included into the models on guideline-adherent recommendation was noted.

The dichotomized inclusion of caseloads presented a rather inconsistent picture: only a dichotomization at eight was able to predict two out of six endpoints. Respondents from clinics with an annual caseload >8 (compared to ≤8) made guideline-adherent decisions for surgical treatment of primary tumor

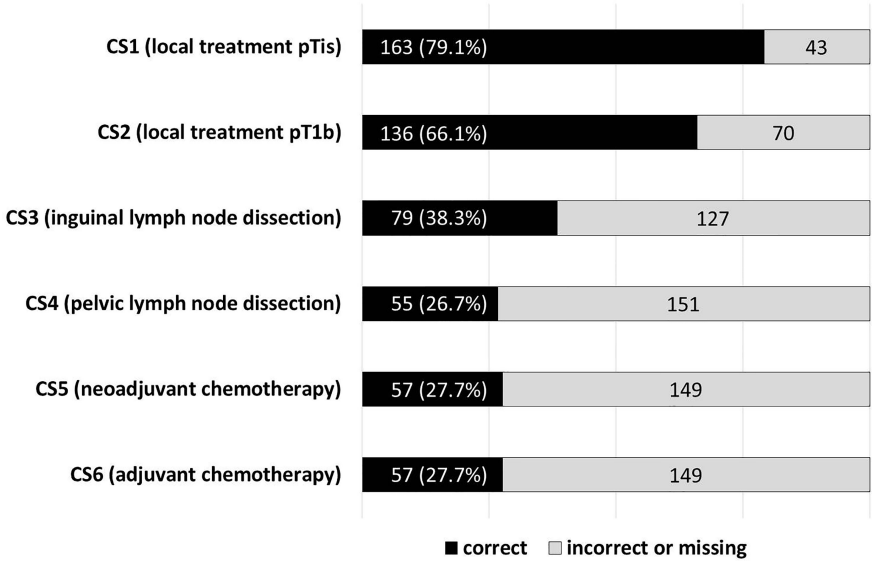

FIGURE 1 | Proportion of treatment recommendations in line with the guidelines in terms of scenarios CS1–CS6. CSn, case scenario.

stages pTis (CS1) and pT1b (CS2) at respectively 3.4 times (p = 0.031) and 2.7 times (p = 0.011) more frequently.

Regarding the indications for pelvic lymphadenectomy and neoadjuvant chemotherapy, an independent influence of the annual caseload on the probability of a guideline-adherent recommendation was not observed, neither for its continuous nor for its dichotomized inclusion (**Table 3**).

Unadjusted and Multivariate Relationship Analyses Between the Level of Care in the Hospital and Guideline-Adherent Treatment Recommendations

In all CS, the level of care (academic vs. non-academic) did not significantly influence the probability of a guideline-adherent treatment recommendation, neither in an unadjusted model nor in a multivariate model (**Table 3**).

DISCUSSION

Satisfactory functional and cosmetic results of primary tumor surgery, as well as lymph node management, are considered essential cornerstones for the quality of surgical treatment in penile cancer.

This study was designed to give an indication of the minimum annual case numbers of specialized penile cancer centers based on international survey results from hospitals in Germany, Austria, Switzerland, and Italy/South Tyrol by using treatment recommendations of clinical decision makers.The qualification of the respondents is shown exemplarily in the high proportion of surgeons (88%) who performed penile cancer procedures independently. In a comprehensive evaluation of 409 procedures for penile cancer (USA 1998–2013) by Matulewicz et al., this only applied to 4.1% (346/8,545) of urologists included (25).

TABLE 1 | Unadjusted relationship between number of cases and guideline-adherent treatment recommendation.

		Scenarios					
Inclusion		**CS1**	**CS2**	**CS3**	**CS4**	**CS5**	**CS6**
Continuous	*p*	0.001	0.016	n.s.	n.s.	n.s.	n.s.
	Rho	0.221	0.168				
Dicho ≤5 vs. >5	*p*	0.001	n.s.	n.s.	n.s.	n.s.	n.s.
	Phi	0.225					
Dicho ≤7 vs. >7	*p*	0.004	n.s.	n.s.	n.s.	n.s.	n.s.
	Phi	0.199					
Dicho ≤8 vs. >8	*p*	0.007	0.009	n.s.	n.s.	n.s.	n.s.
	Phi	0.188	0.181				
Dicho ≤9 vs. >9	*p*	0.025	n.s.	n.s.	n.s.	n.s.	n.s.
	Phi	0.156					

Significant results ($p < 0.05$) with indication of the effect size: Rho = effect size rank-sum correlation; Phi = effect size chi² test.
Inclusion, inclusion of case number; CSn, case scenario; n.s., test result not significant.

TABLE 2 | Multivariate relationship between number of cases and guideline-adherent treatment recommendation.

		Scenarios					
Inclusion		**CS1**	**CS2**	**CS3**	**CS4**	**CS5**	**CS6**
Continuous	*p*	0.003	0.023	0.023	n.s.	n.s.	0.012
	OR	1.16	1.08	1.07			1.10
	95% CI	1.05–1.28	1.01–1.15	1.01–1.14			1.02–1.18
Dicho ≤5 vs. >5	*p*	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.
	OR	3.80					
	95% CI	1.79–8.03					
Dicho ≤7 vs. >7	*p*	0.006	n.s.	n.s.	n.s.	n.s.	n.s.
	OR	3.21					
	95% CI	1.40–7.35					
Dicho ≤8 vs. >8	*p*	0.031	0.011	n.s.	n.s.	n.s.	n.s.
	OR	3.38	2.68				
	95% CI	1.12–10.2	1.25–5.74				
Dicho ≤9 vs. >9	*p*	n.s.	n.s.	0.018	n.s.	n.s.	n.s.
	OR			2.44			
	95% CI			1.17–5.10			

Significant results ($p < 0.05$) with odds ratio (OR) and 95% CI.
Inclusion, inclusion of case number; CSn, case scenario; n.s., test result not significant.

Despite this selection bias, the descriptive results (**Figure 1**) clearly show that four out of five respondents gave a guideline-adherent recommendation for the treatment of a pTis primary tumor, while in the case of a pT1b tumor, it was two out of three. In view of possibly considerable psychosocial implications of primary tumor treatment in penile cancer, this represents a worrisome finding (26). Although organ-sparing surgery carries an inherent increased risk of recurrence, they are not *per se* associated with compromised overall survival rates, thus providing a strong rationale for complying with guidelines to perform organ preservation when feasible (27).

Kirrander et al. showed a 5-year survival rate of 82% (95% CI 78%–85%) for penile cancer irrespective of tumor stage, which decreased to 46% (95% CI 36%–56%), particularly with the extent of nodal metastases. Nevertheless, guideline adherence recorded in this Swedish registry study on lymph node staging was only 50% in clinically normal inguinal lymph nodes in stages ≥pT1G2 (4). For neoadjuvant chemotherapy of patients with stage cN2-3 lymph node metastases, response rates of 43% (complete remission in 14%) were reported, with sustained remission in the adjuvant setting in a pN2-3 stage reported in 53% (median follow-up 42.6 months) (28–30). Particularly with regard to the prognosis-determining management of inguinal lymph nodes (including perioperative chemotherapy), recommendations that were not in adherence with guidelines predominated in our study. Based on these results, we believe that it is highly unlikely that a multimodal approach with adequate inclusion of neoadjuvant and/or adjuvant chemotherapy would be recommended in a N2-3 stage setting, where less than 30% of respondents actually gave a guideline-adherent treatment recommendation.

Based on the unadjusted and multivariate analyses of the correlation between the annual penile cancer caseload in a hospital and the likelihood of guideline-adherent recommendation, it was not possible to gain a reliable picture on a specific minimum number of cases. Although a statistically significant influence of caseload was shown in four out of six CS, with continuous inclusion (odds ratios of 1.07–1.16), every additional penile cancer patient treated per center translates into a 7%–16% increased chance of a guideline-adherent recommendation. At least regarding primary tumor treatment (pTis and pT1b), a clear increase in guideline-adherent recommendations was shown, where the annual caseload was ≥8 penile cancer patients. Although university hospitals treated significantly more penile cancer patients, the degree of care (academic vs. non-academic) demonstrated no influence on guideline-adherent recommendations.

Naturally, the results of a survey study set out here are not without important limitations. Even in a population-based sample area of 4 million and an annual minimum of 25 penile cancer patients, Kilsdonk et al. were unable to demonstrate a reliable effect on overall survival (15). In our own study group, 84% of respondents treated a maximum of 10 penile cancer patients annually in their hospital; another 14% treated 11 to 20, and only 2% treated 25 patients. None of the participating centers treated more than 25 penile cancer patients. In this respect, the number of patients in our study (n = 45 European hospitals, including 19 university hospitals) is probably too low to reliably demonstrate that guideline-adherent treatment recommendations correlate with case numbers. However, these caseloads do reflect a real-life scenario in countries where there is no legally underpinned centralization of penile cancer patients.

From a methodological point of view, regression models basically contain the risk of overfitting. Taking into account the relationship between predictors and number of events, step-by-step procedures, in particular the backward elimination, are a feasible way of counteracting overfitting.

In addition, we considered fictitious CS where each respondent was given individual recommendations; we did not consider treatments that were actually carried out. In daily clinical routine, it can be assumed that corresponding decisions are made by a number of qualified specialists or in an

TABLE 3 | Summary of the regression models on the influence of the annual caseload and other predictor variables on the probability of guideline-adherent treatment recommendations in the queried case scenarios.

Variable		Case scenario					
		CS1	CS2	CS3	CS4	CS5	CS6
Number of penile cancer patients treated in 2017 (cont.)	Elimination	No	No	No	Step 5	Step 5	No
	p	0 .003	0.023	0.023	n.a.	n.a.	0.012
	OR	1.16	1.08	1.07			1.10
	95% CI	1.05–1.28	1.01–1.15	1.01–1.14			1.02–1.18
Academic centers (vs. non-academic)	Elimination	Step 7	Step 3	Step 5	Step 2	Step 2	Step 6
	p	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	OR						
	95% CI						
In-house patient capacity per department (cont.)	Elimination	Step 6	Step 4	Step 3	Step 7	Step 4	Step 4
	p	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	OR						
	95% CI						
Number of urologists in the department (cont.)	Elimination	Step 4	Step 8	Step 2	Step 6	Step 6	No
	p	n.a.	n.a.	n.a.	n.a.	n.a.	0.055
	OR						0.95
	95% CI						0.90–1.01
Urologists performing chemotherapy for penile cancer patients (vs. urologists not performing chemotherapy)	Elimination	No	Step 7	Step 6	Step 8	No	Step 2
	p	0.103	n.a.	n.a.	n.a.	0.030	n.a.
	OR	0.55				2.06	
	95% CI	0.26–1.13				1.07–3.96	
Surgical organ-preserving treatments in penile cancer patients are provided (vs. no)	Elimination	Step 2	Step 2	No	Step 4	Step 7	Step 7
	p	n.a.	n.a.	0.016	n.a.	n.a.	n.a.
	OR			0.26			
	95% CI			0.09–0.78			
Local laser therapies in penile cancer patients are provided (vs. no)	Elimination	Step 5	Step 5	Step 4	Step 3	Step 3	Step 5
	p	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	OR						
	95% CI						
Local radiotherapies in penile cancer patients are provided (vs. no)	Elimination	Step 3	Step 6	No	No	No	Step 3
	p	n.a.	n.a.	0.089	0.064	0.028	n.a.
	OR			0.57	1.80	0.49	
	95% CI			0.30–1.09	0.97–3.37	0.25–0.92	

Green highlight = significant in the last step. Yellow highlight = no elimination, but insignificant in the last step. Orange highlight = elimination before the last step.
CSn, case scenario; OR, odds ratio; n.a., not applicable.

interdisciplinary tumor panel. Bearing this in mind, it might be further assumed that the proportion of guideline-adherent treatment decisions might be higher in reality.

Although it is hypothesized that the correlation between annual caseload, guideline-adherent treatment decisions, and functional as well as oncological outcomes might be confirmed in the case of penile cancer, solid and robust evidence underpinning the guidelines is somewhat limited (3, 14). The introduction of the EAU guidelines on penile cancer rightly points this out: "It must be emphasised that clinical guidelines present the best evidence available to the experts but following guideline recommendations will not necessarily result in the best outcome. Guidelines can never replace clinical expertise when making treatment decisions for individual patients, but rather help to focus decisions - also taking personal values and preferences/individual circumstances of patients into account. Guidelines are not mandates and do not purport to be a legal standard of care" (23).

CONCLUSIONS

With a median annual caseload of six penile cancer patients per hospital, an indication for a significant correlation between the number of cases and guideline-adherent treatment recommendations could be hypothesized. However, in significantly larger study groups, no clear and significant effect of treatment centralization on penile cancer patients' overall survival could be demonstrated, even in hospitals with a minimal annual caseload of 25.

Thus, the results of our study call for a stronger centralization of diagnosis and treatment strategies regarding penile cancer. This goal of course must not be compromised by possibly higher

costs of travelling to high-volume centers, nor by personal, institutional, or even material interests.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the University of Regensburg. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors participated in the conceptualization and writing of this paper, have seen and approved the final manuscript, and approved its submission.

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REFERENCES

1. European Cancer Information System(2020). Available at: https://ecis.jrc.ec.europa.eu.
2. Protzel C, Hakenberg OW. Penile Cancer: Diagnosis and Treatment. *Urol A* (2020) 59(2):209–18. doi: 10.1007/s00120-020-01128-6
3. Hakenberg OW, Dräger DL, Erbersdobler A, Naumann CM, Jünemann KP, Protzel C. The Diagnosis and Treatment of Penile Cancer. *Dtsch Arztebl Int* (2018) 115(39):646–52. doi: 10.3238/arztebl.2018.0646
4. Kirrander P, Sherif A, Friedrich B, Lambe M, Håkansson U, Steering Committee of the Swedish National Penile Cancer Register. Swedish National Penile Cancer Register: Incidence, Tumour Characteristics, Management and Survival. *BJU Int* (2016) 117(2):287–92. doi: 10.1111/bju.12993
5. Mistretta FA, Mazzone E, Palumbo C, Knipper S, Tian Z, Nazzani S, et al. Adherence to Guideline Recommendations for Lymph Node Dissection in Squamous Cell Carcinoma of the Penis: Effect on Survival and Complication Rates. *Urol Oncol* (2019) 37(9):578.e11–9. doi: 10.1016/j.urolonc.2019.05.024
6. Couapel JP, Bensalah K, Bernhard JC, Pignot G, Zini L, Lang H, et al. Is There a Volume-Outcome Relationship for Partial Nephrectomy? *World J Urol* (2014) 32(5):1323–9. doi: 10.1007/s00345-013-1213-1
7. Groeben C, Koch R, Baunacke M, Wirth MP, Huber J. High Volume Is the Key for Improving in-Hospital Outcomes After Radical Prostatectomy: A Total Population Analysis in Germany From 2006 to 2013. *World J Urol* (2017) 35(7):1045–53. doi: 10.1007/s00345-016-1982-4
8. Porter MP, Gore JL, Wright JL. Hospital Volume and 90-Day Mortality Risk After Radical Cystectomy: A Population-Based Cohort Study. *World J Urol* (2011) 29(1):73–7. doi: 10.1007/s00345-010-0626-3
9. Hemschemeier H. *Mindestmengen Im Krankenhaus - Bilanz Und Neustart* (2019). Available at: https://www.sciencemediacenter.de/alle-angebote/investigative/details/news/mindestmengen-im-krankenhaus-bilanz-und-neustart/.
10. Williams SB, et al. Impact of Centralizing Care for Genitourinary Malignancies to High-Volume Providers: A Systematic Review. *Eur Urol Oncol* (2019) 2(3):265–73. doi: 10.1016/j.euo.2018.10.006
11. Kamel MH. Should the Care of Penile Cancer be Confined to Centralized Centers of Excellence? *Eur Urol Focus* (2019) 5(5):735–6. doi: 10.1016/j.euf.2019.01.013
12. Cindolo L, Spiess PE, Bada M, Chipollini JJ, Nyirády P, Chiodini P, et al. Adherence to EAU Guidelines on Penile Cancer Translates Into Better Outcomes: A Multicenter International Study. *World J Urol* (2019) 37 (8):1649–57. doi: 10.1007/s00345-018-2549-3
13. Pettaway CA. Penile Cancer Management in the United States: Regional Centers of Expertise Are Needed! *Ann Surg Oncol* (2019) 26(4):928–9. doi: 10.1245/s10434-019-07178-1
14. Vanthoor J, Thomas A, Tsaur I, Albersen M. Making Surgery Safer by Centralization of Care: Impact of Case Load in Penile Cancer. *World J Urol* (2020) 38(6):1385–90. doi: 10.1007/s00345-019-02866-9
15. Kilsdonk MJ, Siesling S, van Dijk B, Wouters MW, van Harten WH. What Drives Centralisation in Cancer Care? *PloS One* (2018) 13(4):e0195673. doi: 10.1371/journal.pone.0195673
16. Ayres BE, Hounsome L, Alnajjar H, Sharma D, Verne J, Watkin NA, et al. 50 Has Centralisation of Penile Cancer Services in the United Kingdom Improved Survival? *Eur Urol Suppl* (2014) 13(1):e50. doi: 10.1016/S1569-9056(14)60052-X
17. Bayles AC, Sethia KK. The Impact of Improving Outcomes Guidance on the Management and Outcomes of Patients With Carcinoma of the Penis. *Ann R Coll Surg Engl* (2010) 92(1):44–5. doi: 10.1308/003588410X12518836439047
18. Bada M, Berardinelli F, Nyirády P, Varga J, Ditonno P, Battaglia M, et al. Adherence to the EAU Guidelines on Penile Cancer Treatment: European, Multicentre, Retrospective Study. *J Cancer Res Clin Oncol* (2019) 145(4):921–6. doi: 10.1007/s00432-019-02864-9
19. Distler FA, Pahernik S, Gakis G, Hutterer G, Lebentrau S, Rink M, et al. Adherence to the EAU Guideline Recommendations for Systemic Chemotherapy in Penile Cancer: Results of the E-PROPS Study Group Survey. *World J Urol* (2020) 38 (10):2523–30. doi: 10.1007/s00345-019-03052-7
20. Lebentrau S, Wolff I, Hempel MC, Haccius M, Kluth LA, Pycha A, et al. [Knowledge of German-Speaking Urologists Regarding the Association Between Penile Cancer and Human Papilloma Virus: Results of a Survey of the European PROspective Penile Cancer Study (E-PROPS)]. *Aktuelle Urol* (2019) 10.1055/a-1032-8086. doi: 10.1055/a-1032-8086
21. Suarez-Ibarrola R, Zengerling F, Haccius M, Lebentrau S, Schmid HP, Bier M, et al. Adherence to European Association of Urology and National Comprehensive Cancer Network Guidelines Criteria for Inguinal and Pelvic Lymph Node Dissection in Penile Cancer Patients-A Survey Assessment in German-Speaking Countries on Behalf of the European Prospective Penile Cancer Study Group. *Eur Urol Focus* (2021) 7(4):843–9. doi: 10.1016/j.euf.2020.02.005
22. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. *Int J Surg* (2014) 12(12):1495–9. doi: 10.1016/j.ijsu.2014.07.013
23. Hakenberg OW, Compérat EM, Minhas S, Necchi A, Protzel C, Watkin N, et al. EAU Guidelines on Penile Cancer: 2014 Update. *Eur Urol* (2015) 67 (1):142–50. doi: 10.1016/j.eururo.2014.10.017
24. Cohen J. *Statistical Power Analysis for the Behavioral Sciences. 2nd ed.* Hillsdale, N.J: L. Erlbaum Associates (1988). xxi, 567 pages.
25. Matulewicz RS, Flum AS, Helenowski I, Jovanovic B, Palis B, Bilimoria KY, et al. Centralization of Penile Cancer Management in the United States: A Combined Analysis of the American Board of Urology and National Cancer Data Base. *Urology* (2016) 90:82–8. doi: 10.1016/j.urology.2015.12.058
26. Dräger DL, Milerski S, Sievert KD, Hakenberg OW. Psychosocial Effects in Patients With Penile Cancer : A Systematic Review. *Urol A* (2018) 57(4):444–52. doi: 10.1007/s00120-018-0603-9
27. Kamel MH, Tao J, Su J, Khalil MI, Bissada NK, Schurhamer B, et al. Survival Outcomes of Organ Sparing Surgery, Partial Penectomy, and Total Penectomy in Pathological T1/T2 Penile Cancer: Report From the National Cancer Data Base. *Urol Oncol* (2018) 36(2):82.e7–82.e15. doi: 10.1016/j.urolonc.2017.10.017

28. Aziz A, Milerski S, Kernig K, Protzel C, Hakenberg OW. Lymph-Node-Positive Penile Cancer : Management and Systemic Therapy. *Urol A* (2018) 57 (4):440–3. doi: 10.1007/s00120-018-0601-y
29. Giannatempo P, Paganoni A, Sangalli L, Colecchia M, Piva L, Catanzaro M, et al. (2014). Survival Analyses of Adjuvant or Neoadjuvant Combination of a Taxane Plus Cisplatin and 5-Fluorouracil (T-PF) in Patients With Bulky Nodal Metastases From Squamous Cell Carcinoma of the Penis (PSCC): Results of a Single High-Volume Center. *J Clin Oncol* (2014) 32(4_suppl):377–377.
30. Pagliaro L, Williams D, Daliani D. Neoadjuvant Paclitaxel, Ifosfamide, and Cisplatin Chemotherapy for Metastatic Penile Cancer: A Phase II Study. *J Clin Oncol* (2010) 28:3851–7. doi: 10.1200/JCO.2010.29.5477

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Development and Validation of a Prognostic Nomogram for Predicting Cancer-Specific Survival in Patients With Lymph Node Positive Bladder Cancer: A Study Based on SEER Database

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Purpose: To construct a prognostic model to predict the cancer-specific survival (CSS) for bladder cancer patients with lymph node-positive.

Patients and Methods: We enrolled 2,050 patients diagnosed with lymph node-positive bladder cancer from the Surveillance Epidemiology and End Results (SEER) database (2004–2015). All patients were randomly split into development cohort (n = 1,438) and validation cohort (n = 612) at a ratio of 7:3. The univariate and multivariate Cox regression analysis were performed to identify prognostic factors. A nomogram predicting CSS was established based on the results of multivariate Cox analysis. Its performance was evaluated by calibration curves, the receiver operating characteristic (ROC) curves, and the concordance index (C-index). Internal verification was performed in the validation cohort. The Kaplan–Meier method with the log-rank test was applied in the different risk groups.

Results: The nomogram incorporated summary stage, tumor size, chemotherapy, regional nodes examined and positive lymph nodes. The C-index of the nomogram in the development cohort was 0.716 (0.707–0.725), while the value of the C-index was 0.691 (0.689–0.693) in the validation cohort. The AUC of the nomogram was 0.803 for 3-year and 0.854 for 5-year in the development cohort, while was 0.773 for 3-year and 0.809 for 5-year in the validation cohort. Calibration plots for 3-year and 5-year CSS showed good concordance. Significant differences were observed between high, medium, and low risk groups (P <0.001).

Conclusions: We have established a prognostic nomogram providing an accurate individualized probability of cancer-specific survival in bladder cancer patients with

lymph node-positive. The nomogram could contribute to patient counseling, follow-up scheduling, and selection of treatment.

Keywords: lymph node-positive, bladder cancer, SEER, prognosis, nomogram

INTRODUCTION

Bladder cancer (BC) is a common malignancy globally, with an estimated 500,000 new cases and 200,000 deaths worldwide in 2018 (1, 2). In addition, bladder cancer is also a severe and heterogeneous disease with a poor prognosis, especially for those patients with lymph node-positive (3). A retrospective study showed that approximately 25–30% of BC patients undergoing radical cystectomy presented with lymph node-positive after pathologic examination. Moreover, only a 25% disease-free survival rate was observed in these patients (4). Several retrospective studies had confirmed the poor prognosis of the higher recurrence and poorer survival rate in node-positive patients compared with those without (4–7). For example, a survey demonstrated that up to 70–80% of node-positive patients experienced disease recurrence, while this data was only 30% in patients with negative pathological nodes.

Over the course of the past years, some urologists were committed to stratifying patients with lymph node metastasis because a few studies suggested that a part of node-positive patients was still potentially curable (8). Jensen revealed better prognosis was observed in patients with a single node-positive compared with those patients with multiple (9). Meanwhile, a more prolonged overall survival (OS) and cancer-specific survival (CSS) were seen in patients staged N1 in comparison to patients with more extensive node involvement, according to the results of some retrospective studies (10). All these studies intended to meticulously stratify node-positive patients and pick out patients with better prognosis to take more suitable treatment. The eighth edition TNM system of the American Joint Committee on Cancer (AJCC), which divided node-positive patients into N1, N2, and N3 stages was used widely to simply evaluate the prognosis (2). However, lack of high accuracy and vital tumor characteristics like the number of positive nodes were its limitations. When compared with the conventional TNM system, a few studies suggested that the number of positive nodes seemed to be a more promising predictor of the outcome for node-positive BC patients (3, 11). Thus, it is imperative to build an exact model to evaluate the prognosis of BC patients with node-positive.

Nomogram is a visible and trustworthy statistical prediction tool, which was utilized widely to provide tailored individual prognostic information. Nomogram was composed of fundamental variables like demographics, tumor characteristics, and treatment features (12). Rink had constructed a nomogram that included gender, T stage, margin status, LN-density, and adjuvant chemotherapy to predict recurrence and cancer-specific survival for patients with a single lymph node metastasis (13). Meanwhile, a nomogram integrating multiple molecular markers was constructed to access disease recurrence and cancer-specific mortality for BC patients with locally advanced and node-positive (14). However, these models failed to obtain high accuracy (C-index 0.63 and 0.66 for Rink's model) and incorporate variables not easily available. Moreover, the models were not specially designed for all bladder cancer patients with positive lymph nodes. To our knowledge, it is the first study to construct a prognostic nomogram to predict cancer-specific survival (CSS) in all node-positive patients.

In our study, we searched patients with node-positive and collected all information available from the Surveillance, Epidemiology, and End Results (SEER) database from 2004 to 2015. We were committed to establishing a prognostic nomogram that incorporated significant factors to estimate the CSS and make direct decisions on treatment for those patients with node-positive. In addition, the performance of the nomogram was evaluated, and an assessment of applicability with internal verification was also performed in this study.

PATIENTS AND METHOD

Data Source and Patient Selection

All patients were collected from the Surveillance, Epidemiology, and End Results (SEER), which included particular patient demographic and cancer information of the US population. The following inclusion criteria were applied: (1) diagnosed from 2004 to 2014; (2) number of positive lymph nodes more than 1; (3) surgical approach to confirm positive lymph nodes: partial cystectomy, radical cystectomy and pelvic exenteration (Code 30,50,61,62,63,64,71,72,73,74,80). (4) Histology behavior: Transitional cell carcinoma. The exclusion criteria were as follows: (1) race unknown (n = 8); (2) grade unknown (n = 241); (3) Tx (n = 15); (4) Nx (n = 4); (5) chemotherapy unknown (n = 814); (6) tumor size unknown (n = 598); (7) marital status unknown (n = 78); (8) M1 and Mx stage (n = 317).

Variables Defined and End Point

The variables in the selected cohorts included: demographic characteristics (age, sex, race, marital status), tumor characteristics (tumor size, grade, histology, T stage, N stage, summary stage), treatment information (chemotherapy and radiotherapy), and other variables (regional nodes examined and positive lymph nodes). The prime endpoint in this study was cancer-specific mortality (CSM), which referred to the death of bladder cancer.

For conveniently analyzing, we had processed some variables in the SEER database. Some continuous variables, namely, age, tumor size, regional nodes examined and positive lymph nodes were transformed into categorical variables: age (<60, 60–70, 70–80, >80); tumor size (<3 cm, ≥3 cm); positive lymph nodes (1, 2–10, >10). Sex was divided into male and female, and race

included white, black, others which contained American, Indian, Alaska, Native, Asian, and Pacific Island. We defined marital status as married, separated, divorced or widowed (SDW), and single. Our study only was committed to the common histology with transitional cell carcinoma (TCC) and papillary transitional cell carcinoma (PTCC). Grades I and II were combined, considering the small sample size. According to the sixth edition of the AJCC stages, precise information on the TMN system was recorded in this study.

Statistical Analysis

We randomly split the study population into development and validation cohorts based on the ratio of 7:3. The Student's t-test and Chi-square test were performed for continuous and categorical variables, respectively, to explore the baseline characteristics of patients in the two groups. Categorical variables were presented as frequencies and their proportions, while continuous variables were the mean ± Standard Deviation (SD). In the development cohort, the univariate Cox regression analysis was applied to recognize potential significant prognostic factors. They were incorporated in the multiple Cox proportional hazards regression model when their *P*-value was under 0.05. All results were shown as hazards ratios (HR) and 95% confidence intervals (95%CI).

A nomogram incorporated the selected variables from the multiple Cox model, and the critical *P*-value was 0.05. the nomogram was built for visualized prediction of 3- and 5-year survival probability in the development cohort. We used Harrell's concordance-index (C-index) and the receiver operating characteristic (ROC) curves with the calculated area under the curve (AUC) to assess the performances of the model. Moreover, the consistency of predicted and actual outcomes of 3- and 5-year survival time was evaluated by the calibration plots, and it was performed with the package of *rms* in Rstudio. Patients in the development cohort were divided into three levels of risk group based on the total obtaining points. Meanwhile, the Kaplan–Meier method with the log-rank test was applied to analyze the differences of CSS between the three risk groups. SPSS 22.0 (IBM Corp, Armonk, NY) and R version 3.6.3 (https://cran.r-project.org/bin/windows/base/old/3.6.3) were utilized for all statistic analysis.

RESULTS

Characteristics of Study Population

Finally, 2,050 patients with lymph node-positive were enrolled in our study, and 1,438 patients (70%) were distributed into the development cohort while 612 patients (30%) into the validation cohort. Baseline demographical and clinicopathological characteristics of the study population are shown in **Table 1**. There were statistical differences between development and validation cohorts on the grade ($P = 0.013$), and patients in the development cohort tended to have a higher proportion of distant stage (43.9% vs 30.6%, $P < 0.001$). Statistical differences on other variables between the two groups were failed to observe. The 3- and 5-year CSS rates were 43.17% (n = 885) and 37.56% (n = 770) in total cohort, respectively, while 43.6% (n = 627) and 37.83% (n = 544) in the development cohort, respectively. The mean survival time was 34.16, 35.12, and 31.9 months in the total cohort, development cohort, and validation cohort, respectively.

Prognostic Factors of Node-Positive Patients in Development Cohort

Ultimately, five factors, namely, summary stage, tumor size, chemotherapy, regional nodes examined and positive lymph nodes were selected from the multivariate cox model. Five independent factors were determined as following: distant stage (HR = 3.927, 95%CI: 3.393–4.545, $P < 0.001$); tumor size >3 cm (HR = 1.240, 95%CI: 1.075–1.430, $P = 0.003$); receiving chemotherapy (HR = 0.684, 95%CI: 0.594–0.787, $P < 0.001$); regional nodes examined of 20–30 (HR = 0.784, 95%CI: 0.638–0.963, $P = 0.021$), >30 (HR = 0.673, 95%CI: 0.545–0.830, $P < 0.001$); positive lymph nodes of 2–10 (HR = 1.234, 95%CI: 1.018–1.496, $P = 0.032$), >10 (HR = 1.687, 95%CI: 1.219–2.336, $P = 0.002$) (**Table 2**).

Prognostic Nomogram for OS

A nomogram predicted the 3- and 5-year CSS of node-positive patients based on the Cox regression models (**Figure 1**). All variables in the nomogram were assigned a corresponding score of 0 to 100 based on the contribution to this nomogram (**Table 3**). Each patient could obtain a total score by adding scores in every subgroup. The nomogram revealed that the summary stage was the most significant contributor to the prognosis model of CSS.

Validation of the Nomogram

The C-index of this nomogram for CSS was 0.716 (0.707–0.725) in the development cohort, which was more significant than 0.605 of the TNM system ($P < 0.05$). Meanwhile, the discriminative ability of the nomogram was evaluated by ROC curves. The AUC of the nomogram was significantly higher than the TMN system both for 3-year (0.803 vs 0.675) and 5-year (0.854 vs 0.669) CSS prediction (all $P < 0.05$) (**Figures 3A, B**). The calibration plots of the development cohort for 3-year and 5-year all demonstrated good agreement between actual observations and predicted outcomes (**Figures 2A, B**) All these results suggested that better performance of our model in comparison to the traditional TNM system. In addition, internal verification of the nomogram was performed in the validation cohort to evaluate the applicability. The C-index of this nomogram was 0.691 (0.689–0.693), and AUC was 0.773 and also 0.809 for 3-year and 5-year, respectively (**Figures 3C, D**). The calibration curve of the validation cohort all gained good correlation between nomogram prediction and actual outcomes, especially for 5-year prediction. The results of internal validation suggested that this nomogram had satisfying applicability for node-positive patients (**Figures 2C, D**).

Survival Curve for Nomogram

All variables in the nomogram have authorized a score based on the contribution to the CSS, and we provided a corresponding score of 3-year and 5-year cancer-specific mortality probability,

TABLE 1 | Baseline demographical and clinicopathological characteristics of patients.

Characteristics	Total cohort *N* (%)	Development cohort *N* (%)	Validation cohort *N* (%)	*p*-value
Number of patients	2,050	1,438 (70%)	612 (30%)	
Median age (25th–75th percentile)	67.5 (62–77.5)	67.5 (62–77.5)	67.5 (62–77.5)	0.328
Mean age	67.85	67.68	68.25	0.253
Age				0.310
<60	463 (22.6%)	332 (23.1%)	131 (21.4%)	
60–70	683 (33.3%)	472 (32.8%)	211 (34.5%)	
70–80	595 (29.0%)	428 (29.8%)	167 (27.3%)	
>80	309 (15.1%)	206 (14.3%)	103 (16.8%)	
Sex				0.092
Female	544 (26.5%)	397 (27.6%)	147 (24.0%)	
male	1,506 (73.5%)	1,041 (72.4%)	465 (76.0%)	
Race				0.935
White	1,806 (88.1%)	1,265 (88.0%)	541 (88.4%)	
Black	137 (6.7%)	98 (6.8%)	39 (6.4%)	
others	107 (5.2%)	75 (5.2%)	32 (5.2%)	
Marital status				0.854
Married	1,275 (62.2%)	900 (62.6%)	375 (61.3%)	
SDW	524 (25.6%)	364 (25.3%)	160 (26.1%)	
Single	251 (12.2%)	174 (12.1%)	77 (12.6%)	
Histology				0.793
TCC	1,452 (70.8%)	1,021 (71.0%)	431 (70.4%)	
PTCC	598 (29.2%)	417 (29.0%)	181 (29.6%)	
Grade				0.013※
Grade I or II	31 (1.5%)	27 (1.9%)	4 (0.7%)	
Grade III	576 (28.1%)	422 (29.3%)	154 (25.2%)	
Grade IV	1,443 (70.4%)	989 (68.8%)	454 (74.2%)	
T stage				0.809
T1	25 (1.2%)	16 (1.1%)	9 (1.5%)	
T2	394 (19.2%)	281 (19.5%)	113 (18.5%)	
T3	1,046 (51.0%)	736 (51.2%)	310 (50.7%)	
T4	585 (28.5%)	405 (28.2)	180 (29.4%)	
N stage				0.834
N1	978 (47.7%)	680 (47.3%)	298 (48.7%)	
N2	1,033 (50.4%)	730 (50.8%)	303 (49.5%)	
N3	39 (1.9%)	28 (1.9%)	11 (1.8%)	
Summary Stage				<0.001※
Regional	1,232 (60.1%)	807 (56.1%)	425 (69.4%)	
Distant	818 (39.9%)	631 (43.9%)	187 (30.6%)	
Tumor size				0.216
<3 cm	670 (32.7%)	482 (33.5%)	188 (30.7%)	
>3 cm	1,380 (67.3%)	956 (66.5%)	424 (69.3%)	
Chemotherapy				0.411
No	823 (40.1%)	569 (39.6%)	254 (41.5%)	
Yes	1,227 (59.9%)	869 (60.4%)	358 (58.5%)	
Radiotherapy				0.391
No	1,930 (94.1%)	1,358 (94.4%)	572 (93.5%)	
Yes	120 (5.9%)	80 (5.6%)	40 (6.5%)	
Regional nodes examined				0.455
<10	603 (29.4%)	420 (29.2%)	183 (29.9%)	
10–20	784 (38.2%)	544 (37.8%)	240 (39.2%)	
20–30	348 (17.0%)	241 (16.8%)	107 (17.5%)	
>30	315 (15.4%)	233 (16.2%)	82 (13.4%)	
Positive lymph nodes				0.721
1	850 (41.5%)	588 (40.9%)	262 (42.8%)	
2–10	1,086 (53.0%)	769 (53.5%)	317 (51.8%)	
>10	114 (5.6%)	81 (5.6%)	33 (5.4%)	
Survival time(month)				
mean	34.16	35.12	31.9	0.056
median (25th–75th percentile)	20 (10–47)	20 (10–49)	18 (9–42)	0.051

Other race, American/Indian/Alaska/Native/Asian/Pacific Islands; SDW, separated, divorced or widowed; TCC, Transitional cell carcinoma; PTCC, papillary Transitional cell carcinoma.
※:Statistical significance.

TABLE 2 | Univariate and multivariate regression analyses for CSM.

Characteristics	Univariate analysis HR (95%CI)	*p*-value	Multivariate analysis HR (95%CI)	*p*-value
Age				
<60	Ref.		Ref.	
60–70	1.082 (0.917–1.277)	0.351	0.884 (0.737–1.060)	0.184
70–80	1.390 (1.178–1.640)	<0.001※	1.062 (0.884–1.277)	0.519
>80	1.815 (1.493–2.205)	<0.001※	1.140 (0.908–1.433)	0.259
Sex				
Female	Ref.			
male	1.112 (0.973–1.270)	0.119		
Race				
White	Ref.			
Black	1.113 (0.861–1.439)	0.412		
others	0.898 (0.671–1.201)	0.468		
Marital status				
Married	Ref.			
SDW	1.133 (0.973–1.319)	0.108		
Single	1.108 (0.908–1.350)	0.312		
Histology				
TCC	Ref.			
PTCC	0.868 (0.752–1.003)	0.055		
Grade				
Grade I or II	Ref.			
Grade III	0.862 (0.548–1.357)	0.522		
Grade IV	0.782 (0.501–1.220)	0.278		
T stage				
T1	Ref.		Ref.	
T2	1.306 (0.576–2.960)	0.523	0.879 (0.386–2.001)	0.759
T3	2.455 (1.097–5.494)	0.029※	1.377 (0.612–3.097)	0.440
T4	3.800 (1.693–8.531)	0.001※	1.718 (0.760–3.882)	0.193
N stage				
N1	Ref.		Ref.	
N2	1.397 (1.225–1.593)	<0.001※	1.056 (0.877–1.272)	0.563
N3	1.599 (1.020–2.506)	0.041※	1.066 (0.663–1.713)	0.792
Summary Stage				
Regional	Ref.		Ref.	
Distant	4.413 (3.827–5.091)	<0.001※	3.927 (3.393–4.545)	<0.001※
Tumor size				
<3 cm	Ref.		Ref.	
>3 cm	1.339 (1.165–1.539)	<0.001※	1.240 (1.075–1.430)	0.003※
Chemotherapy				
No	Ref.		Ref.	
Yes	0.705 (0.619–0.804)	<0.001※	0.684 (0.594–0.787)	<0.001※
Radiotherapy				
No	Ref.		Ref.	
Yes	1.378 (1.058–1.794)	0.017※	1.285 (0.981–1.684)	0.069
Regional nodes examined				
<10	Ref.			
10–20	0.918 (0.785–1.072)	0.279	0.916 (0.782–1.073)	0.279
20–30	0.782 (0.641–0.955)	0.016	0.784 (0.638–0.963)	0.021※
>30	0.684 (0.557–0.839)	<0.001※	0.673 (0.545–0.830)	<0.001※
Positive lymph nodes				
1				
2–10	1.456 (1.269–1.671)	<0.001※	1.234 (1.018–1.496)	0.032※
>10	2.014 (1.535–2.643)	<0.001※	1.687 (1.219–2.336)	0.002※

CSM, Cancer-specific mortality; Other race, American/Indian/Alaska/Native/Asian/Pacific Islands; SDW, separated, divorced or widowed; TCC, Transitional cell carcinoma, PTCC, papillary Transitional cell carcinoma.

※*:Statistical significance.*

respectively. The lymph node-positive patients were divided into three risk subgroups according to the total points obtained: Low risk group: >198; medium risk group: 148–198; high risk group: <148. As **Figure 4** showed, significant differences in CSS were observed between the three risk subgroups (P <0.001).

DISCUSSION

Lymph node-positive bladder cancer was considered as a severe stage associated with a high recurrence rate and mortality rate (8, 14). However, a part of patients with node metastasis still could

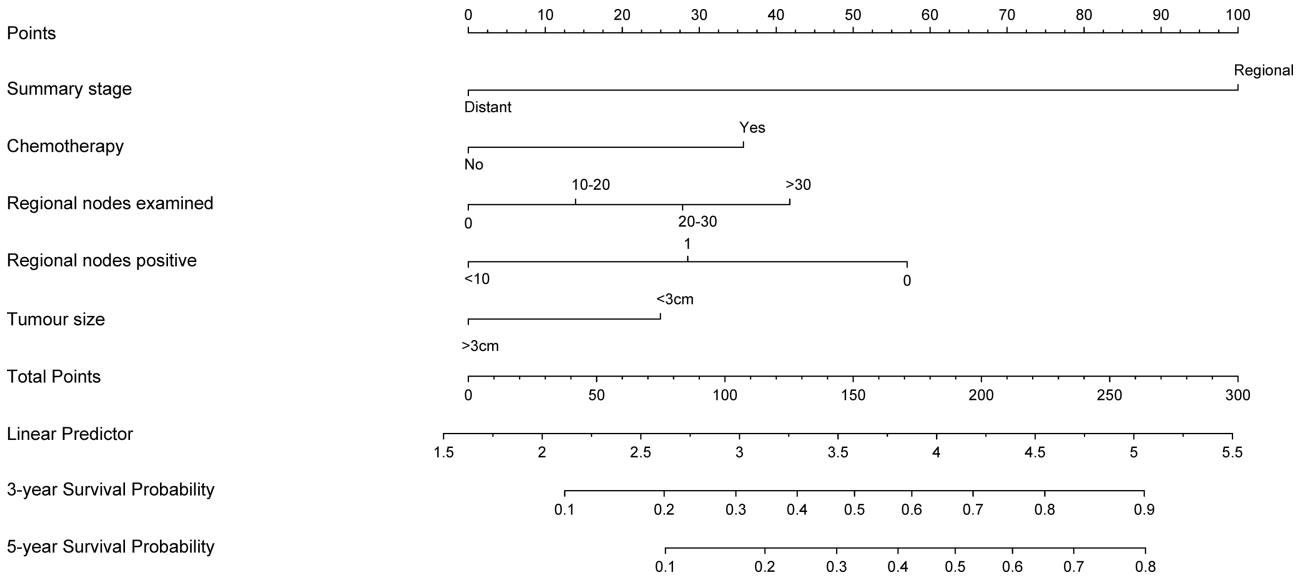

FIGURE 1 | Nomogram predicting 3- and 5-year cancer-specific survival probability for bladder cancer patients with lymph node-positive. use: locate patient values at each axis. Draw a vertical line to the "Point" axis to determine how many points are attributed for each variable value. Sum the points for all variables. Locate the sum on the "Total Points" line. Draw a vertical line towards the 3Yrs.Surv. Prob. and 5Yrs.Surv. Prob, Prob. axes to determine respectively the 3-, and 5-year survival probabilities.

be curable after active treatment (13). In addition, with the development of the treatment for bladder cancer patients, a lot of novel treatments such extend lymph node dissection, neoadjuvant chemotherapy, and targeted molecular therapy were proposed, and they acquired better prognosis possible for part node-positive patients (8, 14, 15). However, the prognostic stratification for patients with node-positive is still lacking. Therefore, it is urgent to establish an accurate and suitable predictive model for patients with lymph node metastasis.

This study comprehensively explored the effect of all factors available in the SEER database in CSS in node-positive patients. Meanwhile, we constructed and internally validated a relatively accurate and discriminating nomogram for the prediction of CSS by incorporating variables from the multivariate cox model. This

TABLE 3 | Nomogram scoring system.

Variables	Points	Variables	Points
Summary stage		Regional nodes positive	
Regional	100	1	94.04
Distant	0	2–10	47.02
Chemotherapy		3	0
No	0	Regional nodes examined	
Yes	65.52	<10	0
Tumor size		10–20	18.67
<3 cm	25	20–30	37.35
≥3 cm	0	>30	56.02
3-Year CSM probability	Points	5-Year CSM probability	Points
0.1	39	0.1	78
0.2	73	0.2	119
0.3	107	0.3	148
0.4	129	0.4	169
0.5	151	0.5	190
0.6	173	0.6	218
0.7	198	0.7	238
0.8	224	0.8	264
0.9	262	0.9	

SDW, separated, divorced or widowed; STBS, Systemic therapy before surgery; STAS, Systemic therapy after surgery; IST, Intraoperative systemic therapy; CSM, Cancer-specific mortality.

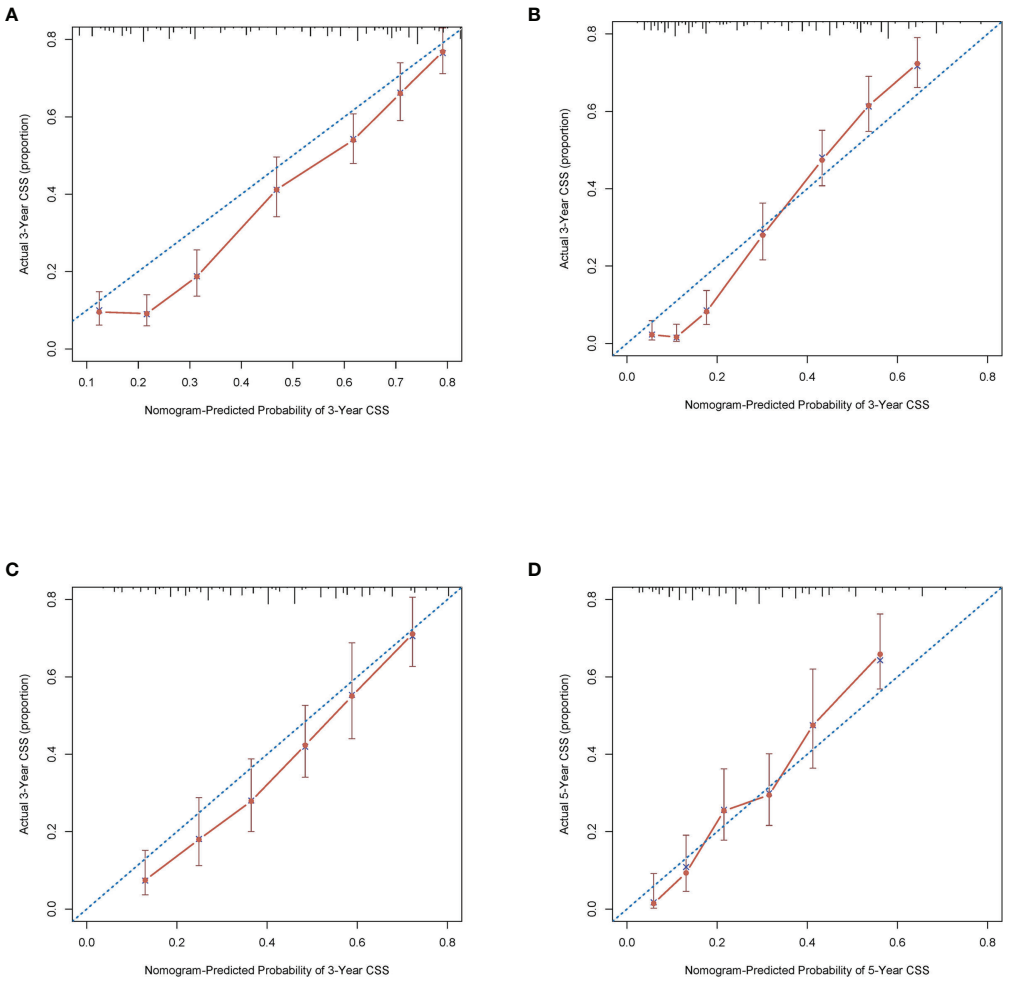

FIGURE 2 | Calibration plots of the nomogram describing 3- **(A)** and 5-year **(B)** CSS in the development cohort; 3- **(C)** and 5-year **(D)** CSS in the validation cohort.

approach produced a relatively easy and accurate tool, which only incorporated the significant variables associated with survival outcome but without sacrificing accuracy. The final survival nomogram yielded highly accurate prediction far exceeded the accuracy of individual predictors. In addition, the other advantage of nomogram over standard multivariate regression model was providing the individual probability of survival outcome at specific time points instead of a relative risk concept. Meanwhile, using Harrell's concordance index, which was a global measure of model accuracy to evaluate the accuracy of the nomogram, was also the advantage compared to conventional Cox regression models (12, 16–18). Furthermore, different levels of risk groups could be constructed based on the points of the nomogram, and individual patient counseling and follow-up scheduling were tailored for different risk groups (16).

We had compared our nomogram with the traditional AJCC TNM classification on clinical performance by the C-index and AUC. The results showed that our model obtained a greater C-index and AUC composed to the TNM system in the development cohort. Bruins et al. retrospectively enrolled 146 node-positive patients to evaluate the effect of the TNM system and failed to obtain differences on overall survival and disease-free survival (DFS) between patients staging N1–3 (19). Meanwhile, Jensen had constructed a nomogram based on 381 pN1 patients, namely, gender, T stage, margin status, LN density, and adjuvant chemotherapy. However, only focusing on pN1 patients and excluding patients with neoadjuvant chemotherapy limited its applicability in all node-positive patients. Moreover, the C-index of the model was 0.66 and 0.63, respectively, and it seemed to not be enough to satisfy the accuracy of the model (9). A nomogram in the combination of multiple molecular markers incorporating p53, pRB, p21, and p27 applied for predicting recurrence and cancer-specific survival (CSS) in pT3–4 or node-positive patients (14). Nevertheless, adding the molecular markers to the model failed to significantly improve the performance of outcome prediction (3.9% for recurrence, 4.3% for CSS) (20). Moreover, the application of molecular marker was still limited on account of ambiguously effect and expensive

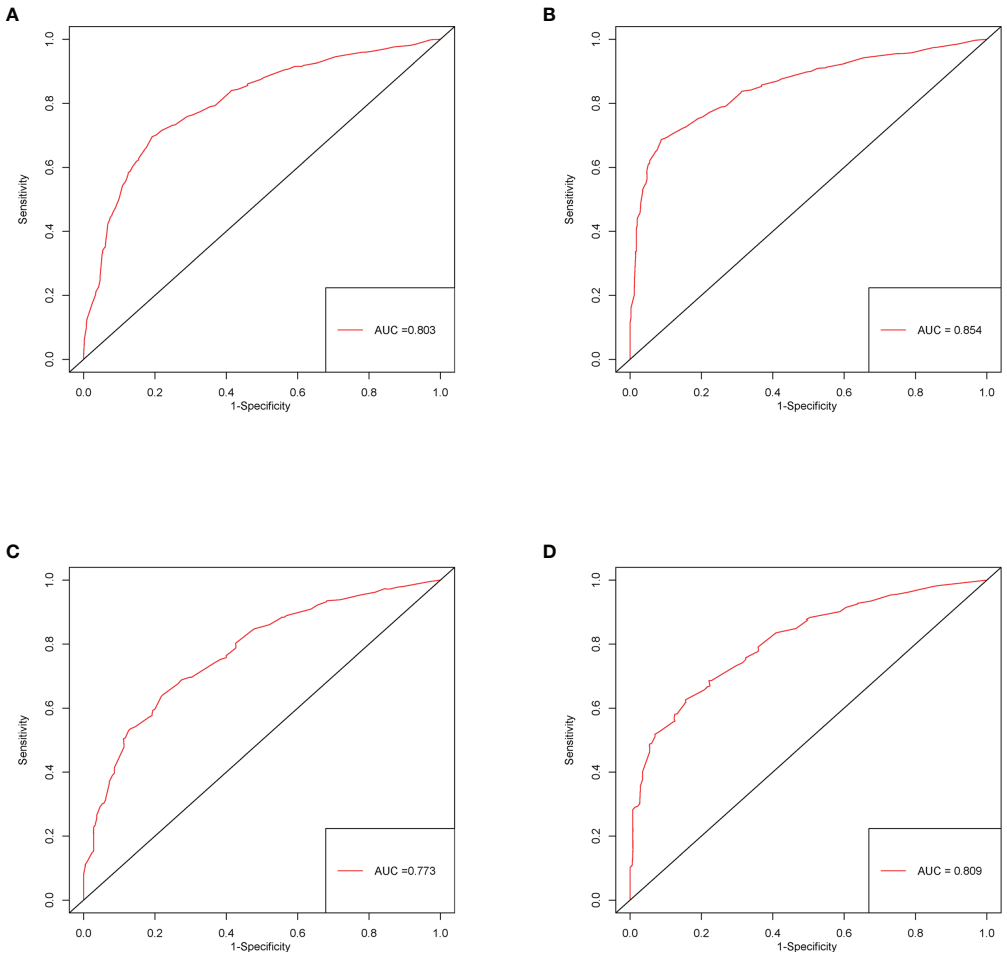

FIGURE 3 | ROC curves of the nomogram predicting 3-year **(A)** and 5-year **(B)** CSS in the development cohort; 3- **(C)** and 5-year **(D)** CSS in the validation cohort.

cost. The nomogram in this study had a great clinical performance in CSS prediction and variables incorporated relatively easily accessible in most hospitals. In detail, the good discriminative ability and accuracy of the nomogram were confirmed with the relatively high C-index and AUC of 3-year and 5-year in development and validation cohorts. The calibration curves also revealed a perfect consistency between the prediction of the nomogram and the actual outcome.

This novel nomogram for CSS probability prediction incorporated five factors, which included summary stage, regional nodes positive, tumor size, regional nodes examined, and chemotherapy. Studies suggested that a number of positive nodes seem to be a more promising predictor of outcome in node-positive patients than the conventional TNM system (3). In addition, some researchers found significant differences in disease outcome between patients with one and more nodes positive (9, 13, 21). Meanwhile, a retrospective study with 244 node-positive patients obtained the result that the 10-year disease-free survival rate in patients with eight or fewer positive nodes was significantly higher than those with greater than eight positive nodes. The degree of the number of positive nodes had been confirmed to strongly associate with prognosis in node-positive patients. Furthermore, receiving chemotherapy was shown as a protective factor for patients with node-positive. Several retrospective studies enrolling bladder cancer patients with node-positive observed higher overall survival and cancer-specific survival rates in patients with chemotherapy than those patients without (9, 15, 21). Therefore, chemotherapy might be a suitable and meaningful treatment for patients with node-positive.

Several significant advantages were worth noting in this study. First, it is the first study, up to our knowledge, to perform a prognostic nomogram for the prediction of CSS for all bladder cancer patients with lymph node-positive. Then, the number of patients in this study was relatively great and enough to construct a prognostic nomogram with good performance (n = 2,050). Finally, the variables in the nomogram were easily available in most hospitals, and the good applicability was obtained in our nomogram. Meanwhile, we divided study population into three risk groups based on the prognostic nomogram, and it was easier to detect patients with worse

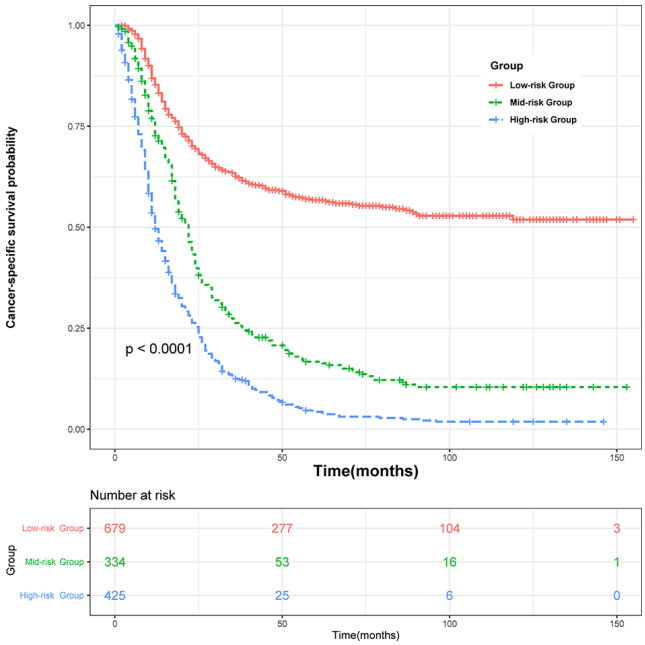

FIGURE 4 | Survival curves stratified by the score calculated by the nomogram. low-risk group (score >198); medium group (score 148–198); high-risk group (score <148).

survival outcomes. Nevertheless, some limitations in this study should be noticed. First of all, this is a retrospective study based on the SEER database, which means the results of this study were inevitably influenced by selection biases. In addition, we excluded patients with unknown variable information, and it was also a significant source of selection biases. Second, there were some limitations in the SEER database. Such as the SEER database collected massive information of patients from multiple regions and hospitals, and it seemed impossible to balance the differences in treatment and pathological evaluation standards. Moreover, some vital factors like drugs of chemotherapy and course of treatment of radiotherapy, which were also vital for node-positive patients, were lacking in the SEER database. Simultaneously, novel treatment such as target therapy is a growing field, and they need more research to verify the effect (8). Finally, although internal verification was performed in the validation cohort, the result of this verification method was not perfect because the patients in the development and validation came from the same database. Therefore, a large prospective clinical trial was demanded for external validation.

CONCLUSION

The study based on the SEER database revealed several demographics, lymph node characteristics, and therapeutic features, which were significantly associated with the cancer-specific survival of bladder cancer patients with lymph node-positive. A prognostic nomogram was constructed and validated to predict the individualized probability of cancer-specific survival at the time of 3- and 5-year. The nomogram could contribute to patient counseling, follow-up scheduling, and selection of treatment. Nonetheless, external and prospective validation was demanded for widely applying.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The data from SEER is publicly available and de-identified. This study was approved by the institutional. This study was approved by the institutional of the First Affiliated Hospital of Nanchang University.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

1. Lenis AT, Lec PM, Chamie K, Mshs MD. Bladder Cancer: A Review. *JAMA* (2020) 324(19):1980–91. doi: 10.1001/jama.2020.17598
2. Cornejo KM, Rice-Stitt T, Wu CL. Updates in Staging and Reporting of Genitourinary Malignancies. *Arch Pathol Lab Med* (2020) 144(3):305–19. doi: 10.5858/arpa.2019-0544-RA
3. Vanb S, Costello AJ, Vanp H. Prognosis of Node-Positive Bladder Cancer in 2016. *Minerva Urol Nefrol* (2016) 68(2):125–37.
4. Herr HW, Bochner BH, Dalbagni G, Donat SM, Reuter VE, Bajorin DF. Impact of the Number of Lymph Nodes Retrieved on Outcome in Patients With Muscle Invasive Bladder Cancer. *J Urol* (2002) 167(3):1295–8. doi: 10.1016/S0022-5347(05)65284-6
5. Lerner SP, Skinner DG, Lieskovsky G, Boyd SD, Groshen SL, Ziogas A, et al. The Rationale for En Bloc Pelvic Lymph Node Dissection for Bladder Cancer Patients With Nodal Metastases: Long-Term Results. *J Urol* (1993) 149 (4):758–64; discussion 64-5. doi: 10.1016/S0022-5347(17)36200-6
6. Skinner DG. Management of Invasive Bladder Cancer: A Meticulous Pelvic Node Dissection can Make a Difference. *J Urol* (1982) 128(1):34–6. doi: 10.1016/S0022-5347(17)52740-8
7. Abdollah F, Sun M, Schmitges J, Djahangirian O, Tian Z, Jeldres C, et al. Stage-Specific Impact of Pelvic Lymph Node Dissection on Survival in Patients With non-Metastatic Bladder Cancer Treated With Radical Cystectomy. *BJU Int* (2012) 109(8):1147–54. doi: 10.1111/j.1464-410X.2011.10482.x
8. Pedrosa JA, Koch MO, Cheng L. Lymph Node-Positive Bladder Cancer: Surgical, Pathologic, Molecular and Prognostic Aspects. *Expert Rev Anticancer Ther* (2013) 13(11):1281–95. doi: 10.1586/14737140.2013.850847
9. Jensen JB, Ulhøi BP, Jensen KM. Evaluation of Different Lymph Node (LN) Variables as Prognostic Markers in Patients Undergoing Radical Cystectomy and Extended LN Dissection to the Level of the Inferior Mesenteric Artery. *BJU Int* (2012) 109(3):388–93. doi: 10.1111/j.1464-410X.2011.10369.x
10. Tilki D, Brausi M, Colombo R, Evans CP, Fradet Y, Fritsche HM, et al. Lymphadenectomy for Bladder Cancer at the Time of Radical Cystectomy. *Eur Urol* (2013) 64(2):266–76. doi: 10.1016/j.eururo.2013.04.036
11. Pedrosa JA, Kaimakliotis HZ, Monn MF, Cary KC, Masterson TA, Rice KR, et al. Critical Analysis of the 2010 TNM Classification in Patients With Lymph Node-Positive Bladder Cancer: Influence of Lymph Node Disease Burden. *Urol Oncol* (2014) 32(7):1003–9. doi: 10.1016/j.urolonc.2014.04.002
12. Iasonos A, Schrag D, Raj GV, Panageas KS. How to Build and Interpret a Nomogram for Cancer Prognosis. *J Clin Oncol: Off J Am Soc Clin Oncol* (2008) 26(8):1364–70. doi: 10.1200/JCO.2007.12.9791
13. Rink M, Hansen J, Cha EK, Green DA, Babjuk M, Svatek RS, et al. Outcomes and Prognostic Factors in Patients With a Single Lymph Node Metastasis at Time of Radical Cystectomy. *BJU Int* (2013) 111(1):74–84. doi: 10.1111/j.1464-410X.2012.11356.x
14. Shariat SF, Chade DC, Karakiewicz PI, Ashfaq R, Isbarn H, Fradet Y, et al. Combination of Multiple Molecular Markers can Improve Prognostication in Patients With Locally Advanced and Lymph Node Positive Bladder Cancer. *J Urol* (2010) 183(1):68–75. doi: 10.1016/j.juro.2009.08.115
15. Lee EK, Herr HW, Dickstein RJ, Kassouf W, Munsell MF, Grossman HB, et al. Lymph Node Density for Patient Counselling About Prognosis and for Designing Clinical Trials of Adjuvant Therapies After Radical Cystectomy. *BJU Int* (2012) 110(11 Pt B):E590–5. doi: 10.1111/j.1464-410X.2012.11325.x
16. Shariat SF, Karakiewicz PI, Palapattu GS, Amiel GE, Lotan Y, Rogers CG, et al. Nomograms Provide Improved Accuracy for Predicting Survival After Radical Cystectomy. *Clin Cancer Res* (2006) 12(22):6663–76. doi: 10.1158/1078-0432.CCR-06-0372
17. Yang Z, Bai Y, Liu M, Hu X, Han P. Development and Validation of a Prognostic Nomogram for Predicting Cancer-Specific Survival After Radical Cystectomy in Patients With Bladder Cancer:A Population-Based Study. *Cancer Med* (2020) 9(24):9303–14. doi: 10.1002/cam4.3535
18. Bochner BH, Kattan MW, Vora KC. Postoperative Nomogram Predicting Risk of Recurrence After Radical Cystectomy for Bladder Cancer. *J Clin Oncol: Off J Am Soc Clin Oncol* (2006) 24(24):3967–72. doi: 10.1200/JCO.2005.05.3884
19. Bruins HM, Dorin RP, Rubino B, Miranda G, Cai J, Daneshmand S, et al. Critical Evaluation of the American Joint Committee on Cancer TNM Nodal Staging System in Patients With Lymph Node-Positive Disease After Radical Cystectomy. *Eur Urol* (2012) 62(4):671–6. doi: 10.1016/j.eururo.2012.04.050
20. Sanguedolce F, Cormio A, Bufo P, Carrieri G, Cormio L. Molecular Markers in Bladder Cancer: Novel Research Frontiers. *Crit Rev Clin Lab Sci* (2015) 52 (5):242–55. doi: 10.3109/10408363.2015.1033610
21. Horn T, Schmid SC, Seitz AK, Grab J, Wolf P, Haller B, et al. Clinical Prognosticators of Survival in Patients With Urothelial Carcinoma of the Bladder and Lymph Node Metastases After Cystectomy With Curative Intent. *World J Urol* (2015) 33(6):813–9. doi: 10.1007/s00345-014-1355-9

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CASE REPORT
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Efficacy and Biomarker Exploration of Sintilimab Combined With Chemotherapy in the Treatment of Advanced Penile Squamous Cell Carcinoma—A Report of Two Cases

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Penile squamous cell carcinoma is a rare malignant tumor of the male reproductive system. We report two cases of advanced penile squamous cell carcinoma with persistent partial response/complete response after sintilimab combined with chemotherapy and analyze the relevant tumor biomarkers.

Keywords: penile cancer, immune checkpoint inhibitors, biomarkers, tumor immune microenvironment, case report

INTRODUCTION

Penile cancer is considered a rare malignancy, with an incidence of penile squamous cell carcinoma in the United States ranging from 0.1 to 1 per 10,0000 (1). Squamous cell carcinoma is the most common histological type, and its pathogenesis is closely related to chronic human papillomavirus (HPV) infection. Immunotherapy has made progress in many malignant tumors, especially in squamous cell carcinomas, such as head and neck squamous cell carcinoma, lung squamous cell carcinoma, and esophageal squamous cell carcinoma. The predictors of efficacy in immunotherapy for these squamous cell carcinomas are unclear. The two most commonly used biomarkers are programmed death ligand-1 (PD-L1) expression and tumor mutation burden (TMB); however, neither PD-L1 expression nor TMB is a definite biomarker for immune therapy prediction in squamous cell carcinoma, as responses are also observed in PD-L1-negative and low TMB patients (2). In penile squamous cell carcinoma, the expression of PD-L1 has been reported to range from 40% to 62% (3). Studies have shown that PD-L1 expression is independent of patient age, tumor location, histological subtype, tumor stage, anatomical depth of tumor invasion, and tumor grade in patients with advanced penile squamous cell carcinoma (4). HPV-negative tumors have a significant proportion of PD-L1 expression compared with HPV-positive tumors (5). Tumor PD-L1 expression is significantly associated with lymph node metastasis. Tumors with diffuse positivity for PD-L1 show a higher probability of lymph node metastasis than tumors with a marginal expression of PD-L1 or tumors with negative expression of PD-L1, present a higher risk of disease-specific death (6, 7). High expression of PD-L1 is associated with short survival, so it can be used as a factor to identify a poor prognosis in penile cancer (3). TMB is defined as the total number of somatic/acquired mutations per coding area of a tumor genome; in penile cancer, TMB values between 3.6 and 4.5 mutations/Mb have been reported (5).

Immunotherapy for penile squamous cell carcinoma is currently under clinical study; some results showed that immunotherapy could effectively improve the disease control and prognosis (8, 9). Sintilimab is an immune checkpoint inhibitor that acts on programmed cell death-1 (PD-1). It has been confirmed to have a good efficacy in a variety of advanced tumors, such as lung cancer, esophageal cancer, and gastric cancer. Here, we report two patients with advanced penile squamous cell carcinoma who were administered chemotherapy combined with sintilimab and discuss associated tumor biomarkers.

CASE REPORTS

Patient A, a 63-year-old man, was diagnosed with well-differentiated squamous cell carcinoma of the penis in February 2014. He received partial penectomy without postoperative chemoradiotherapy. In December 2018, the patient presented a local recurrence of penile cancer and received a further partial penectomy. The postoperative pathology showed moderate to poorly differentiated squamous cell carcinoma of the penis invading the corpus cavernosum. The patient did not receive chemoradiotherapy after surgery. In November 2019, lung metastases were found, and the TNM stage of which was T3NxM0. From November 2019 to March 2020, the patient received paclitaxel 180 mg + nedaplatin 120 mg + sintilimab 200 mg for 6 cycles, and the treatment efficacy indicated a partial response. From April 2020 to March 2021, the patient was given maintenance immunotherapy with sintilimab 200 mg for 1 year. The sustained partial response was achieved during this period. The drug was subsequently discontinued due to a grade III immune-related rash. The patient was followed up regularly and currently maintains a persistent partial response.

Patient B, a 39-year-old man, underwent partial penectomy due to moderately differentiated squamous cell carcinoma of the penis in February 2016. The postoperative pathology revealed the tumor had invaded into the corpus cavernosum in the absence of chemoradiotherapy. In February 2017, the patient was found to have lymph node metastasis in the inguinal region, and the TNM stage of which was T3N3M0. He then received 60 GY/30 F three-dimensional modulated intensity radiotherapy for cavernous metastasis, bilateral inguinal, right pelvic enlarged lymph nodes, and corresponding lymphatic drainage area, and received concurrently, 2 cycles of TPF chemotherapy (docetaxel + cisplatin + tegafur). A partial response was achieved after 2 cycles, and 4 cycles of TPF chemotherapy were continued after the concurrent chemoradiotherapy. The patient achieved a progression-free survival of approximately 35.9 months after the first-line treatment. In March 2019, the patient's lymph nodes in the inguinal region of the right radiation field continued to expand, and the puncture confirmed metastatic squamous cell carcinoma. The patient received 2 cycles of second-line chemotherapy with nedaplatin + capecitabine, and in June 2019, the disease progressed, although multiple ulcers on the skin of the right abdominal region were observed, accompanied by pain and exudation. From June 2019 to September 2019, the patient was treated with a third-line regimen of sintilimab 200 mg + ifosfamide 2 g + estradiol 40 mg + mesna 0.4 g for 5 cycles. A partial response was evaluated after 2 cycles, and a complete response was achieved after 4 cycles. From November 2019 to July 2021, the patient received maintenance immunotherapy with sintilimab 200 mg. Currently, the patient has completed a 2-year treatment with sintilimab. Treatment has been stopped, and the patient is being followed up regularly. The evaluation shows a sustained complete response (**Figures 1**, **2**).

TUMOR BIOMARKER DETECTION

Immunohistochemical examinations were performed using surgically excised specimens obtained from 2 patients. Patient A was wild-type p53, which is established as a potent tumor suppressor; whereas, patient B was mutant p53, which is often

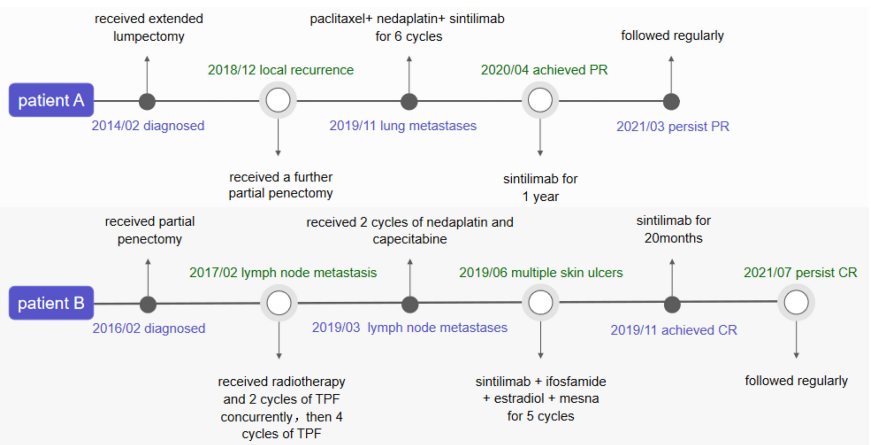

FIGURE 1 | Timeline of the two patients's therapy and effect of therapy.

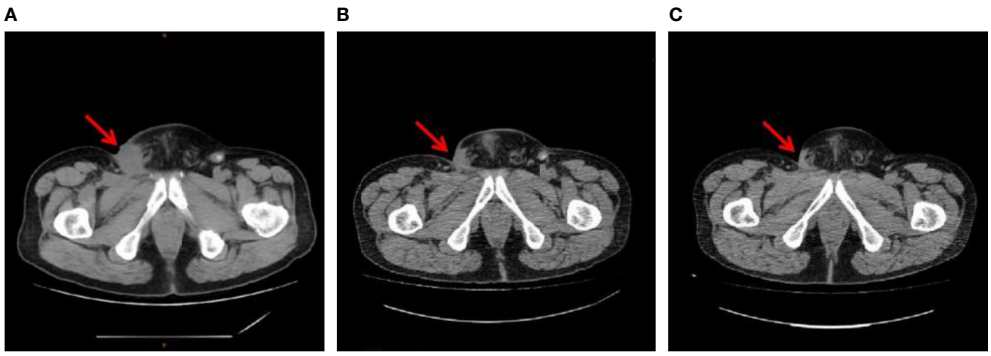

FIGURE 2 | Inguinal metastasis imaging of patient B **(A)** before sintilimab plus chemotherapy (June 13, 2019), **(B)** 6 months after sintilimab plus chemotherapy (December 20 2019), and **(C)** 2 months after the end of sintilimab maintenance therapy (September 08, 2021).

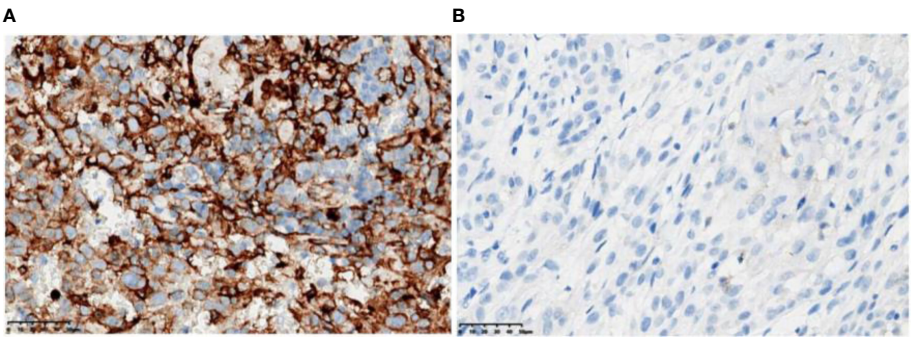

FIGURE 3 | PD-L1 immunohistochemistry test (antibody model: SP263). **(A)** Patient A has a positive PD-L1 immunohistochemistry with 50% to 60% PD-L1-positive tumor cells (TC) and 15% PD-L1-positive tumor-associated immune cells (IC). **(B)** Patient B was negative for PD-L1 immunohistochemistry with <1% TC and 1% IC.

associated with tumor malignancy and therapy resistance. Both patients were P16 negative, HPV negative, and proficient for mismatch repair proteins (MMR proficient). P16 has been used as a surrogate marker for active HPV infection (10). MMR proficient and microsatellite stable have been shown in clinical studies for colorectal cancer to be associated with limited immunotherapy efficacy (11).

The entire exon regions of 310 genes and the hotspot mutation regions of 210 genes were evaluated by probe hybridization and high-throughput sequencing in primary tumor tissue samples from the two patients. TMB values were calculated, and microsatellite instability was detected. The VENTANA PD-L1 (SP263) assay (Roche) (Ventana Medical Systems, Inc., Tucson, AZ, USA) was used to determine the PD-L1 expression. Multiplex fluorescence immunohistochemistry was used to detect the expression of CD8, CD3, PD-L1, and PD-1 in tumor tissue samples. Mantra System (PerkinElmer, Waltham, MA, USA) was used for imaging studies. InForm image analysis software (Version 2.4, PerkinElmer, Waltham, MA, USA) was used for the identification of tumor tissues and cells. The density and percentage of positive cells with different markers were calculated in the tumor parenchyma and stroma, respectively, to evaluate the tumor immune microenvironment (**Figure 3** and **Table 1**).

No genetic mutations of definite clinical significance were detected in the two patients, but 3 genetic mutations with potential clinical significance were found in patient A. Studies of relevant tumor biological markers for the two patients showed that both were HPV negative, but the expression levels of PD-L1 and TMB levels were very different. The reality is that both are effective for immunotherapy and show sustained partial response/complete response. By analyzing the tumor immune microenvironment (TIME), it is found that both TIME have high expressions of $CD3^+/CD8^+$ cells in tumor parenchyma and tumor stroma.

DISCUSSION

The TIME is a key target for immunotherapy in cancer patients. The expression of tumor-related immune markers to define the TIME can be divided into subtypes according to the expression levels of PD-L1 and the presence of tumor-infiltrating lymphocytes (TIL): immune neglect type ($B7^-H1^-/TIL^-$), adaptive immune

TABLE 1 | Detection of biomarkers in patients A and B.

Test items		Patient A	Patient B
Gene mutations with clinically significant		HRAS LRP1B TERT	Null
Microsatellite instability		Microsatellite stable	Microsatellite stable
TMB		17.95 mutations /Mb	0 mutations /Mb
PD - L1 expression			
PD-L1 positive tumor cells(TC)		50%-60%	<1%
PD-L1 positive tumor associated immune cells(IC)		15%	1%
Tumor Parenchyma			
$CD8^+$T cells	Density	121 cells/mm^2	201 cells/mm^2
	Positive rate	1.70%	3.28%
$CD3^+$T cells	Density	439 cells/mm^2	315 cells/mm^2
	Positive rate	6.19%	5.14%
$PD\text{-}L1^+$T cells	Density	1268 cells/mm^2	261 cells/mm^2
	Positive rate	17.88%	4.26%
$PD\text{-}1^+$T cells	Density	8 cells/mm^2	18 cells/mm^2
	Positive rate	0.12%	0.30%
Tumor Stroma			
$CD8^+$T cells	Density	837 cells/mm^2	801 cells/mm^2
	Positive rate	8.06%	11.14%
$CD3^+$T cells	Density	2599 cells/mm^2	1455 cells/mm^2
	Positive rate	25.02%	20.24%
$PD\text{-}L1^+$T cells	Density	1302 cells/mm^2	367 cells/mm^2
	Positive rate	12.53%	5.10%
$PD\text{-}1^+$T cells	Density	141 cells/mm^2	95 cells/mm^2
	Positive rate	1.36%	1.33%

tolerance type ($B7^-H1^+/TIL^+$), other pathway-dependent immune evasion types ($B7^-H1^-/TIL^+$), and primary induced expression type ($B7^-H1^+/TIL^-$) (12). Immunotherapy is most likely to be effective in the presence of TILs and B7-H1 expression. Little is known about the use of TIME in patients with penile cancer. In this study, it is found that the TIME of both is considered an adaptive immune tolerance type, which may provide explain the good efficacy of immunotherapy. We speculate that the presence of TILs in penile squamous cell carcinoma can be used as potent predictive biomarkers for the efficacy of immunotherapy, so TIME can be used to develop immunotherapeutic targets or evaluate whether immunotherapy is effective. These are just two cases, but since penile cancer is a rare tumor, further research can be carried out based on this study's result.

In head and neck squamous cell carcinoma, a higher degree of T-cell infiltration was found in HPV-positive tumors compared with HPV-negative tumors, and involved $CD8^+$ T cells within the tumor and stroma (13), with an increased rate of $CD8^+$ T-cell infiltration in the tumor-associated stroma being significantly associated with lymph node metastasis. HPV-positive tumors are characterized by a rich TIME, which has a better prognosis. Furthermore, patients with positive PD-L1 expression in tumor cells and higher levels of $CD8^+$ TILs presented a shorter cancer-specific survival (14). In patients with penile squamous cell carcinoma, the relationship between HPV infection status, PD-L1 expression, and the TIME warrants further investigation and may help predict immunotherapy efficacy and screening effective populations.

Currently, clinical studies are being conducted to evaluate the use of immune checkpoint inhibitors in advanced penile cancer, and the exploration of biomarkers is also a focus of intense research. Our findings relative to the detection of tumor molecular markers and successful treatment of two patients with penile squamous cell carcinoma may provide new insights.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Fuyang Cancer Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XM and YYZ took the lead in drafting the manuscript and provided tumor biomarker detection data. YRZ, JL, and CJ were involved in drafting the manuscript. YD and HQ provided supervision and participated in the literature review. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.823459/full#supplementary-material

REFERENCES

1. Thomas A, Necchi A, Muneer A, Tobias-Machado M, Tran ATH, Van Rompuy A-S, et al. Penile Cancer. *Nat Rev Dis Primers* (2021) 7:11. doi: 10.1038/s41572-021-00246-5
2. Zhu J, Zhang T, Li J, Lin J, Liang W, Huang W, et al. Association Between Tumor Mutation Burden (TMB) and Outcomes of Cancer Patients Treated With PD-1/PD-L1 Inhibitions: A Meta-Analysis. *Front Pharmacol* (2019) 10:673. doi: 10.3389/fphar.2019.00673
3. Davidsson S, Carlsson J, Giunchi F, Harlow A, Kirrander P, Rider J, et al. PD-L1 Expression in Men With Penile Cancer and its Association With Clinical Outcomes. *Eur Urol Oncol* (2019) 2:214–21. doi: 10.1016/j.euo.2018.07.010
4. De Bacco MW, Carvalhal GF, Macgregor B, Marçal JMB, Wagner MB, Sonpavde GP, et al. PD-L1 and P16 Expression in Penile Squamous Cell Carcinoma From an Endemic Region. *Clin Genitourin Cancer* (2020) 18: e254–e9. doi: 10.1016/j.clgc.2019.10.014
5. De Vries H-M, Ottenhof SR, Horenblas S, van der Heijden MS, Jordanova ES. Defining the Tumor Microenvironment of Penile Cancer by Means of the Cancer Immunogram. *Eur Urol Focus* (2019) 5:718–21. doi: 10.1016/j.euf.2019.02.019
6. Udager AM, Liu TY, Skala SL, Magers MJ, Mcdaniel AS, Spratt DE, et al. Frequent PD-L1 Expression in Primary and Metastatic Penile Squamous Cell Carcinoma: Potential Opportunities for Immunotherapeutic Approaches. *Ann Oncol: Off J Eur Soc Med Oncol* (2016) 27:1706–12. doi: 10.1093/annonc/mdw216
7. Chu C, Yao K, Lu J, Zhang Y, Chen K, Lu J, et al. Immunophenotypes Based on the Tumor Immune Microenvironment Allow for Unsupervised Penile Cancer Patient Stratification. *Cancers* (2020) 12:1796. doi: 10.3390/cancers12071796
8. Vanthoor J, Vos G, Albersen M. Penile Cancer: Potential Target for Immunotherapy? *World J Urol* (2021) 39:1405–11. doi: 10.1007/s00345-020-03510-7
9. Han H, An X, Yan R, Guo S, Xue C, Liu T, et al. Immune Checkpoint Inhibitor Plus Anti-EGFR Target Therapy Plus Chemotherapy in Patients With Locally Advanced Penile Squamous Cell Carcinoma. *J Clin Oncol* (2021) 39:e17016–e. doi: 10.1200/JCO.2021.39.15_suppl.e17016
10. Sand FL, Rasmussen CL, Frederiksen MH, Andersen KK, Kjaer SK. Prognostic Significance of HPV and P16 Status in Men Diagnosed With Penile Cancer: A Systematic Review and Meta-Analysis. *Cancer Epidemiol Biomarkers Prevention: Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol* (2018) 27:1123–32. doi: 10.1158/1055-9965.EPI-18-0322
11. Ciardiello D, Vitiello PP, Cardone C, Martini G, Troiani T, Martinelli E, et al. Immunotherapy of Colorectal Cancer: Challenges for Therapeutic Efficacy. *Cancer Treat Rev* (2019) 76:22–32. doi: 10.1016/j.ctrv.2019.04.003
12. Sanmamed MF, Chen L. A Paradigm Shift in Cancer Immunotherapy: From Enhancement to Normalization. *Cell* (2018) 175:313–26. doi: 10.1016/j.cell.2018.09.035
13. Solomon B, Young RJ, Rischin D. Head and Neck Squamous Cell Carcinoma: Genomics and Emerging Biomarkers for Immunomodulatory Cancer Treatments. *Semin Cancer Biol* (2018) 52:228–40. doi: 10.1016/j.semcancer.2018.01.008
14. Deng C, Li Z, Guo S, Chen P, Chen X, Zhou Q, et al. Tumor PD-L1 Expression Is Correlated With Increased TILs and Poor Prognosis in Penile Squamous Cell Carcinoma. *Oncoimmunology* (2017) 6:e1269047. doi: 10.1080/2162402X.2016.1269047

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ORIGINAL RESEARCH
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Radioisotope-Guided Sentinel Lymph Node Biopsy in Penile Cancer: A Long-Term Follow-Up Study

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Lymph node (LN) management is critical for survival in patients with penile cancer. However, radical inguinal lymphadenectomy carries a high risk of postoperative complications such as lymphedema, lymphocele, wound infection, and skin necrosis. The European Association of Urology guidelines therefore recommend invasive LN staging by modified inguinal lymphadenectomy or dynamic sentinel node biopsy (DSNB) in clinically node-negative patients (cN0) with intermediate- and high-risk tumors (≥ T1G2). However, the timing of DSNB (simultaneous vs. subsequent to partial or total penile resection) is controversial and the low incidence of penile cancer means that data on the long-term outcomes of DSNB are limited. The present study aimed to analyze the reliability and morbidity of DSNB in patients with penile cancer during long-term follow-up. This retrospective study included 41 patients (76 groins) who underwent radioisotope-guided DSNB simultaneously or secondarily after penile surgery from June 2004 to November 2018. In total, 193 sentinel LNs (SLNs) and 39 non-SLNs were removed. The median number of dissected LNs was 2.5 (interquartile range 2–4). Histopathological analysis showed that five of the 76 groins (6.6%) contained metastases. None of the non-SLNs were tumor-positive. In accordance with the guidelines, all inguinal regions with positive SLNs underwent secondary radical inguinal lymphadenectomy, which revealed three additional metastases in one groin. Regional LN recurrence was detected in three patients (four groins) during a median follow-up of 70 months, including two patients in whom DSNB had been performed secondarily after repetitive penile tumor resections. DSNB-related complications occurred in 15.8% of groins. Most complications were mild (Clavien–Dindo grade I; 50%) or moderate (II; 25%), and invasive intervention was only required in 3.9% of groins (IIIa: $n = 1$; IIIb: $n = 2$). In summary, this study suggests that the current radioisotope-guided DSNB procedure may reduce the complication rate of inguinal lymphadenectomy in patients with cN0 penile cancer. However, DSNB and penile surgery should be performed simultaneously to minimize the false-negative rate. Recent advances, such as new tracers and imaging techniques, may help to reduce the false-negative rate of DSNB further.

Keywords: penile cancer, sentinel lymph node, dynamic sentinel node biopsy, inguinal lymphadenectomy, lymph node metastases

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INTRODUCTION

Penile cancer is a rare disease with an overall incidence of less than one case in 100,000 persons worldwide (1). Lack of knowledge about the disease and the feeling of embarrassment often lead to a delay in diagnosis (2). The most important prognostic factor in patients with penile carcinoma is the presence of lymph node (LN) metastases (3, 4). Metastatic spread of penile cancer typically occurs in a stepwise fashion with the inguinal LNs affected first, followed by spread to the pelvic and distant LNs (5). An analysis of 944 patients with penile squamous cell carcinoma revealed that patients without nodal involvement had a 5-year cancer-specific survival rate of 90%, but this rate was considerably reduced to 56% in patients with LN metastases (6). Further studies showed that early "prophylactic" inguinal lymphadenectomy improved survival compared with delayed lymphadenectomy when metastases became clinically evident (7, 8). The management of regional LNs is thus essential in the treatment of penile cancer.

According to the European Association of Urology (EAU) guidelines on penile cancer, the management of LNs depends on the clinical LN status (9). Patients with palpable inguinal LNs are at high risk of lymphatic spread, and radical inguinal lymphadenectomy is therefore indicated in these patients. However, the optimal management of regional LNs in patients with clinically normal LNs (cN0) is more controversial. Approximately 20%–25% of these patients harbor occult LN metastases (10–12). Unfortunately, current imaging modalities, such as computed tomography, positron emission tomography/computed tomography, and magnetic resonance imaging cannot reliably detect micrometastases (13). Clinical surveillance of cN0 patients carries the risk of not detecting metastases until a later stage, with a negative effect on patient prognosis (7, 8). In contrast, radical inguinal lymphadenectomy is associated with a high rate of complications, such as skin-edge necrosis, wound infection, seroma, and lymphedema, and may result in overtreatment in 75%–80% of these patients (14, 15). The EAU guidelines thus recommend invasive LN staging by either modified inguinal lymphadenectomy or dynamic sentinel node biopsy (DSNB) for cN0 patients with intermediate- (pT1G2) or high-risk (≥ T1G3) tumors.

Modified inguinal lymphadenectomy aims to reduce the morbidity associated with radical inguinal lymphadenectomy by limiting the dissection area and preserving the saphenous vein (16–23). However, the false-negative rate of modified inguinal lymphadenectomy is unknown (9).

The concept of sentinel node biopsy was first described by Cabañas more than 40 years ago (24). This method relies on the principle that the first LNs on the direct drainage pathway of a tumor, referred to as the sentinel LNs (SLNs), will be the first sites of metastasis. Based on this assumption, a negative SLN biopsy indicates the absence of lymphatic spread and radical inguinal lymphadenectomy can thus be avoided. Using lymphangiography, Cabañas identified a LN at the anterior or medial aspect of the superficial epigastric vein as the SLN for the penis. However, consideration of this static model resulted in a large number of false-negative results (25–27). In 2000, the concept of DSNB was introduced in cases of penile cancer (28, 29). DSNB enabled the individual patient's SLNs to be identified by peritumoral injection of a radioactive tracer, preoperative lymphoscintigraphy, and intraoperative detection of radioactive LNs using a gamma probe. Continuous improvements of this method have reduced the complication and false-negative rates of DSNB to 5.7% and 4.8%, respectively (30). The reliability and morbidity of this technique have also been investigated in several other studies; however, most have included small patient numbers and reported highly variable complication and false-negative rates (31–37). The timing of DSNB is controversial. Two single-center studies suggested that DSNB was a reliable procedure for LN staging in cN0 patients after previous resection of the primary tumor (38, 39), while other authors observed regional recurrence after secondary, but not after primary DSNB, arguing against this hypothesis (40, 41).

We previously reported an initial experience of radioisotope-guided DSNB in patients with penile cancer in our center (42). A retrospective analysis of 32 patients with a median follow-up of 30.5 months revealed a complication rate of 11.1%, with no nodal recurrence. The present study aimed to update the outcomes of patients with penile cancer undergoing DSNB at our hospital, and to evaluate the reliability and morbidity of radioisotope-guided DSNB in a larger cohort with long-term follow-up.

MATERIALS AND METHODS

Patients

Fifty-three patients with intermediate- or high-risk penile cancer (≥ T1G2) underwent radioisotope-guided DSNB at the University Hospital for Urology in Oldenburg, Germany, between July 2004 and November 2018. All patients were informed about DSNB verbally and in writing and provided signed consent. Four patients were excluded from this study because they did not want to participate. Another eight patients were excluded because they could not be followed up for at least 2 years or until regional recurrence or death. None of these patients developed tumor recurrence during follow-up. A total of 41 patients were left for analysis (**Figure 1**). Pre-existing cardiovascular diseases in the patient cohort were chronic rheumatic heart diseases, hypertension, coronary heart disease, myocardial infarction, peripheral atrial disease, cerebrovascular disease, stroke, and atrial fibrillation.

Of the 41 patients included in this study, 38 patients were newly diagnosed with penile cancer and three patients presented with recurrent tumors of the penis. The histological subtypes of penile tumors were categorized according to the respective current World Health Organization classification. Thirty-eight patients underwent surgical treatment of the primary tumor at our hospital, while three patients were initially treated in another hospital and referred to us for DSNB. DSNB was either performed during surgery for the primary tumor (n = 24) or as a secondary procedure (n = 17). Thirty-five patients underwent bilateral DSNB, and the remaining six patients received unilateral DSNB and unilateral modified or radical inguinal lymphadenectomy due to ipsilateral suspicious LNs (n = 2), histologically confirmed LN metastases (n = 2), or non-visualization of SLNs during DSNB (n = 2) in the same operation.

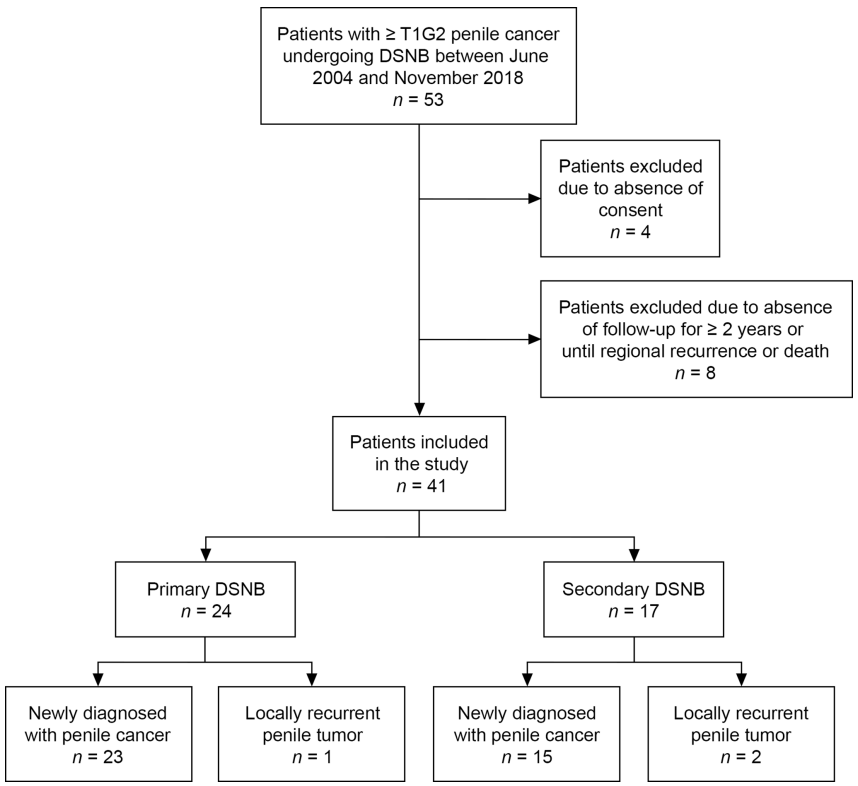

FIGURE 1 | Flowchart of included and excluded patients.

This study is registered in an international clinical trials register (Research Registry, researchregistry7492).

Dynamic Sentinel Node Biopsy and Surgical Treatment

All patients underwent preoperative ultrasonography of both inguinal regions. ^{99m}Technetium (^{99m}Tc) nanocolloid (radioactivity ca. 30 Mbq) was injected peritumorally or in a two-step procedure into the resection area approximately 4 hours before surgery. Preoperative visualization of SLNs was achieved by lymphoscintigraphy. SLNs were detected intraoperatively using a gamma probe (C-Trak System, Care Wise, Morgan Hill, CA, USA; Crystal Probe SG04, Crystal Photonics GmbH, Berlin, Germany). Non-SLNs directly adjoining SLNs were also removed if *in situ* separation was not possible. Intraoperative palpation of the wound was also performed to identify and dissect clinically suspicious LNs.

In accordance with the EAU guidelines, patients with at least one positive LN were offered secondary ipsilateral radical inguinal lymphadenectomy and patients with at least two positive LNs were offered additional ipsilateral pelvic lymphadenectomy.

Histopathological Examination

All dissected LNs were fixed in formalin, embedded in paraffin, and cut into 3-mm transverse sections. After deparaffinization and rehydration, 4- to 5-µm sections were stained with hematoxylin-eosin and examined by one of three pathologists with high experience in urological malignancies. If conventional histology was inconclusive, immunohistochemistry with a pancytokeratin antibody (AE1/AE3) was carried out using a DAKO Autostainer (Agilent Technologies, Santa Clara, CA, USA). In cases of false-negative DSNB results, SLNs were histopathologically reexamined by one pathologist.

Definitions of Tumor Recurrences and Follow-Up

Tumor recurrences were classified into local, regional and distant recurrences. Local recurrence was defined as recurrent disease on the penis, regional recurrence was defined as recurrent disease in inguinal and/or pelvic LNs, and distant recurrence was defined as recurrent disease in distant LNs or other organs.

Follow-up was performed by resident urologists on an outpatient basis. Control visits were carried out at 3-, 6-, or 12-month intervals. Local or regional recurrence was detected by physical examination of the penis and groins, with ultrasound, computed tomography, or magnetic resonance imaging if indicated. The time of follow-up was defined as the time from DSNB to the latest follow-up, regional recurrence, or death of the patient. DSNB-related complications were assessed by analyzing hospital and outpatient clinical records and questionnaires completed by the patients and urologists. All complications were categorized according to the Clavien–Dindo classification (43).

Analysis

DSNB was defined as a false-negative procedure if all SLNs were negative but non-SLNs were positive, or if regional recurrence occurred after a negative DSNB procedure without evidence of a new primary tumor or local recurrence. We calculated the false-negative rate according to the standard formula: false-negative rate = false-negative procedures/(true-positive procedures + false-negative procedures).

RESULTS

Patient Characteristics and Histopathological Analysis

This study included 41 patients with penile cancer who underwent radioisotope-guided DSNB. The patient and tumor characteristics, including potential risk factors for postoperative complications after inguinal lymphadenectomy, such as obesity (BMI > 25), diabetes mellitus and cardiovascular disease (15, 44, 45), are listed in **Table 1**. Among the 76 groins that received DSNB, a total of 193 SLNs and 39 non-SLNs were removed. The median number of dissected LNs (SLNs + non-SLNs) per groin was 2.5 (interquartile range 2–4). Two patients had radioactive LNs located in the pelvis that were not dissected because they were not accessible through the incision of inguinal DSNB and considered second echelon LNs.

Histopathological examination revealed that five of the 76 groins (6.6%) contained metastases. Three patients had unilateral and one patient had bilateral LN involvement. One patient with unilateral metastatic disease had two positive SLNs. Two of the four patients with LN metastases underwent primary DSNB and two underwent secondary DSNB. None of the non-SLNs harbored metastases. In accordance with the EAU guidelines, all groins with positive SLNs underwent secondary radical inguinal lymphadenectomy, which revealed three additional metastases in one patient with unilateral nodal involvement. This patient had a transient ischemic attack 1 month after radical inguinal lymphadenectomy and died a few months later, and no pelvic lymphadenectomy was therefore performed. None of the other patients showed further metastases at complementary radical inguinal lymphadenectomy. A summary of the histopathological findings is presented in **Table 2**. The histopathological results of the six groins that underwent radical or modified inguinal lymphadenectomy are given in **Table 3**.

TABLE 1 | Patient and tumor characteristics.

Characteristic	
Patients, *n*	41
Median age, years (interquartile range)	67 (61–73)
BMI, kg/m^2, *n*	
< 25	13
> 25	28
Diabetes mellitus, *n*	
Yes	5
No	36
Cardiovascular disease, *n*	
Yes	26
No	15
Previous inguinal surgery, *n*	
Yes	5
No	36
Histological type of penile cancer, *n*	
Squamous cell carcinoma, usual type (with or without verrucous areas)	38
Papillary squamous cell carcinoma	1
Mixed squamous cell carcinoma	1
Papillary-basaloid carcinoma (HPV-related)	1
Tumor stage, *n*	
pT1G2	23
pT1G3	3
pT2G1	1
pT2G2	11
pT3G2	1
pT3G3	2
Surgical treatment of primary tumor, *n*	
Circumcision	4
Local excision at the penis shaft	2
Glansectomy with or without circumcision	16
Partial penectomy	19

False-Negative Procedures and False-Negative Rate

The median follow-up was 70 (range 6–158, interquartile range 36–96) months. In total, three patients with bilateral negative DSNB developed regional recurrence. One patient underwent local tumor excision (R1 resection) and partial penectomy (R0 resection) before referral to our hospital for DSNB. Bilateral inguinal metastases and systemic metastatic disease were detected 32 months after DSNB and the patient died of penile cancer 4 months later. Another patient underwent partial penectomy simultaneously with DSNB. However, due to inaccessibility, a radioactive pelvic LN remained and the patient was diagnosed with left-sided inguinal and iliac LN metastases and pulmonary metastatic disease 7 months after DSNB. A third patient underwent radical circumcision and circular re-resection of the penile shaft skin in another hospital. He presented with an enlarged LN in the right groin 12 months after negative DSNB. This LN was dissected 2 months later and histopathological analysis revealed metastasis. The patient declined complementary radical inguinal lymphadenectomy because of the high morbidity risk, but he remained alive without evidence of disease at 13 months. The clinical and pathological characteristics of the false-negative patients are

TABLE 2 | Histopathological results of DSNB.

Tumor stage	pN0 (*n* = 37)	pN+ inguinal (*n* = 4)	pN+ pelvic (*n* = 0)
pT1G2	21	2	0
pT1G3	3	0	0
pT2G1	1	0	0
pT2G2	10	1*	0
pT3G2	1	0	0
pT3G3	1	1	0

**Patient with bilateral LN metastases.*

TABLE 3 | Histopathological results of unilateral modified or radical inguinal lymphadenectomy in six patients.

Patient No.	Tumor stage	Type of lymphadenectomy	Groin	Reason	Number of dissected LNs	Number of positive LNs	LN status
2	pT3G3	Radical inguinal	Right	Suspicious LNs	3	0	pN0
25	pT1G3	Radical inguinal (+ pelvic)	Left	LN metastasis	5 (+ 11)	2 (+ 3)	pN+ inguinal (+ pelvic)
29	pT2G2	Modified inguinal	Left	Non-visualization	5	0	pN0
35	pT2G2	Radical inguinal (+ pelvic)	Left	Suspicious LNs	8 (+ 3)	2 (+ 0)	pN+ inguinal
38	pT1G2	Modified + radical inguinal	Left	Non-visualization	0 + 12	0 + 0	pN0
41	pT1G3	Radical inguinal	Right	LN metastasis	8	1	pN+ inguinal

shown in **Table 4**. None of these patients had locally recurrent penile tumors or previous inguinal surgeries.

In summary, we encountered four true-positive and three false-negative patients (false-negative rate of 42.9%). However, two of the three false-negative patients had repetitive penile tumor resections prior to DSNB.

Histopathological Re-Evaluation of SLNs in False-Negative Cases

Histopathological reexamination of the SLNs from the false-negative groins revealed normal lymphatic tissue. In the third false-negative patient (regional recurrence on the right side), one previously undetected micrometastasis (2 mm) was found in an SLN from the left groin. The results of the histopathological reexamination are summarized in **Table 5**.

Follow-Up

During follow-up, four patients developed distant recurrences. Two of these patients had false-negative DSNB results (**Table 4**, patients 17 and 39). The third patient underwent unilateral DSNB (right groin, pN0) and unilateral radical inguinal and pelvic lymphadenectomy due to histologically confirmed lymph node metastasis (left groin) (**Table 3**, patient 25). 10 months after DSNB and radical inguinal lymphadenectomy, he presented with distant metastases. However, he did not show recurrent disease in inguinal or pelvic LNs of the right side and was therefore not classified false-negative. The fourth patient received unilateral DSNB (right groin, pN0) and unilateral modified inguinal lymphadenectomy due to non-visualization of SLNs (left groin, pN0) (**Table 3**, patient 29). 14 months later, he was diagnosed with retroperitoneal metastasis on the left side. The modified inguinal lymphadenectomy procedure was therefore considered false-negative.

Five other patients presented with local relapse and underwent further surgery (glansectomy or partial penectomy). One of these patients received a second bilateral DSNB, which did not reveal metastases. However, this patient was lost to follow-up after the hospital stay. Another patient with local recurrence was subsequently diagnosed with LN metastasis in the right groin and underwent radiotherapy.

Nine patients died during follow-up. The median follow-up of these patients was 33 (range 6-132, interquartile range 17–55) months. Two of the patients with distant metastases died of penile cancer (**Tables 3**, **4**, patients 17 and 25), one patient with systemic metastatic disease died 1 day after retroperitoneal tumor extirpation (R2 resection) due to pre-existing conditions (**Table 3**, patient 29), three patients died from causes unrelated to penile cancer (advanced lung cancer, renal cell carcinoma, pulmonary emphysema), and three other patients died of unknown causes.

Complications

Postoperative complications after DSNB occurred in 12 groins, with a morbidity rate of 15.8% per inguinal region. Most complications were mild or moderate and non-invasive or invasive intervention was only required in six groins (7.9%). No patient died from complications. The DSNB-related complications graded according to Clavien–Dindo are shown in **Table 6**.

DISCUSSION

In this retrospective study, we investigated the reliability and morbidity of radioisotope-guided DSNB in a cohort of patients with penile cancer who underwent long-term follow-up in a tertiary referral hospital. This study represents the largest German series of penile cancer patients treated with DSNB to date. Notably, unlike other European countries, the treatment of penile cancer in Germany is not centralized. The current analysis revealed that DSNB was associated with a low complication rate of 15.8%. In total, we encountered four true-positive and three false-negative patients in our cohort of 41 patients; however, two of the three false-negative patients underwent repetitive penile tumor resections prior to DSNB.

The optimal management of regional LNs in cN0 patients with penile cancer has been controversial for many years. Clinical surveillance carries the risk of detecting metastases at a later stage, thereby compromising the oncological outcome (7, 8), whereas radical inguinal lymphadenectomy is associated with high morbidity and may result in overtreatment in 75%–80% of patients (14, 15). To reduce the morbidity associated with inguinal lymphadenectomy, the EAU guidelines recommend invasive LN staging by modified inguinal lymphadenectomy or DSNB in cN0 patients with ≥ T1G2 tumors (9).

In the present study, we reported a complication rate of 15.8% for radioisotope-guided DSNB, which was considerably lower than most of the published contemporary complication rates for radical inguinal lymphadenectomy ranging between 49% and 58% (14, 15, 46). Only one study by Koifman et al. revealed a lower complication rate of 10.3% (47). DSNB thus seems to be a suitable procedure for decreasing the morbidity risk in patients with cN0 penile cancer.

TABLE 4 | Clinical and pathological characteristics of patients with false-negative DSNB results.

Patient No.	Age	Tumor stage	Surgical treatment of primary tumor	Timing of DSNB	Dissected LNs		LN status	Regional recurrence	Time to regional recurrence (months)	Distant recurrence	Status (time after recurrence, months)
					Left groin	Right groin					
17	81	pT2G2	Local excision + partial penectomy	Secondary	1 SLN + 1 non-SLN	1 SLN	pN0	Bilateral	32	Yes	Died of penile cancer (4)
39	66	pT3G2	Partial penectomy	Primary	1 SLN + 1 non-SLN	2 SLNs	pN0	Left groin	7	Yes	Unknown (0)
40	51	pT2G2	Radical circumcision + re-resection of penile shaft skin	Secondary	2 SLNs	1 SLN	pN0	Right groin	12	No	Alive without evidence of disease (13)

TABLE 5 | Results of histopathological reexamination of SLNs from false-negative patients.

Patient No.	False-negative groin	Metastasis	
		Left groin	Right groin
17	Left + right	No	No
39	Left	No	No
40	Right	Yes (micrometastasis, 2 mm)	No

TABLE 6 | DSNB-related complications.

Complication	No. of DSNB procedures (n = 76)	Clavien–Dindo classification, grade
Lymphocele/seroma (no intervention)	4	I
Hematoma (no intervention)	2	I
Wound infection requiring antibiotics	3	II
Lymphocele requiring drainage	1	IIIa
Wound infection requiring revision operation	2	IIIb
Total (%)	12 (15.8%)	

Compared to the complication rate of 10–45% for modified inguinal lymphadenectomy, our complication rate for DSNB was similar (19, 21, 48). Previous studies showed high variability in complication rates for DSNB. A two-center study of 323 patients from the Netherlands and England found a morbidity rate of 4.7%, with most of the complications being transient and managed conservatively (49). Lam et al. found DSNB-related complications in 20 of 264 patients (7.6%), including lymphocele, wound infection, hematoma, penoscrotal lymphedema, and wound bleeding (35). In contrast, Dimopoulos et al. reported a higher overall morbidity rate of 21.4%, although, similar to the current study, most of the complications were categorized as Clavien-Dindo grade I–II (36). The apparently large variability in morbidity rates may be due to underreporting or differences in the definitions of complications (e.g., exclusion of complications without intervention). Although DSNB may avoid overtreatment in patients with penile cancer, it carries the risk of false-negative results, and a delayed detection of LN metastases may have a negative effect on patient survival (7, 8).

Several studies have investigated the reliability of DSNB in patients with penile cancer. The Netherlands Cancer Institute, which introduced DSNB in penile cancer, reported an initial false-negative rate of 19.2%–22% (30, 50). The initial DSNB procedure consisted of preoperative lymphoscintigraphy, sentinel node biopsy after peritumoral injection of blue dye, and histopathological examination. Detailed analysis of the false-negative cases led to several procedural modifications, including the addition of preoperative ultrasonography with fine needle aspiration cytology of suspicious LNs, followed by radical inguinal lymphadenectomy if the results were positive. In addition, scintigraphically non-visualized groins were surgically explored, the wound was intraoperatively palpated, and histopathological analysis was extended by serial sectioning and immunohistochemistry. These modifications reduced the false-negative rate to 4.8% per groin (30). A prospective study by

Lam et al. analyzed 500 groins that underwent DSNB and found a false-negative rate of 5% per inguinal region (35). Two European multicenter studies reported false-negative rates of 7% and 10.8% per groin, respectively (37, 49). However, in line with our results, some other studies showed considerably higher false-negative rates. Using the isolated gamma probe technique, Gonzaga-Silva et al. found only one patient with LN metastases in a cohort of 27 patients, but three patients with a negative DSNB procedure developed regional recurrence during a mean follow-up of 36 months, resulting in a false-negative rate of 75% per patient. The authors concluded that the isolated gamma probe technique was not reliable for detecting LN metastases in patients with penile cancer (31). A study of 21 patients by Spiess et al. found a false-negative rate of 28.6% per groin (33). A recent review and meta-analysis of 27 articles on radioisotope-guided DSNB in penile cancer reported pooled sensitivity and negative-predictive values of 88% and 99%, respectively (51). The large variability in false-negative rates may be explained by the small patient cohorts, heterogeneity of DSNB protocols, and different levels of experience with the technique.

There are several possible reasons for false-negative DSNB results. One possibility is that histopathological analysis may fail to detect micrometastases; however, pathological reevaluation of the SLNs from the four false-negative groins in the current study revealed normal lymphatic tissue. False-negative results may also be due to tumor blockage, in which lymphatic drainage is obstructed by tumor cells leading to rerouting of the radioactive tracer to a "neo-SLN" (52). DSNB is thus not recommended in patients with palpable LNs because of the high risk of LN metastases and thus tumor blockage (9). False-negative procedures may also be caused by alteration of the lymphatic drainage as a result of the previous removal of the primary tumor. In the present study, two of the three patients with false-negative results had multiple primary tumor resections prior to DSNB. Graafland et al. investigated the reliability of postresection DSNB in a cohort of 40 patients and found no regional recurrence after a median follow-up of 28 months (38). In a study by Omorphos et al., one of 92 patients who underwent secondary DSNB developed regional recurrence during a median follow-up of 22 months, and the false-negative rate was 11.1% per patient (39). The results of these studies indicate that DSNB is reliable after previous removal of the primary tumor. In contrast, however, Fuchs et al. and Lützen et al. only observed regional recurrence after secondary but not after primary DSNB, which argues against this hypothesis (40, 41). Similarly, it is unclear whether DSNB is reliable in patients with local recurrence or previous inguinal surgeries who may have an altered lymphatic drainage, e.g. due to scarring. In the present study, none of the patients with locally recurrent tumors or previous groin surgeries developed regional recurrence. However, further studies with larger patient cohorts and long-term follow-up are needed to confirm or disprove the reliability of DSNB after surgical treatment of the primary tumor, local recurrence or previous inguinal surgeries.

In addition to the above reasons, several studies have suggested that the false-negative rate of DSNB depends on the protocol used. Dimopoulos et al. compared the results of 1- and 2-day protocols for DSNB in patients with penile cancer. The 1-day protocol resulted in harvesting of significantly more LNs than the 2-day protocol, with false-negative rates of 0% and 6.8%, respectively, suggesting that the 1-day protocol may be more reliable for the detection of LN metastases in patients with cN0 penile cancer (36). Moreover, preoperative ultrasonography and intraoperative palpation of the wound are suggested to improve the false-negative rate by identifying suspect LNs that are not visualized due to tumor blockage (30). In contrast, fine needle aspiration cytology is no longer recommended in cN0 patients because of its low sensitivity of 39% (9, 53). Many groups performed additional injection of blue dye to visualize the SLNs; however, several studies using a combination of ^{99m}Tc nanocolloid and blue dye found no SLNs that were stained with blue dye but were not radioactive (32, 34, 54), suggesting that the addition of blue dye may not reduce the false-negative rate of DSNB. Our DSNB procedure included preoperative ultrasonography, ^{99m}Tc nanocolloid injection on the day of surgery, and palpation of the exposed wound, and this protocol therefore cannot explain the high false-negative rate in our study.

Recent efforts have been made to further refine the DSNB technique in patients with penile cancer. The introduction of the hybrid radioactive and fluorescent tracer indocyanine green-^{99m}Tc nanocolloid significantly improved the optical detection of SLNs compared with blue dye (55). Dell'Oglio et al. recently confirmed the reliability of indocyanine green-^{99m}Tc nanocolloid for DSNB in a large cohort of 400 patients and reported false-negative rates of 10% per patient and 8.9% per groin (56). Moreover, initial results indicated that magnetometer-guided DSNB using superparamagnetic iron oxide nanoparticles was a feasible, radiation-free technique for the identification of SLNs in penile cancer (57, 58). Another recent study investigated the use of intraoperative freehand magnetic particle imaging together with a hybrid indocyanine green–superparamagnetic iron oxide nanoparticle tracer for intraoperative SLN detection (59). The feasibility of this method was confirmed in *ex vivo* human skin transplants and in a porcine model, but the results need to be verified in human patients. Further refinements of the DSNB technique will hopefully reduce the false-negative rate of the procedure in the future. Apart from that, Choo et al. recently reported that adding postoperative adjuvant concurrent radiotherapy and chemotherapy may have a therapeutic benefit and may help to further improve survival in patients with penile cancer and regional LN metastases (60).

The present study had some limitations. One limitation was the retrospective nature of the study with all its drawbacks, such as a possible information bias due to incomplete data in medical records. Moreover, our analysis relied on a single center and included a relatively small number of patients because of the low incidence of penile cancer and the non-centralized treatment of penile cancer in Germany. These issues should be taken into account when interpreting the results of the present study. Only nine groins that underwent DSNB

contained LN metastases (true-positive + false-negative procedures), and a single false-negative event thus had a great impact on the false-negative rate. Nonetheless, our study represents the largest German series of the use of DSNB in patients with penile cancer published to date. The study was also limited by the follow-up time; although 86.1% of regional recurrences occur within 2 years after primary treatment (61), we cannot rule out the possibility that further false-negative procedures would come to light in the future.

In conclusion, the results of this study suggest that radioisotope-guided DSNB may reduce the morbidity of inguinal lymphadenectomy in patients with cN0 penile cancer. However, DSNB and primary tumor resection should be performed simultaneously to avoid false-negative results. Recent advances, such as new tracers and imaging techniques, may help to further reduce the false-negative rate of DSNB.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of the University of Oldenburg. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AW and FW conceived the study. LN, AV, L-MM, and AW acquired and analyzed the data. All authors contributed to the interpretation of data. R-PH carried out the histopathological reexaminations of the lymph nodes. LN wrote a first draft of the manuscript. AW, FW, AV, BM, and SE revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Cardona CEM, García-Perdomo HA. Incidence of Penile Cancer Worldwide: Systematic Review and Meta-Analysis. *Rev Panam Salud Pública* (2017) 41: e117. doi: 10.26633/RPSP.2017.117
2. De Rose AF, Mantica G, Gallo F, Dotta F, Testino N. Risk Factors for the Delay in the Diagnosis of Penile Lesions: Results From a Single Center in Italy. *Minerva Urol Nefrol* (2019) 71:258–63. doi: 10.23736/S0393-2249.18.03189-2
3. Horenblas S, van Tinteren H. Squamous Cell Carcinoma of the Penis. IV. Prognostic Factors of Survival: Analysis of Tumor, Nodes and Metastasis Classification System. *J Urol* (1994) 151:1239–43. doi: 10.1016/S0022-5347(17)35221-7
4. Ficarra V, Akduman B, Bouchot O, Palou J, Tobias-Machado M. Prognostic Factors in Penile Cancer. *Urology* (2010) 76:S66–73. doi: 10.1016/j.urology.2010.04.008
5. Wood HM, Angermeier K. Anatomic Considerations of the Penis, Lymphatic Drainage, and Biopsy of the Sentinel Node. *Urol Clin North Am* (2010) 37:327–34. doi: 10.1016/j.ucl.2010.04.013
6. Djajadiningrat RS, Graafland NM, van Werkhoven E, Meinhardt W, Bex A, van der Poel HG, et al. Contemporary Management of Regional Nodes in Penile Cancer-Improvement of Survival? *J Urol* (2014) 191:68–73. doi: 10.1016/j.juro.2013.07.088
7. McDougal WS. Carcinoma of the Penis: Improved Survival by Early Regional Lymphadenectomy Based on the Histological Grade and Depth of Invasion of the Primary Lesion. *J Urol* (1995) 154:1364–6. doi: 10.1016/s0022-5347(01)66863-0
8. Kroon BK, Horenblas S, Lont AP, Tanis PJ, Gallee MPW, Nieweg OE. Patients With Penile Carcinoma Benefit From Immediate Resection of Clinically Occult Lymph Node Metastases. *J Urol* (2005) 173:816–9. doi: 10.1097/01.ju.0000154565.37397.4d
9. Hakenberg OW, Compérat E, Minhas S, Necchi A, Protzel C, Watkin N. *EAU Guidelines on Penile Cancer*. Arnhem, The Netherlands: EAU Guidelines Office (2018).
10. Ficarra V, Zattoni F, Cunico SC, Galetti TP, Luciani L, Fandella A, et al. Lymphatic and Vascular Embolizations Are Independent Predictive Variables of Inguinal Lymph Node Involvement in Patients With Squamous Cell Carcinoma of the Penis: Gruppo Uro-Oncologico Del Nord Est (Northeast Uro-Oncological Group) Penile Cancer Data Base Data. *Cancer* (2005) 103:2507–16. doi: 10.1002/cncr.21076
11. Graafland NM, Lam W, Leijte JAP, Yap T, Gallee MPW, Corbishley C, et al. Prognostic Factors for Occult Inguinal Lymph Node Involvement in Penile Carcinoma and Assessment of the High-Risk EAU Subgroup: A Two-Institution Analysis of 342 Clinically Node-Negative Patients. *Eur Urol* (2010) 58:742–7. doi: 10.1016/j.eururo.2010.08.015
12. Winters BR, Mossanen M, Holt SK, Lin DW, Wright JL. Predictors of Nodal Upstaging in Clinical Node Negative Patients With Penile Carcinoma: A National Cancer Database Analysis. *Urology* (2016) 96:29–34. doi: 10.1016/j.urology.2016.06.033
13. Bloom JB, Stern M, Patel NH, Zhang M, Phillips JL. Detection of Lymph Node Metastases in Penile Cancer. *Transl Androl Urol* (2018) 7:879–86. doi: 10.21037/tau.2018.08.01
14. Bevan-Thomas R, Slaton JW, Pettaway CA. Contemporary Morbidity From Lymphadenectomy for Penile Squamous Cell Carcinoma: The M.D. Anderson Cancer Center Experience. *J Urol* (2002) 167:5. doi: 10.1016/S0022-5347(05)65169-5
15. Stuiver MM, Djajadiningrat RS, Graafland NM, Vincent AD, Lucas C, Horenblas S. Early Wound Complications After Inguinal Lymphadenectomy in Penile Cancer: A Historical Cohort Study and Risk-Factor Analysis. *Eur Urol* (2013) 64:486–92. doi: 10.1016/j.eururo.2013.02.037
16. Catalona WJ. Modified Inguinal Lymphadenectomy for Carcinoma of the Penis With Preservation of Saphenous Veins: Technique and Preliminary Results. *J Urol* (1988) 140:306–10. doi: 10.1016/S0022-5347(17)41589-8
17. Lopes A, Rossi BM, Fonseca FP, Morini S. Unreliability of Modified Inguinal Lymphadenectomy for Clinical Staging of Penile Carcinoma. *Cancer* (1996) 77:2099–102. doi: 10.1002/(SICI)1097-0142(19960515)77:10<2099::AID-CNCR20>3.0.CO;2-P
18. Parra RO. Accurate Staging of Carcinoma of the Penis in Men With Nonpalpable Inguinal Lymph Nodes by Modified Inguinal Lymphadenectomy. *J Urol* (1996) 155:560–3. doi: 10.1016/S0022-5347(01)66449-8
19. Coblentz TR, Theodorescu D. Morbidity of Modified Prophylactic Inguinal Lymphadenectomy for Squamous Cell Carcinoma of the Penis. *J Urol* (2002) 168:1386–9. doi: 10.1097/01.ju.0000028090.39287.68
20. d'Ancona CAL, De Lucena RG, De Oliveira Querne FA, Martins MHT, Denardi F, Netto NR. Long-Term Followup of Penile Carcinoma Treated With Penectomy and Bilateral Modified Inguinal Lymphadenectomy. *J Urol* (2004) 172:498–501. doi: 10.1097/01.ju.0000132239.28989.e1

21. Yao K, Tu H, Li Y-H, Qin Z-K, Liu Z-W, Zhou F-J, et al. Modified Technique of Radical Inguinal Lymphadenectomy for Penile Carcinoma: Morbidity and Outcome. *J Urol* (2010) 184:546–52. doi: 10.1016/j.juro.2010.03.140
22. Tsaur I, Biegel C, Gust K, Huesch T, Borgmann H, Brandt MPJK, et al. Feasibility, Complications and Oncologic Results of a Limited Inguinal Lymph Node Dissection in the Management of Penile Cancer. *Int Braz J Urol* (2015) 41:486–95. doi: 10.1590/S1677-5538.IBJU.2014.0304
23. Nerli RB, Sharma M, Patel P, Ghagane SC, Patil SD, Gupta P, et al. Modified Inguinal Lymph Node Dissection in Groin-Negative Patients of Penile Cancer: Our Experience. *Indian J Surg Oncol* (2021) 12:229–34. doi: 10.1007/s13193-021-01285-w
24. Cabanas R. An Approach for the Treatment of Penile Carcinoma. *Cancer* (1977) 39:456–66. doi: 10.1002/1097-0142(197702)39:2<456::aid-cncr2820390214>3.0.co;2-i
25. Perinetti E, Crane DB, Catalona WJ. Unreliability of Sentinel Lymph Node Biopsy for Staging Penile Carcinoma. *J Urol* (1980) 124:734–5. doi: 10.1016/S0022-5347(17)55633-5
26. Wespes E, Simon J, Schulman CC. Cabanas Approach: Is Sentinel Node Biopsy Reliable for Staging Penile Carcinoma? *Urology* (1986) 28:278–9. doi: 10.1016/0090-4295(86)90004-x
27. Pettaway CA, Pisters LL, Dinney CPN, Jularbal F, Swanson DA, von Eschenbach AC, et al. Sentinel Lymph Node Dissection for Penile Carcinoma: The M. D. Anderson Cancer Center Experience. *J Urol* (1995) 154:1999–2003. doi: 10.1016/S0022-5347(01)66671-0
28. Horenblas S, Jansen L, Meinhardt W, Hoefnagel CA, Jong DD, Nieweg OE. Detection of Occult Metastasis in Squamous Cell Carcinoma of the Penis Using a Dynamic Sentinel Node Procedure. *J Urol* (2000) 163:100–4. doi: 10.1016/S0022-5347(05)67982-7
29. Wawroschek F, Vogt H, Bachter D, Weckermann D, Hamm M, Harzmann R. First Experience With Gamma Probe Guided Sentinel Lymph Node Surgery in Penile Cancer. *Urol Res* (2000) 28:246–9. doi: 10.1007/s002400000125
30. Leijte JAP, Kroon BK, Valdés Olmos RA, Nieweg OE, Horenblas S. Reliability and Safety of Current Dynamic Sentinel Node Biopsy for Penile Carcinoma. *Eur Urol* (2007) 52:170–7. doi: 10.1016/j.eururo.2007.01.107
31. Gonzaga-Silva LF, Tavares JM, Freitas FC, Tomas Filho ME, Oliveira VP, Lima MV. The Isolated Gamma Probe Technique for Sentinel Node Penile Carcinoma Detection Is Unreliable. *Int Braz J Urol* (2007) 33:58–63. doi: 10.1590/S1677-55382007000100009
32. Hadway P, Smith Y, Corbishley C, Heenan S, Watkin NA. Evaluation of Dynamic Lymphoscintigraphy and Sentinel Lymph-Node Biopsy for Detecting Occult Metastases in Patients With Penile Squamous Cell Carcinoma. *BJU Int* (2007) 100:561–5. doi: 10.1111/j.1464-410X.2007.07013.x
33. Spiess PE, Izawa JI, Bassett R, Kedar D, Busby JE, Wong F, et al. Preoperative Lymphoscintigraphy and Dynamic Sentinel Node Biopsy for Staging Penile Cancer: Results With Pathological Correlation. *J Urol* (2007) 177:2157–61. doi: 10.1016/j.juro.2007.01.125
34. Kirrander P, Andrén O, Windahl T. Dynamic Sentinel Node Biopsy in Penile Cancer: Initial Experiences at a Swedish Referral Centre. *BJU Int* (2013) 111: E48–53. doi: 10.1111/j.1464-410X.2012.11437.x
35. Lam W, Alnajjar HM, La-Touche S, Perry M, Sharma D, Corbishley C, et al. Dynamic Sentinel Lymph Node Biopsy in Patients With Invasive Squamous Cell Carcinoma of the Penis: A Prospective Study of the Long-Term Outcome of 500 Inguinal Basins Assessed at a Single Institution. *Eur Urol* (2013) 63:657–63. doi: 10.1016/j.eururo.2012.10.035
36. Dimopoulos P, Christopoulos P, Shilito S, Gall Z, Murby B, Ashworth D, et al. Dynamic Sentinel Lymph Node Biopsy for Penile Cancer: A Comparison Between 1- and 2-Day Protocols. *BJU Int* (2016) 117:890–6. doi: 10.1111/bju.13389
37. Jakobsen JK, Krarup KP, Sommer P, Nerstrøm H, Bakholdt V, Sørensen JA, et al. Dapeca-1: Diagnostic Accuracy of Sentinel Lymph Node Biopsy in 222 Patients With Penile Cancer at Four Tertiary Referral Centres - A National Study From Denmark. *BJU Int* (2016) 117:235–43. doi: 10.1111/bju.13127
38. Graafland NM, Valdés Olmos RA, Meinhardt W, Bex A, van der Poel HG, van Boven HH, et al. Nodal Staging in Penile Carcinoma by Dynamic Sentinel Node Biopsy After Previous Therapeutic Primary Tumour Resection. *Eur Urol* (2010) 58:748–51. doi: 10.1016/j.eururo.2010.06.036
39. Omorphos S, Saad Z, Arya M, Freeman A, Malone P, Nigam R, et al. Feasibility of Performing Dynamic Sentinel Lymph Node Biopsy as a Delayed Procedure in Penile Cancer. *World J Urol* (2016) 34:329–35. doi: 10.1007/s00345-015-1577-5
40. Fuchs J, Hamann MF, Schulenburg F, Knüpfer S, Osmonov D, Lützen U, et al. Sentinel Lymph Node Biopsy for Penile Carcinoma. Assessment of Reliability. *Urol* (2013) 52:1447–50. doi: 10.1007/s00120-013-3166-9
41. Lützen U, Naumann CM, Dischinger J, Marx M, Baumann R, Zhao Y, et al. 10-Year Experience Regarding the Reliability and Morbidity of Radio Guided Lymph Node Biopsy in Penile Cancer Patients and the Associated Radiation Exposure of Medical Staff in This Procedure. *BMC Urol* (2016) 16:47. doi: 10.1186/s12894-016-0166-2
42. Schubert T, Uphoff J, Henke R-P, Wawroschek F, Winter A. Reliability of Radioisotope-Guided Sentinel Lymph Node Biopsy in Penile Cancer: Verification in Consideration of the European Guidelines. *BMC Urol* (2015) 15:98. doi: 10.1186/s12894-015-0093-7
43. Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications: A New Proposal With Evaluation in a Cohort of 6336 Patients and Results of a Survey. *Ann Surg* (2004) 240:205–13. doi: 10.1097/01.sla.0000133083.54934.ae
44. Hinten F, van den Einden LCG, Hendriks JCM, van der Zee AGJ, Bulten J, Massuger LFAG, et al. Risk Factors for Short- and Long-Term Complications After Groin Surgery in Vulvar Cancer. *Br J Cancer* (2011) 105:1279–87. doi: 10.1038/bjc.2011.407
45. Poos HPAM, Kruijff S, Bastiaannet E, van Ginkel RJ, Hoekstra HJ. Therapeutic Groin Dissection for Melanoma: Risk Factors for Short Term Morbidity. *Eur J Surg Oncol* (2009) 35:877–83. doi: 10.1016/j.ejso.2008.10.012
46. Spiess PE, Hernandez MS, Pettaway CA. Contemporary Inguinal Lymph Node Dissection: Minimizing Complications. *World J Urol* (2009) 27:205–12. doi: 10.1007/s00345-008-0324-6
47. Koifman L, Hampl D, Koifman N, Vides AJ, Ornellas AA. Radical Open Inguinal Lymphadenectomy for Penile Carcinoma: Surgical Technique, Early Complications and Late Outcomes. *J Urol* (2013) 190:2086–92. doi: 10.1016/j.juro.2013.06.016
48. Bouchot O, Rigaud J, Maillet F, Hetet JF, Karam G. Morbidity of Inguinal Lymphadenectomy for Invasive Penile Carcinoma. *Eur Urol* (2004) 45:761–6. doi: 10.1016/j.eururo.2003.12.003
49. Leijte JAP, Hughes B, Graafland NM, Kroon BK, Olmos RAV, Nieweg OE, et al. Two-Center Evaluation of Dynamic Sentinel Node Biopsy for Squamous Cell Carcinoma of the Penis. *J Clin Oncol* (2009) 27:3325–9. doi: 10.1200/JCO.2008.20.6870
50. Tanis PJ, Lont AP, Meinhardt W, Horenblas S. Dynamic Sentinel Node Biopsy for Penile Cancer: Reliability of a Staging Technique. *J Urol* (2002) 168:76–80. doi: 10.1016/S0022-5347(05)64835-5
51. Zou Z, Liu Z, Tang L, Wang Y, Liang J, Zhang R, et al. Radiocolloid-Based Dynamic Sentinel Lymph Node Biopsy in Penile Cancer With Clinically Negative Inguinal Lymph Node: An Updated Systematic Review and Meta-Analysis. *Int Urol Nephrol* (2016) 48:2001–13. doi: 10.1007/s11255-016-1405-x
52. Leijte JAP, van der Ploeg IMC, Valdés Olmos RA, Nieweg OE, Horenblas S. Visualization of Tumor Blockage and Rerouting of Lymphatic Drainage in Penile Cancer Patients by Use of SPECT/CT. *J Nucl Med* (2009) 50:364–7. doi: 10.2967/jnumed.108.059733
53. Kroon BK, Horenblas S, Deurloo EE, Nieweg OE, Teertstra HJ. Ultrasonography-Guided Fine-Needle Aspiration Cytology Before Sentinel Node Biopsy in Patients With Penile Carcinoma. *BJU Int* (2005) 95:517–21. doi: 10.1111/j.1464-410X.2005.05330.x
54. Perdonà S, Autorino R, De Sio M, Di Lorenzo G, Gallo L, Damiano R, et al. Dynamic Sentinel Node Biopsy in Clinically Node-Negative Penile Cancer Versus Radical Inguinal Lymphadenectomy: A Comparative Study. *Urology* (2005) 66:1282–6. doi: 10.1016/j.urology.2005.06.085
55. Brouwer OR, van den Berg NS, Mathéron HM, van der Poel HG, van Rhijn BW, van Tinteren H, et al. Horenblas s. A Hybrid Radioactive and Fluorescent Tracer for Sentinel Node Biopsy in Penile Carcinoma as a Potential Replacement for Blue Dye. *Eur Urol* (2014) 65:600–9. doi: 10.1016/j.eururo.2013.11.014
56. Dell'Oglio P, de Vries HM, Mazzone E, KleinJan GH, Donswijk ML, van der Poel HG, et al. Hybrid Indocyanine Green–99mtc-Nanocolloid for Single-Photon Emission Computed Tomography and Combined Radio- and Fluorescence-Guided Sentinel Node Biopsy in Penile Cancer: Results of 740

Inguinal Basins Assessed at a Single Institution. *Eur Urol* (2020) 78:865–72. doi: 10.1016/j.eururo.2020.09.007
57. Cleaveland P, Lau M, Parnham A, Murby B, Ashworth D, Manohoran P, et al. Testing the Feasibility of Sentimag/Sienna+ for Detecting Inguinal Sentinel Nodes in Penile Cancer (Sentipen): An Eurogen and National Cancer Research Institute Trial. *Eur Urol* (2019) 76:874–5. doi: 10.1016/j.eururo.2019.09.007
58. Winter A, Kowald T, Engels S, Wawroschek F. Magnetic Resonance Sentinel Lymph Node Imaging and Magnetometer-Guided Intraoperative Detection in Penile Cancer, Using Superparamagnetic Iron Oxide Nanoparticles: First Results. *Urol Int* (2020) 104:177–80. doi: 10.1159/000502017
59. Azargoshasb S, Molenaar L, Rosiello G, Buckle T, van Willigen DM, van de Loosdrecht MM, et al. Advancing Intraoperative Magnetic Tracing Using 3D Freehand Magnetic Particle Imaging. *Int J Comput Assist Radiol Surg* (2022) 17(1):211–8. doi: 10.1007/s11548-021-02458-2
60. Choo R, Nehra A, Zattoni F, Pagliaro LC, Karnes RJ. Is There Any Benefit in Adding Postoperative Adjuvant Concurrent Radiotherapy and Chemotherapy for Penile Cancer With Regional Lymph Node Metastasis? *Minerva Urol Nefrol* (2020) 72:474–81. doi: 10.23736/S0393-2249.19.03387-3
61. Leijte JAP, Kirrander P, Antonini N, Windahl T, Horenblas S. Recurrence Patterns of Squamous Cell Carcinoma of the Penis: Recommendations for Follow-Up Based on a Two-Centre Analysis of 700 Patients. *Eur Urol* (2008) 54:161–9. doi: 10.1016/j.eururo.2008.04.016

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CASE REPORT
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Primary Leiomyosarcoma of the Glans

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Penile leiomyosarcoma isz an extremely uncommon entity that rarely occurs in the glans. Due to the limited number of cases described in literature, guidelines regarding non-surgical treatment, prognosis, and management remain equivocal. Among the mesenchymal tumors of the penis, leiomyosarcoma has the highest propensity for recurrence. It originates in the smooth muscle cells from two distinct locations: superficial and deep. The deep subtype is the most aggressive and has the highest potential for metastasis. Surgical treatment should be implemented early and must be locally aggressive. Herein, we present a rare case of a 54-year-old patient with deep localized leiomyosarcoma of the glans, albeit with superficial characteristics. A review of the main histopathological, clinical, immunohistochemical, and therapeutic aspects of this unusual entity is presented.

Keywords: leiomyosarcoma, penile tumors, immunohistochemistry, histopathological, glans

INTRODUCTION

In the penis, squamous cell carcinoma (SCC) is by far the most common neoplasm, accounting for 95% of all neoplasms. Mesenchymal tumors are extremely rare, representing <5% of all types of malignant tumors (1). According to the World Health Organization, leiomyosarcoma is the second most common penile sarcoma after Kaposi's sarcoma. The patients' age at leiomyosarcoma diagnosis ranges from 6 (2) to 80 (1) years, and the most prevalent age group is 42–63 years (mean age, 52 years). Pathologically, leiomyosarcomas are classified into superficial and deep lesions (1, 2). The superficial type usually appears in areas of integumentary support, and the deep type originates from the support structures of the corpora cavernosa and corpus spongiosum (3). We herein report a rare case of leiomyosarcoma of

the glans in a 54-year-old patient who underwent surgical resection of the lesion. In addition, we review the histopathological, immunohistochemical, clinical, and therapeutic aspects of this unusual entity.

CASE REPORT

A 54-year-old male patient visited the urology department with a 6-month history of a "nodule (similar to callus)" on the glans. The lesion was exophytic and grew gradually and painlessly. The patient had no history of bleeding or weight loss. A physical examination identified a nodule at the tip of the penis, measuring 2.0 × 1.0 cm (**Figure 1**). Inguinal lymphadenopathy was not detected. On palpation, the nodule had a soft consistency. The lesion was excised, and histopathological examination of the specimen under a microscope revealed a morphology similar to that of malignant spindle cell neoplasm, with high mitoses and atypical mitoses. The surgical margin had neoplasm. However, neither angiolymphatic nor perineural invasion were observed. The corpus spongiosum, tunica albuginea, corpora cavernosa, and urethra were all free from neoplasia. A focal area compatible with Penile intraepithelial neoplasia (PeIN) was also observed, with epithelial layers showing immunostaining for P16, P53, and Ki67 (**Figure 2**).

Immunohistochemical tests were positive and diffuse for muscle antigens, specifically smooth muscle actin (SMA), calponin, and HHF35. Tumor cells were negative for, CD34, CD31, v-ets erythroblastosis virus E26 oncogene like isoform 2 (ERG), and FLI1 (**Figure 3**). Polymerase chain reaction assay for human papillomavirus (HPV) in two fresh samples of the invasive tumor showed no viral infection. Eventually, a case of high-grade penile leiomyosarcoma was diagnosed with a focus on PeIN.

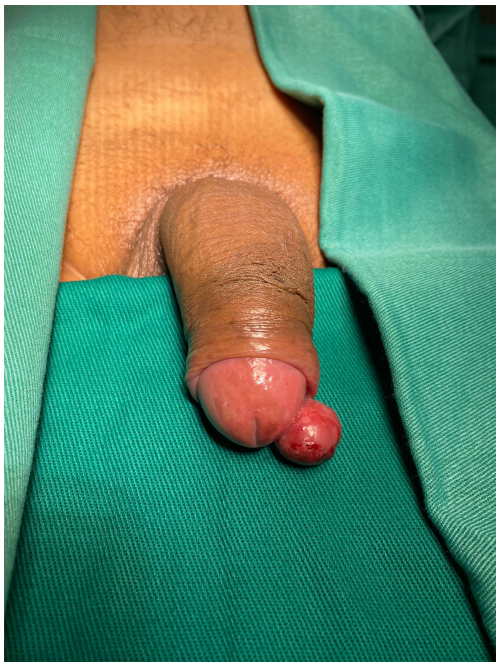

FIGURE 1 | A 54-years-old patient with a pedunculated nodule at the tip of glans that was show to be leiomyosarcoma on histopathological exam.

At 5 months since presentation, the patient shows no signs of recurrence. The patient has been advised a reoperation to enlarge the compromised surgical margin. However, so far, the patient refuses to resurgery.

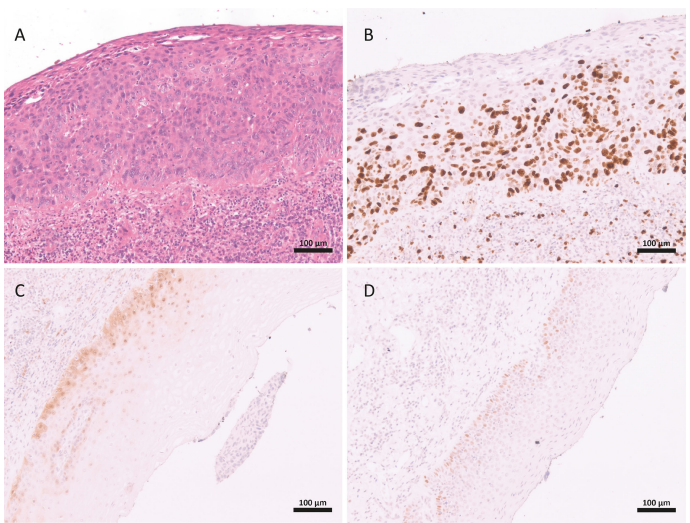

FIGURE 2 | Features of Penile intraepithelial neoplasia in the glans of a patient with leiomyosarcoma. Dysplastic epithelial cell in all squamous layers **(A)**. Immunohistochemistry staing for: Ki67 in suprabasal layers **(B)**, p16 **(C)** and p53 **(D)**.

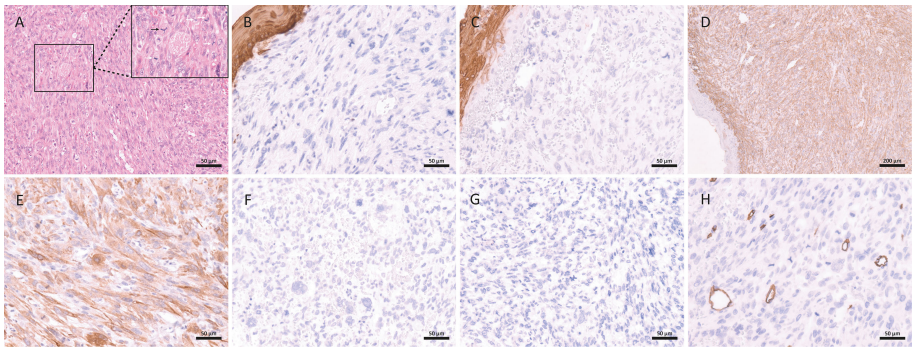

FIGURE 3 | Histopathological and immunohistochemical aspects of leiomyosarcoma of the penis: spindle cell malignant neoplasm with a high number of mitoses (white arrow) and atypical mitoses (black arrow) **(A)**; the neoplasm was negative for epithelial markers: epithelial membrane antigen **(B)** and pan-cytokeratin **(C)**; the immunohistochemical expression was strong and diffuse for smooth muscle markers: alpha-smooth muscle actin **(D)** and calponin **(E)**; the neoplasm is also negative expression for: p16 **(F)**, S100 **(G)** and CD34 **(H)**.

DISCUSSION

Primary mesenchymal tumors of the penis are among the least common tumors of the male genitourinary tract (1, 4, 5). The first case of penile leiomyosarcoma was described by Levi in 1930 (1). The total number of superficial leiomyosarcoma reported cases was 30 in 2013 (6), and although current data regarding the exact number of cases superficial and deep are disparate, there are certainly fewer than 60 cases reported to date (7–9).

There are two types of leiomyosarcomas of the penis that are classified into two clinically and pathologically distinct entities: superficial and deep leiomyosarcomas (1). Superficial leiom yosarcomas usually manifest as lesions in the distal and dorsal regions of the penis. They typically occur in young patients, are asymptomatic, and show slow tumor growth and low metastatic potential but are locally aggressive. The origin of these superficial neoplasms is presumably the piloerector muscle of the skin or smooth muscle elements of the subcutaneous cellular tissue (1, 5).

Deep leiomyosarcomas are most frequently found in the glans and proximal region of the corpora cavernosa and corpus spongiosum and occur in older adult patients. Unlike superficial tumors, these deep lesions have a higher metastatic potential, with a worse prognosis, increased aggressiveness, and a tendency to infiltrate the urethra and produce symptoms, such as urethrocutaneous fistula and urinary obstruction. These deep lesions can result from the progression of initially superficial lesions or from the smooth muscle cells of the corpora cavernosa and corpus spongiosum (5).

Leiomyosarcoma is described as a mesenchymal tumor prone to recurrence, which becomes increasingly histologically undifferentiated after each recurrence (3, 10). Our patient presented with a leiomyosarcoma that behaved like a superficial leiomyosarcoma, but in a region of the penis more characteristic of a deep leiomyosarcoma. This suggests that deep leiomyosarcomas can result from the progression of an initially superficial lesion (5). Additionally, the lesion was located in the glans. Of a total of 30 cases leimyosarcoma superficial reviewed, in only 3 cases, it occurred exclusively in the glans (6). As in our case, the three cases of glans tumors did not show recurrence or metastasis. Despite being considered a deep lesion, leiomyosarcomas that affect the glans seem to behave as superficial lesions.

Differential diagnoses of this neoplasm include sarcomatoid SCC, neurogenic sarcoma, angiosarcoma, fibrosarcoma, and, most importantly, Kaposi's sarcoma. Immunohistochemistry is essential for a definitive diagnosis. Leiomyosarcoma is distinguished from sarcomatoid carcinoma by its negative immunoreactivity to cytokeratin's. Kaposi's sarcoma has a prominent lymphoplasmacytic infiltrate and is immunoreactive for CD31, CD34, and HHV8. Fibrosarcomas are rare in the penis and are smooth muscle actin-negative. In our case, the tumor cells were positive for SMA (strong and diffuse), calponin (strong and diffuse), and HHF35, which are muscle antigens, and negative for pan-cytokeratin (AE1AE3), CK18 and EMA (all epithelial markers), s100 (neural/melanocytic marker), and CD31, CD34, ERG, and FLI1 (all endothelial/vascular markers).

Surprisingly, our patient also had a focal area of PeIN, with immunoreactivity for p16, p53, and Ki67, but with no human papillomavirus (HPV) infection. However, HPV research was not performed around the *in situ* lesions. Due to the high frequency of HPV and associated lesions in our region, the PeIN is likely a fortuitous finding. However, the presence this lesion makes it essential to completely exclude the possibility of the sarcomatoid variant of the SCC through a wide panel of epithelial markers, as performed in our patient. Interestingly, to date, this is the only case of a penile leiomyosarcoma associated with PeIN reported in the literature. Of all cases included in our records from 2004 to date (>600), this is the first case of a non-epithelial neoplasm (11, 12).

Local recurrence is frequent in penile leiomyosarcoma ranging from 23% to 29% of cases depending on the deepness of the primary lesion (3) and if there is no distant metastasis, surgery, if possible, would be the preferential salvage approach. The extension of the procedure (amputation vs partial penectomy) depends on the location and extension of the lesion on the penis

an invasion of adjacent tissues and structures. Radiotherapy would be reserved to palliation in inoperable tumors, since leiomyosarcomas are not listed at as the most sensitives sarcomas to radiation therapy (13).

Because of the high risk of distant metastasis (50%) of deep primary tumors (3), in these cases we could consider neoadjuvant chemotherapy as an option in large and more aggressive recurrent lesions in order to select *in vivo* good responders and avoid futile aggressive surgery in patients who would inevitably progress to distant metastatic disease. However, these are data extrapolated from extremities high grade leiomyosarcomas, since there is no available data from prospective studies of chemotherapy in penile leiomyosarcomas dur to its rarity (14, 15).

In conclusion, penile leiomyosarcoma has rarely been described in the literature. Hence, reporting this neoplasm will improve recognition and management of this occurrence. This leiomyosarcoma can behave mildly aggressive, with local growth, and indolent, like a superficial leiomyosarcoma, or more aggressive, with rapid progression, and potentially metastatic, like a deep leiomyosarcomas. An accurate histopathological diagnosis, from macroscopy to microscopy, complemented by immunohistochemistry, is essential to avoid misdiagnosis and, consequently, incorrect treatment, especially in regions with a high incidence of penile cancer, such as in our case. Surgical treatment must be locally aggressive to prevent recurrence.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The study was reviewed and approved by the Research Ethics Committee on Humans from the University Hospital of the Federal University of Maranhão (CEP-HUUFMA), approval term No. 4.228.789 (CAAE No. 30760420.3.0000.5086). Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

RNGdCJ, AALTJ, and GEBS are the principal investigators and wrote the first version of the manuscript. TMSR, TBMS, AMAJ, FSMSN, JRRC, and RCS participated in data collection, care and management of the patient. LdOL and SPdCM performed pathological analysis and interpretation. RdGCFC contribute to critical revision of important intellectual content of the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Pratt RM, Ross RT. Leiomyosarcoma of the Penis. A Report of a Case. *Br J Surg* (1969) 56(11):870–2. doi: 10.1002/bjs.1800561122
2. Escudero C, Miralles EJ, Robles A, Rufo MC. Leiomiosarcoma De Pene *Rev Esp Patol,* (2000) 33(3):221–5.
3. Fetsch JF, Davis CJJr, Miettinen M, Sesterhenn IA. Leiomyosarcoma of the Penis: A Clinicopathologic Study of 14 Cases With Review of the Literature and Discussion of the Differential Diagnosis. *Am J Surg Pathol* (2004) 28 (1):115–25. doi: 10.1097/00000478-200401000-00014
4. Glucker E, Hirshowitz B, Gellei B. Leiomyosarcoma of the Glans Penis. Case Report. *Plast Reconstr Surg* (1972) 50(4):406–8. doi: 10.1097/00006534-197210000-00026
5. Pow-Sang MR, Orihuela E. Leiomyosarcoma of the Penis. *J Urol* (1994) 151 (6):1643–5. doi: 10.1016/s0022-5347(17)35328-4
6. Cigna E, Maruccia M, Parisi P, Soda G, Nasca MR, Micali G, et al. Superficial Leiomyosarcoma of the Glans: Report of a Case and Literature Review. *Aesthetic Plast Surg* (2013) 37(5):1052–8. doi: 10.1007/s00266-013-0199-9
7. Nanri M, Kondo T, Okunda H, Tanabe K, Toma H. A Case of Leiomyosarcoma of the Penis. *Int J Urol* (2006) 13(5):655–8. doi: 10.1111/j.1442-2042.2006.01376.x
8. Rabinovich J. Leiomyosarkom Der Vorhaut : Ein Fall Des Seltenen Mesenchymalen Vorhauttumors [Leiomyosarcoma of the Foreskin : A Rare Case of Mesenchymal Foreskin Tumor]. *Urologe A* (2018) 57(5):591–3. doi: 10.1007/s00120-018-0577-7. German.
9. Venyo KGA. *Primary Leiomyosarcoma of the Penis: A Review and Update of the Literature*. Edelweiss: Edelweiss Cancer OA (2019) 1 p. 43–50. doi: 10.33805/2689-6737.110
10. Valadez RA, Waters WB. Leiomyosarcoma of Penis. *Urology* (1986) 27 (3):265–7. doi: 10.1016/0090-4295(86)90287-6
11. Vieira CB, Feitoza L, Pinho J, Teixeira-Júnior A, Lages J, Calixto J, et al. Profile of Patients With Penile Cancer in the Region With the Highest Worldwide Incidence. *Sci Rep* (2020) 10:2965. doi: 10.1038/s41598-020-59831-5
12. Coelho RWP, Pinho JD, Moreno JS, Garbis DVEO, do Nascimento AMT, Larges JS, et al. Penile Cancer in Maranhão, Northeast Brazil: The Highest Incidence Globally? *BMC Urol* (2018) 18(1):50. doi: 10.1186/s12894-018-0365-0
13. Yang GQ, Yuan ZM, Welsh E, Ahmed KA, Torres-Roca JF, Naghavi AO. Intrinsic Radiosensitivity Index Differences of Sarcoma and the Potential for Genome-Adjusted Radiation Dosing. *Int J Radiat Oncol* (2019) 105:sup E812. doi: 10.1016/j.ijrobp.2019.06.2525
14. Gronchi A, Ferrari S, Quagliuolo V, Broto JM, Lopez Pousa A, Grignani G, et al. Neoadjuvant Chemotherapy in High-Risk Soft Tissue Sarcomas: A Randomised Clinical Trial From the Italian Sarcoma Group (ISG), the Spanish Sarcoma Group (GEIS), the French Sarcoma Group (FSG) and the Polish Sarcoma Group (PSG). *J Clin Oncol* (2017) 38:2178–86. doi: 10.1200/JCO.19.03289
15. Hensley ML, Maki R, Venkatraman E, Geller G, Lovegren M, Aghajanian C, et al. Gemcitabine and Docetaxel in Patients With Unresectable Leiomyosarcoma: Results of a Phase II Trial. *J Clin Oncol* (2002) 20:2824–31. doi: 10.1200/JCO.2002.11.050

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REVIEW
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Non-Coding RNA in Penile Cancer

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Penile cancer (PC) still presents a health threat for developing countries, in particular Brazil. Despite this, little progress has been made on the study of markers, including molecular ones, that can aid in the correct management of the patient, especially concerning lymphadenectomy. As in other neoplasms, non-coding RNAs (ncRNAs) have been investigated for penile cancer, with emphasis on microRNAs, piRNAs (PIWI-interacting small RNAs), and long non-coding RNAs (LncRNAs). In this context, this review aims to assemble the available knowledge on non-coding RNA linked in PC, contributing to our understanding of the penile carcinogenesis process and addressing their clinical relevance. ncRNAs are part of the novel generation of biomarkers, with high potential for diagnosis and prognosis, orientating the type of treatment. Furthermore, its versatility regarding the use of paraffin samples makes it possible to carry out retrospective studies.

Keywords: non coding RNAs (ncRNAs), penile cancer, biomarkers, piRNAs, miRNA

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INTRODUCTION

Penile cancer (PC) is highly incident in developing regions such as Asia, Africa, and South America, with Brazil having the highest incidence rate in the world, 6.15/100,000 inhabitants (1, 2). The etiology of penile cancer is not fully understood, but some risk factors have been strongly associated with this malignant neoplasm. Among them stand out the presence of phimosis, poor hygiene of the organ, and infection by the Human Papilloma Virus (HPV) (1).

HPV prevalence in male genital cancer is highly variable, reflecting differences in sensitivity in the methods used to detect the virus, and also associated with the histological subtype of the tumor, being more frequent in condylomatous and basaloid tumors (3, 4). The global prevalence is 36-40%, with a more significant contribution from subtypes HPV16 and HPV18 (3, 5).

Penectomy is still the "gold standard" for the treatment of primary tumors. It can be partial or total, depending on the extension of the lesion (6, 7). In some patients, lymphadenectomy is essential for surgical management, although it presents risks of complications and has high morbidity. At some health services, this type of procedure has been performed prophylactically, especially in developing regions, where many patients have difficulties maintaining medical care (8). Furthermore, patients without palpable lymph nodes at diagnosis may present micrometastases.

The rate of occurrence of micrometastases is 25%, and the involvement of more than two inguinal lymph nodes is associated with a greater risk of recurrence (9). Therefore, the concern with lymph node involvement is justified by the significant impact on prognosis (6, 9, 10). Thus, biological markers that can predict or assist in diagnosing this phenomenon are of great clinical importance. Some markers based on ncRNAs have been investigated, especially those associated with lymph node metastasis (11, 12), perineural invasion (13), and HPV (14).

For several decades, ncRNAs were considered 'evolutionary junk.' They can be classified according to their size, with those up to 200 nucleotides in length being considered small non-coding RNAs (sncRNA). Those with more than 200 nucleotides are long non-coding RNAs (lncRNA). Among the sncRNAs, we highlight microRNAs, piRNAs, and snoRNAs (Small nucleolar RNAs) (15) (**Figure 1**). When interacting with DNA, RNA, or proteins, ncRNAs have many essential functions, such as epigenetic regulation, chromatin remodeling, protein modification, and RNA degradation. Furthermore, they can function as important regulators of gene expression and play crucial roles in many physiological and pathological processes, so much that the abnormal expression of these sncRNAs is involved in many human diseases, including cancer (16).

ncRNAs are involved in the deregulation of several signaling pathways, similar to miRNAs that have several target genes that regulate the expression of epithelial mesenchymal transition (EMT) transcription factors, and also direct genes involved in the encoding of signaling mediators, adhesion junction and polarity complex proteins (17). LncRNAs have also been documented to be involved in the regulation of key factors such as: oxidative stress and inflammation (18). In addition to these, other ncRNAs have also been considered in crucial processes in cancer, among which we have piRNAs that are involved in apoptosis and proliferation (19), and snoRNAs, involved in invasion and metastasis (20). These data point to the importance of studying ncRNAs as potential biomarkers in PC.

In this context, this review aims to interconnect the information produced on non-coding RNAs addressed in PC, relating them to their clinical importance, with perspectives of use as markers that aid in management, in addition to helping to understand the process of carcinogenesis.

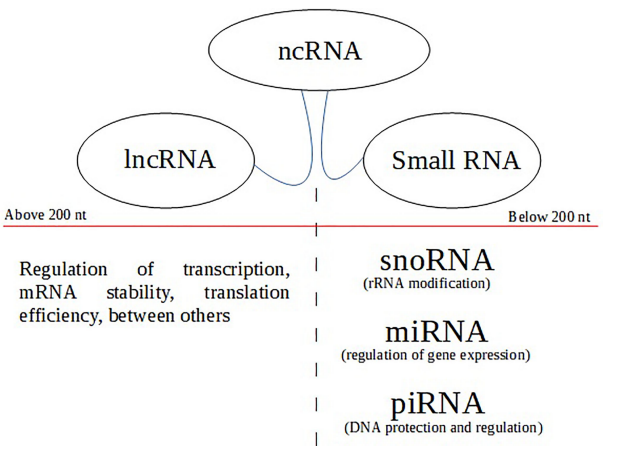

FIGURE 1 | The top of the flowchart (balloons) represents non-coding RNA types based on transcript length (nt). Below the nt size divider (red line) are the subclass with their respective functions.

MicroRNAs and Penile Cancer

MicroRNAs (miRNAs or miRs) are small non-coding RNAs (19-23 nt) involved in regulating gene expression at the transcriptional and post-transcriptional levels. These biomolecules constitute one of the most abundant classes of ncRNAs, being widely studied due to their high mRNA silencing potential, regulating relevant processes of gene expression, such as apoptosis, proliferation, and differentiation (21). Gene regulation and expression occur through the complementarity of microRNA and mRNA in the 3'UTR region, with the consequent degradation or repression of target gene transcripts (22).

The dysregulation in the expression of these biomolecules has been related to different pathologies, including cancer (23, 24). There is evidence that the differential expression allows not only the identification of neoplastic tissue but also the different subtypes of malignant lesions, being also helpful in determining the stage and progression of cancer and prognosis and response to treatment (25). Because of this, microRNAs have been considered potential biomarkers for diagnosis, prognosis, and therapy (22, 24, 25).

It is worth noting that there are two forms of therapeutic approaches based on microRNAs. The first approach aims to inhibit the activity of oncogenic miRNAs using miRNA antagonists such as antagomiRs or mimic miRNAs (25). AntagomiRs act by reducing the levels of intracellular overexpression of miRNAs, through their specific binding to mature target miRNA. Meanwhile, mimic miRNAs or mimics are constructed with the aim of replacing the deleted tumor suppressor miRNA (26). The action of antagomiRs and mimics has already been assessed with promising results in malignant neoplasms, such as leukemia (26) and prostate cancer (27). The second specific microRNA therapeutic strategy can be performed using synthetic oligonucleotides that act as microRNA sponges (28) (**Figure 2**).

Furthermore, microRNAs have been used as biomarkers of metastatic disease, which can be termed as metastamiRs. These microRNAs do not influence the initial steps of tumorigenesis, but regulate processes such as transition-mesenchymal epithelium (TEM), apoptosis and angiogenesis (29).

There are few studies that address the role of microRNAs in PC; mainly, they provide important information about HPV infection and/or worse prognostic factors, which are described in **Table 1**.

The first study showcasing the participation of microRNAs in PC was described by Barzon et al. (30). They observed that miR-218 was down-regulated in those samples from patients with high-risk HPV (hrHPV) and with negative protein expression of

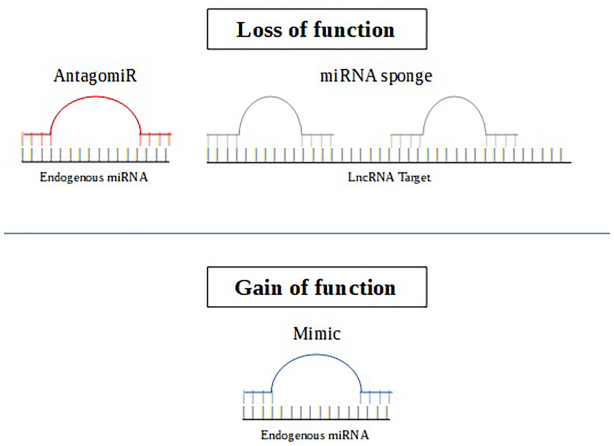

FIGURE 2 | The AntagomiR (in red) is an oligonucleotide sequence complementary to the endogenous target miRNA, leading to functional inhibition. MiRNA sponge (in gray) are RNA transcripts containing binding sites that sequester specific miRNAs to prevent them from interacting with their target sequence. The miRNA mimic (in blue) is an RNA fragment that acts by mimicking an endogenous miRNA that can specifically bind to its target gene.

p53. In oral cancer with HR-HPV+, it has been reported that the dysregulation of miR-218 is mediated by dysregulation oncoprotein E6 (34).

Later, it was also observed that the reduced expression of miR-146a is mediated by oncoprotein E6. The high expression of EGFR (Epidermal Growth Factor Receptor) was associated with the reduced expression (14). The target genes of this microRNA are involved in migration, metastasis formation, and proliferation, such as NOTCH1 (Notch Receptor 1), ROCK1 (Rho Associated Coiled-Coil Containing Protein Kinase 1), and EGFR. The EGFR gene has been extensively studied for PC, and its protein and gene dysregulation has been associated with advanced stage, lower overall survival, and lymph node status. It is, therefore, a vital target marker for therapy (35, 36).

Kuasne et al. (31) found relevant data, who identified some microRNAs with decreased expression (let-7b-5p, miR-185-5p, miR-29b-3p, miR-505-3p miR-146-5p), in a group of seven patients, five of which hrHPV positive. These microRNAs regulate genes; MMP2 (Metalanoprotease 2), MMP9 (Metalanoprotease 9), IGF1R (Insulin Like Growth Factor 1 Receptor), and PTEN (Phosphatase and Tensin homologue), which regulate important mechanisms in the progression of carcinogenesis.In addition to the microRNAs as mentioned above, this same study highlighted three other microRNAs (miR-31-5p, miR-224-5p, and miR-223-3p) that presented high sensitivity and specificity to distinguish between tumoral and non-neoplastic penile tissue. MicroRNA miR-31-5p regulates the AR gene (Androgen Receptor), which is pointed out as the driver gene in penile cancer (37).

Regarding the PTEN gene, it has been reported to be inactivated in several types of cancers (38, 39), including penile cancer (40, 41), either by deletions, mutations, methylation in the promoter region and/or transcriptional post-regulation, through the action of microRNAs (42–44).

As for the relationship between PTEN and microRNAs, it is necessary to mention the data found by Yayu et al. (45), which revealed the increased expression of miR-26a in blood and urine samples from patients with penile cancer. This high expression

TABLE 1 | Main microRNA linked to penile cancer.

MicroRNAs	Function/ Expression	Clinical Significance	Method	References
miR-218	TsmiR/Down	miR-218 was less expressed in hrHPV samples	RT-qPCR	(30)
miR-146a	TsmiR/Down	miR-146a had a decreased expression in hrHPV samples. Its low expression was mediated by oncoprotein E6.	RT-qPCR	(14)
miR-223-3p	oncomiR/Up	Specificity and sensibility to distinct between tumor and non-tumor samples	Microarray/qRT-PCR NGS/qRT-PCR	(31) (32)
		Associated lymph node metastasis.	qRT-PCR	(12)
miR-224-5p	TsmiR/Down	Specificity and sensibility to distinct between tumor and non-tumor samples	Microarray/qRT-PCR	(31)
miR-31-5p	OncomiR/Up	The AR gene is targeted by miR-31-5p. This gene has already been observed as a driver gene in penile cancer.		
miR-145-5p	TsmiR/Down	miR-145-5p targets gene MMP1, which showed increased expression levels in samples from patients with lymph node metastasis	Microarray/qRT-PCR	(31)
		Reduced expression was associated with perineural invasion	qRT-PCR	(13)
miR-1 miR-101 miR-204	TsmiR/Down	The reduced expression of these three microRNAs can predict metastasis.	TaqMan Array	(11)
miR-107	OncomiR/Up	High expression when comparing tumor and non-tumor samples.	NGS/qRT-PCR	(32)
		Associated with worsening of prognosis: histological grade II and III, tumors bigger than 2.0 cm, stage III and IV, and lower disease-free survival	qRT-PCR	(12)
miR-21-5p	OncomiR/Up	Was correlated to the absence of PTEN protein expression	qRT-PCR	(12)
miR-137 miR-328-3p	TsmiR/Down	Reduced expression in patients with lymph node metastasis.	Microarray qRT-PCR	(33)

was associated with low expression of PTEN tumors from HPV-positive patients. The authors suggest that miR-26a can regulate the progression of HPV-positive penile tumors through PTEN modulation.

IGF1R, regulated by the microRNA let-7b-5, is a transmembrane receptor tyrosine kinase that is overexpressed in several malignant neoplasms, including urologic cancers (46). This receptor plays a critical role in cell proliferation, differentiation, and malignant transformation. Protein overexpression of IGF1R has been associated with lower disease-free survival in penile cancer (47).

In PC, the high expression of metalloproteases (MMP2 and MMP9), regulated by miR-29b-3p, correlated with a higher incidence of distant metastasis and lower survival (48).

Only three studies addressed the relationship between alteration in microRNAs expression and lymph node metastasis in PC, as summarized in **Table 1**. Hartz et al. (11) observed that miR-1, miR-101, and miR-204 were under-expressed in penile metastatic tumors. Low expression of miR-1 has been reported for colorectal (49) and cervical (50) cancers. MiR-101 is related to clinical outcomes of worse prognosis in several types of tumors, such as cervical cancer (51) and pancreatic cancer (52), regulating genes such as: mTor (mammalian target of rapamycin), ROCK1, ACKR3 (atypical chemokine receptor 3), MCL1 (MCL1Apoptosis Regulator, BCL2 Family Member) and RAC1 (Rac Family Small GTPase 1), which participate in important pathways in the mechanism of carcinogenesis (53).

In a study carried out by our group, it was possible to identify that the high expression of miR-223-3p is associated with lymph node metastasis. Furthermore, the increased expression of miR-107 and the absence of protein expression of PTEN were observed in patients at more advanced stages of the disease (12). In this same study, we observed that the expression or miR-21 was higher in tumoral samples when compared to non-tumoral ones. According to Gao et al. (54), miR-223-3p can also regulate several pathways in the promotion of tumor metastases, local invasion, transport, extravasation, colonization, and epithelial-mesenchymal transition.

In another study, our group also observed that miR-145-5p is a potential biomarker for perineural invasion (13), an indicator of worse survival in patients with penile cancer (55). MiR-145-5p also has therapeutic potential since the use of mimics of this microRNA in cervical cancer can inhibit cell proliferation (56) and metastasis in ovarian cancer (57).

MiR-21 indirectly modulates PDL-1 expression (58) and miR-145 is able to downregulate the expression of this same marker through its direct binding to 3'UTR (59) PD-L1, which is the main immune checkpoint receptor expressed on cells of the immune system and plays a significant role in cell adhesion, proliferation and cytokine signaling (60). The use of immune checkpoint inhibitors has shown considerable interest as a chemotherapeutic agent in penile cancer and results of clinical trials have provided valuable information for the treatment of aggressive disease (61–63). The use of these two microRNAs can aid in the study and development of these chemotherapeutics, with potential utility in penile cancer, because as we modulate the expression of a microRNA through a single therapeutic approach, the expression of all its target genes returns to baseline.

Recently, Ayoubian et al. (33) identified a low expression of miR-137 and miR-328-3p in usual metastatic penile cancer tumors. Overexpression of miR-137 acts to inhibit tumor growth, in addition to having been assessed as holding therapeutic potential in lung cancer (64). Overexpression of miR-328-3p inhibits cell proliferation, migration, invasion, and transition epithelial-mesenchymal (EMT), acting by inactivating the PI3K/Akt signaling pathway colon-rectal cancer (65).

piRNAs and Penile Cancer

piRNAs are a type of ncRNA, with a size between 26-31nt. They are so named because they interact with members of the Argonaut family, namely the PIWI (P-element-induced wimpy tests) proteins. With PIWI proteins, piRNAs form a gene silencing complex (66). These silencing complexes act by suppressing transposable elements (TE), which are responsible for maintaining the integrity of the genome, in addition to transcriptionally regulating gene expression, inducing chromatin remodeling and repressing mRNAs that harbor transposon sequences in the 3'UTR or regions 5'UTR (67).

In recent years, some studies have shown, mainly in gastric cancer, that abnormal expression of piRNAs is associated with cancer initiation, progression, and metastasis (67–70). In this context, piRNAs can become a diagnostic tool, therapeutic targets, besides being prognostic cancer biomarkers (67). Using next-generation sequencing, the only work with piRNAs for PC highlighted the ten most abundant piRNAs with a difference in expression when comparing tumor tissue with normal tissue (32). Among the piRNAs highlighted in this work, piR-49145 has already been observed with altered expression in gastric cancer samples compared to adjacent tissue (69).

Long Non-Coding RNA in Penile Cancer

LncRNAs are transcribed from non-protein-coding mRNAs greater than 200nt. According to their position relative to the protein-coding genes, the lncRNAs can be divided into; a) sense; b) antisense: transcripts located on the opposite strand of protein-coding genes; c) bidirectional; d) intronic: transcripts that are located within introns of protein-coding genes; e) intergenic: lncRNAs that are located in the region between two protein-coding genes (71).

LncRNAs can regulate gene expression through multiple mechanisms, including epigenetic, transcriptional, and post-transcriptional levels. Furthermore, these biomolecules participate in regulating various cellular activities, such as cell differentiation, proliferation, invasion, apoptosis, and autophagy through interaction with RNA, DNA, or proteins (71).

Several studies have shown that LncRNAs are deregulated in pathologies such as cancer, acting as oncogenes or tumor suppressors. Furthermore, these molecules have been identified as clinically useful diagnostic or prognostic biomarkers or therapeutic targets for cancer (71, 72).

In penile cancer, only a single work refers to alterations in LncRNA. Macedo et al. (73) observed amplification in

LINC00226 and LINC00221. LINC00221 when positively regulated can serve as a potential diagnostic and prognostic biomarker in hepatocellular cancer (74), and its dysregulation has already been associated with a worse prognosis in cisplatin-resistant non-small cell lung cancer (75), evidencing the relevance of this biomolecule for the carcinogenesis process.

Perspectives

ncRNAs comprehend the novel generation of biomarkers, with potential use in diagnosis and prognosis, and possibly even aiding in the choice of treatments, especially those with high sensitivity and specificity in distinguishing different tumor stages. The microRNAs discussed in this article are already known to participate in the carcinogenic process. In the literature, some of these have been investigated in clinical routine, using non-invasive samples (blood and urine), such as miR-145-5p and miR-26a, possible targets to be explored in PC. Embora ainda não haja informações sobre o papel destes ncRNAs

In addition, ncRNAs, especially microRNAs, demonstrate to be resistant to the process of formalin-fixed paraffin inclusion, enabling their study in cases where fresh material was not collected and in studies with a retrospective sampling (76). Finally, it is important to consider the importance of researching other ncRNAs such as; snoRNAs, circRNAs (circular RNAs), siRNAs (small interfering RNAs), which have already been observed altered in gastric cancer (44, 69), cervical cancer (51, 56), hepatocellular carcinoma (74) and vulvar cancer (72) in order to understand the role of these biomolecules in penile carcinogenesis.

AUTHOR CONTRIBUTIONS

Conception and design: JP, GS, AT, and AK. Administrative support: AT, JP. Provision of study materials or patients: AT, JC, and AK. Collection and assembly of data: JP, AT. Data analysis and interpretation: JP, GS, AK, RB. Manuscript writing: All authors. Final approval of manuscript: All authors.

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REFERENCES

1. Coelho RWP, Pinho JD, Moreno JS, Garbis DVO, Nascimento AMT, Larges JS, et al. Penile Cancer in Maranhão, Northeast Brazil: The Highest Incidence Globally? *BMC Urol* (2018) 18(1):50. doi: 10.1186/s12894-018-0365-0
2. Douglawi A, Masterson TA. Penile Cancer Epidemiology and Risk Factors. *Curr Opin Urol* (2019) 29(2):145–49. doi: 10.1097/MOU.0000000000000581
3. Alemany L, Saunier M, Alvarado-Cabrero I, Quirós B, Salmeron J, Shin HR, et al. Human Papillomavirus DNA Prevalence and Type Distribution in Anal Carcinomas Worldwide. *Int J Cancer* (2015) 136(1):98–107. doi: 10.1002/ijc.28963
4. Olesen TB, Sand FL, Rasmussen CL, Albiere V, Toft BG, Norrild B, et al. Prevalence of Human Papillomavirus DNA and P16ink4a in Penile Cancer and Penile Intraepithelial Neoplasia: A Systematic Review and Meta-Analysis. *Lancet Oncol* (2019) 20(1):145–58. doi: 10.1016/S1470-2045(18)30682-X
5. Flaherty A, Kim T, Giuliano A, Magliocco A, Hakky TS, Pagliaro LC, et al. Implications for Human Papillomavirus in Penile Cancer. *Urol Oncol* (2014) 32(1):53.e1–8. doi: 10.1016/j.urolonc.2013.08.010
6. Protzel C, Alcaraz A, Horenblas S, Pizzocaro G, Zlotta A, Hakenberg OW. Lymphadenectomy in the Surgical Management of Penile Cancer. *Eur Urol* (2009) 55:1075–88. doi: 10.1016/j.eururo.2009.02.021
7. O'Neill S, Barns M, Vujovic F, Lozinskiy M. The Role of Penectomy in Penile Cancer-Evolving Paradigms. *Transl Androl Urol* (2020) 9(6):3191–4. doi: 10.21037/tau.2019.08.14
8. Chipollini J, Yan S, Ottenhof SR, Zhu Y, Draeger D, Baugarten A, et al. Surgical Management of Penile Carcinoma in Situ: Results From an International Collaborative Study and Review of the Literature. *BJU Int* (2018) 121(3):393–8. doi: 10.1111/bju.14037
9. Azizi M, Spiess P. Targeted Therapy in Penile Cancer: A New Treatment Paradigm. *Nat Rev Urol* (2018) 15:5–6. doi: 10.1038/nrurol.2017.183
10. Chipollini J, Tang DH, Sharma P, Baumgarten As, Spiess PE. Patterns of Regional Lymphadenectomy for Clinically Node-Negative Patients With Penile Carcinoma: Analysis From the National Cancer Database From 1998 to 2012. *Clin Genitourin Cancer* (2017) 15(6):670–7. doi: 10.1016/j.clgc.2017.04.019
11. Hartz JM, Engelmann D, Furst K, Marquardt S, Spitschak A, Goodv D, et al. Integrated Loss of miR-1/miR-101/miR-204 Discriminates Metastatic From Nonmetastatic Penile Carcinomas and Can Predict Patient Outcome. *J Urol* (2016) 196:570–8. doi: 10.1016/j.juro.2016.01.115
12. Pinho JD, Silva GEB, Teixeira Júnior AAL, Belfort MRC, Mendes JM, da Cunha IW, et al. MIR-107, MIR-223 and MIR-21-5P Reveals Potential Biomarkers in Penile Cancer. *Asian Pac J Can Preven* (2020) 21(2):391–397. doi: 10.31557/APJCP.2020.21.2.391
13. Pinho JD, Silva GEB, Teixeira Júnior AAL, Belfort MRC, Mendes JM, Calixto JRR, et al. Downregulation of miR-145 Is Associated With Perineural Invasion in Penile Carcinoma. *Transl Androl Urol* (2021) 10(5):2019–26. doi: 10.21037/tau-20-1378
14. Peta E, Cappellesso R, Masi G, Sinigaglia A, Trevisan M, Grassi A, et al. Down-Regulation of microRNA-146a Is Associated With High-Risk Human Papillomavirus Infection and Epidermal Growth Factor Receptor Overexpression in Penile Squamous Cell Carcinoma. *Hum Pathol* (2017) 61:33–40. doi: 10.1016/j.humpath.2016.10.019
15. Zhou B, Yang H, Yang C, Bao YL, Yang SM, Liu J, et al. Translation of Noncoding RNAs and Cancer. *Cancer Lett* (2021) 497:89–99. doi: 10.1016/j.canlet.2020.10.002
16. Goodall GJ, Wickramasinghe VO. RNA in Cancer. *Nat Rev Cancer* (2021) 21(1):22–36. doi: 10.1038/s41568-020-00306-0
17. Shenas MHM, Eghbal-Fard S, Mehrisofiani V, Yazdani NA, Farzam OR, Marofi F, et al. MicroRNAs and Signaling Networks Involved in Epithelial–Mesenchymal Transition. *J Cell Physiol* (2019) 234(5):5775–85. doi: 10.1002/jcp.27489
18. Wang X, Shen C, Zhu J, Shen G, Li Z, Dong J. Long Noncoding RNAs in the Regulation of Oxidative Stress. *Oxid Med Cell Longev* (2019) 2019:1318795. doi: 10.1155/2019/1318795
19. Zeng Q, Wan H, Zhao S, Xu H, Tang T, Oware KA, et al. Role of PIWI-Interacting RNAs on Cell Survival: Proliferation, Apoptosis, and Cycle. *IUBMB Life* (2020) 72(9):1870–8. doi: 10.1002/iub.2332
20. Werf JV, Chin CV, Flemming NI. SnoRNA in Cancer Progression, Metastasis and Immunotherapy Response. *Biol (Basel)* (2021) 10(8):809. doi: 10.3390/biology10080809
21. Saliminejad K, Khorram KHR, Fard SS, Ghaffari SH. An Overview of microRNAs: Biology, Functions, Therapeutics, and Analysis Methods. *J Cell Physiol* (2019) 234(5):5451–65. doi: 10.1002/jcp.27486
22. Nazarov PV, Kreis S. Integrative Approaches for Analysis of mRNA and microRNA High-Throughput Data. *Comput Struct Biotechnol J* (2021) 19:1154–62. doi: 10.1016/j.csbj.2021.01.029

23. Shen J, Sanford A, Feng Jaing S. MicroRNAs as Potential Biomarkers in Human Solid Tumors. *Cancer Lett* (2013) 329(2):125–36. doi: 10.1016/j.canlet.2012.11.001
24. Babaei K, Shams S, Keymoradzadeh A, Vahidi S, Hamami P, Khasar R, et al. An Insight of microRNAs Performance in Carcinogenesis and Tumorigenesis; an Overviews of Cancer Therapy. *Life Sci* (2020) 240:117077. doi: 10.1016/j.lfs.2019.117077
25. Lu T, Rothenberg ME. MicroRNA. *J Allergy Clin Immunol* (2020) 141 (4):1202–7. doi: 10.1016/j.jaci.2017.08.034
26. Innao V, Allegra A, Pulvirenti N, Allegra AG, Musolino C. Therapeutic Potential of antagomiRs in Haematological and Oncological Neoplasms. *Eur J Cancer Car (Engl)* (2020) 29(2):e13208. doi: 10.1111/ecc.13208
27. Su JO, Lange T. Role of Metastasis-Related microRNAs in Prostate Cancer Progression and Treatment. *Cancers (Basel)* (2021) 13(17):4492. doi: 10.3390/cancers13174492
28. Chi Y, Wang D, Wang J, Yu W, Yang J. Long Non-Coding RNA in the Pathogenesis of Cancers. *Cells* (2019) 8(9):1015. doi: 10.3390/cells8091015
29. Lopez-Camarillo C, Marchat LA, Arechaga-Ocampo E, Perez-Plasencia C, Moral-Hernandez OD, Castaneda EJ, et al. MetastamiRs: Non-Coding MicroRNAs Driving Cancer Invasion and Metastasis. *Int J Mol Sci* (2012) 13(2):1347–79. doi: 10.3390/ijms13021347
30. Barzon L, Cappelesso R, Peta E, Militello V, Sinigaglia A, Fassan M, et al. Profiling of Expression of Human Papillomavirus-Related Cancer miRNAs in Penile Squamous Cell Carcinomas. *Am J Pathol* (2014) 184(12):3376–83. doi: 10.1016/j.ajpath.2014.08.004
31. Kuasne H, Barros- Filho MC, Busso-Lopes A, Marchi FA, Pinheiro M, Muñoz JJM, et al. Integrative miRNA and mRNA Analysis in Penile Carcinomas Reveals Markers and Pathways With Potential Clinical Impact. *Oncot* (2017) 8 (9):15294–306. doi: 10.18632/oncotarget.14783
32. Zhang L, Wei P, Shen X, Zhang Y, Xu B, Zhou J, et al. MicroRNA Expression Profile in Penile Cancer Revealed by Next-Generation Small RNA Sequencing. *PloS One* (2015) 10(7):e0131336. doi: 10.1371/journal.pone.0131336
33. Ayoubian H, Heinzelmann J, Holters S, Khalmurzaev O, Pryalukin A, Loertzer P, et al. miRNA Expression Characterizes Histological Subtypes and Metastasis in Penile Squamous Cell Carcinoma. *Cancer* (2021) 13 (6):1480. doi: 10.3390/cancers13061480
34. Uesugi A, Kozaki K, Tsuruta T, Furuta M, Morita KI, Imoto I, et al. The Tumor Suppressive microRNA miR-218 Targets the mTOR Component Rictor and Inhibits AKT Phosphorylation in Oral Cancer. *Cancer Res* (2011) 71(17):5765–78. doi: 10.1158/0008-5472.CAN-11-0368
35. Dorff TB, Schuckman AK, Schwartz R, Rashad S, Bulbul A, Cai J, et al. Epidermal Growth Factor Receptor, Excision-Repair Cross-Complementation Group 1 Protein, and Thymidylate Synthase Expression in Penile Cancer. *Clin Genitourin Cancer* (2016) 14(5):450–6.e1. doi: 10.1016/j.clgc.2016.01.013
36. Huang KB, Liu RY, Peng QH, Li ZS, Jiang LJ, Guo SJ, et al. EGFR Mono-Antibody Salvage Therapy for Locally Advanced and Distant Metastatic Penile Cancer: Clinical Outcomes and Genetic Analysis. *Urol Oncol* (2019) 37(1):71–7. doi: 10.1016/j.urolonc.2018.10.016
37. Marchi FA, Martins DC, Barros-Filho MC, Kuasne H, Busso AFL, Brentani H, et al. Multidimensional Integrative Analysis Uncovers Driver Candidates and Biomarkers in Penile Carcinoma. *Sci Rep* (2017) 7(1):6707. doi: 10.1038/s41598-017-06659-1
38. Altınoğlu SA, Wang M, Li KQ, Li Y, Xu Q. Intracellular Delivery of PTEN Protein Using Cationic Lipids for Cancer Therapy. *Bio Sci* (2016) 4(12):1773–80. doi: 10.1039/c6bm00580b
39. Papa A, Wan L, Bonora M, Salmena L, Song MS, Hobbs RM, et al. Cancer-Associated PTEN Mutants Act in a Dominant-Negative Manner to Suppress PTEN Protein Function. *Cell* (2014) 157(3):595–610. doi: 10.1016/j.cell.2014.03.027
40. Stankiewicz E, Prowse DM, Mansum N, Cuzick J, Mesher D, Hiscock F, et al. Alternative HER/PTEN/Akt Pathway Activation in HPV Positive and Negative Penile Carcinomas. *PloS One* (2011) 6(3):e17517. doi: 10.1371/journal.pone.0017517
41. Chaux A, Munari E, Cubilla AL, Lecksell K, Burnett AL, Netto GJ. Immunohistochemical Expression of the Mammalian Target of Rapamycin Pathway in Penile Squamous Cell Carcinomas: A Tissue Microarray Study of 112 Cases. *Histopathology* (2014) 64(6):863–71. doi: 10.1111/his.12338
42. Yang ES, Willey CD, Mehta A, Crowley MR, Crossmam DK, Chen D, et al. Kinase Analysis of Penile Squamous Cell Carcinoma on Multiple Platforms to Identify Potential Therapeutic Targets. *Oncotarget* (2017) 8(13):21710–8. doi: 10.18632/oncotarget.15558
43. De Roock W, Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN Mutations: Implications for Targeted Therapies in Metastatic Colorectal Cancer. *Lancet Oncol* (2011) 12(6):594–603. doi: 10.1016/S1470-2045(10)70209-6
44. Hu M, Zhu S, Xiong S, Xue X, Zhou X. MicroRNAs and the PTEN/PI3K/Akt Pathway in Gastric Cancer. *Oncol Rep* (2019) 41(3):1439–54. doi: 10.3892/or.2019.6962
45. Yayu H, Enping H, Xiagchuan S. Molecular Mechanism of microRNA-26a Regulation of Phosphatase and Tensin Homolog Gene in Condyloma Acuminatum and Penile Squamous Cell Carcinoma. *J Int Med Res* (2021) 49(7):3000605211014379. doi: 10.1177/03000605211014379
46. Gonzalez-Roibon N, Kim JJ, Faraj SF, Chaux A, Bezerra SM, Munari E, et al. Insulin-Like Growth Factor-1 Receptor Overexpression Is Associated With Outcome in Invasive Urothelial Carcinoma of Urinary Bladder: A Retrospective Study of Patients Treated Using Radical Cystectomy. *Urology* (2014) 83(6):1444.e1–6. doi: 10.1016/j.urology.2014.01.028
47. Ball MW, Bezerra SM, Chaux A, Faraj SF, Gonzalez-Roibon N, Munari N, et al. Overexpression of Insulin-Like Growth Factor-1 Receptor Is Associated With Penile Cancer Progression. *Urology* (2016) 92:51–6. doi: 10.1016/j.urology.2016.02.006
48. Campos RS, Lopes A, Guimarães GC, Carvalho AL, Soares FA. E-Cadherin, MMP-2, and MMP-9 as Prognostic Markers in Penile Cancer: Analysis of 125 Patients. *Urology* (2006) 67(4):797–802. doi: 10.1016/j.urology.2005.10.026
49. Wu Y, Pu N, Su W, Yang X, Xing C. Downregulation of miR-1 in Colorectal Cancer Promotes Radioresistance and Aggressive Phenotypes. *J Cancer* (2020) 11(16):4832–40. doi: 10.7150/jca.44753
50. Gao L, Yan P, Guo FF, Liu HJ, Zhao ZF. iR-1-3p Inhibits Cell Proliferation and Invasion by Regulating BDNF-TrkB Signaling Pathway in Bladder Cancer. *Neoplasia* (2018) 65(1):89–96. doi: 10.4149/neo_2018_161128N594
51. Wei H, He WR, Chen KM, Wang XW, Yi CJ. MiR-101 Affects Proliferation and Apoptosis of Cervical Cancer Cells by Inhibition of JAK2. *Eur Rev Med Pharmacol Sci* (2019) 23(13):5640–7. doi: 10.26355/eurrev_201907_18299
52. Zhu L, Chen Y, Nie K, Xiao Y, Yu H. MiR-101 Inhibits Cell Proliferation and Invasion of Pancreatic Cancer Through Targeting STMN1. *Cancer Biomark* (2018) 23(2):301–9. doi: 10.3233/CBM-181675
53. Yi J, Huang WZ, Wen YQ, Yi YC. Effect of miR-101 on Proliferation and Oxidative Stress-Induced Apoptosis of Breast Cancer Cells *via* Nrf2 Signaling Pathway. *Eur Rev Med Pharmacol Sci* (2019) 23(20):8931–9. doi: 10.26355/eurrev_201910_19291
54. Gao Y, Lin L, Li T, Yang J, Wei Y. The Role of miRNA-223 in Cancer: Function, Diagnosis and Therapy. *Gene* (2017) 616:1–7. doi: 10.1016/j.gene.2017.03.021
55. Zhou X, Qi F, Zhou R, Wang S, Wang Y, Wang Y, et al. The Role of Perineural Invasion in Penile Cancer; a Meta-Analysis and Systematic Review. *Biosci Rep* (2018) 38(5):BSR20180333. doi: 10.1042/BSR20180333
56. Li M, Ling-Ling L. miR-145 Contributes to the Progression of Cervical Carcinoma by Directly Regulating Fscn1. *Cell Transplant* (2019) 28(9-10):1299–305. doi: 10.1177/0963689719861063
57. Wang L, Wu X, Wang B, Wang W, Han L. Mechanisms of miR-145 Regulating Invasion and Metastasis of Ovarian Carcinoma. *Am J Transl Res* (2017) 9(7):3443–51.
58. Xi J, Huang Q, Wang L, Ma X, Deng Q, Kumar M, et al. miR-21 Depletion in Macrophages Promotes Tumoricidal Polarization and Enhances PD-1 Immunotherapy. *Oncogene* (2018) 37(23):3151–65. doi: 10.1038/s41388-018-0178-3
59. Zhu J, Li Y, Luo Y, Xu J, Liufu H, Tian Z, et al. A Feedback Loop Formed by ATG7/Autophagy, FOXO3a/miR-145 and PD-L1 Regulates Stem-Like Properties and Invasion in Human Bladder Cancer. *Cancer (Basel)* (2019) 11(3):349. doi: 10.3390/cancers11030349
60. Wu Y, Chen W, Xu ZP, Gu W. PD-L1 Distribution and Perspective for Cancer Immunotherapy-Blockade, Knockdown, or Inhibition. *Front Immunol* (2019) 10:2022. doi: 10.3389/fimmu.2019.02022

61. Gu W, Zhu Y, Ye D. Beyond Chemotherapy for Advanced Disease-the Role of EGFR and PD-1 Inhibitors.Transl. *Androl Urol* (2017) 6(5):848–54. doi: 10.21037/tau.2017.03.92
62. Hu L, Shan X, Han D, Guo Z, Wang H, Xiao Z. Multimodal Treatment Combining Salvage Surgery-Assisted Chemotherapy and Checkpoints Blockade Immunotherapy Achieves Complete Remission on a Recurrent Penile Cancer Patient: A Case Report. *Onco Targets Ther* (2021) 14:4891–6. doi: 10.2147/OTT.S319932
63. Denis C, Sakalihasan S, Frères P, Withofs N, Sautois B. Cemiplimab for Cisplatin Resistant Metastatic Penile Cancer. *Case Rep Oncol* (2021) 14 (2):972–6. doi: 10.1159/000517008
64. Shen H, Wang L, Ge X, Jiang CF, Shi ZM, Li DM, et al. MicroRNA-137 Inhibits Tumor Growth and Sensitizes Chemosensitivity to Paclitaxel and Cisplatin in Lung Cancer. *Oncotarget* (2016) 7(15):20728–42. doi: 10.18632/oncotarget.8011
65. Pan S, Ren F, Li L, Liu D, Li Y, Wang A, et al. MiR-328-3p Inhibits Cell Proliferation and Metastasis in Colorectal Cancer by Targeting Girdin and Inhibiting the PI3K/Akt Signaling Pathway. *Exp Cell Res* (2020) 390 (1):111939. doi: 10.1016/j.yexcr.2020.111939
66. Ozata DM, Gainetdinov I, Zoch A, O'Carroll D, Zamore PD. PIWI-Interacting RNAs: Small RNAs With Big Functions. *Nat Rev Genet* (2019) 20(2):89–108. doi: 10.1038/s41576-018-0073-3
67. Guo B, Li D, Du L, Zhu X. piRNAs: Biogenesis and Their Potential Roles in Cancer. *Cancer Metastasis Rev* (2020) 39(2):567–75. doi: 10.1007/s10555-020-09863-0
68. Fonseca CG, Azevedo SPJ, Vidal AF, Santos S, Ribeiro-dos-Santos Â. piRNAs in Gastric Cancer: A New Approach Towards Translational Research. *Int J Mol Sci* (2020) 21(6):2126. doi: 10.3390/ijms21062126
69. Vinasco-Sandoval T, Moreira FC, Vidal AF, Pinto P, Ribeiro-dos-Santos A, Cruz RLS, et al. Global Analyses of Expressed Piwi-Interacting RNAs in Gastric Cancer. *Int J Mol Sci* (2020) 21(20):7656. doi: 10.3390/ijms21207656
70. Araújo T, Khayat A, Quintana L, Calcagno D, Mourão R, Modesto A, et al. Piwi Like RNA-Mediated Gene Silencing 1 Gene as a Possible Major Player in Gastric Cancer. *World J Gastroenterol* (2018) 24(47):5338–50. doi: 10.3748/wjg.v24.i47.5338
71. Dahariya S, Paddibhatla I, Kumar S, Raghuwanshi S, Pallepati A, Gutti RK. Long Non-Coding RNA: Classification, Biogenesis and Functions in Blood Cells. *Mol Immunol* (2019) 112:82–92. doi: 10.1016/j.molimm.2019.04.011
72. Gao Q, Fang X, Chen Y, Li Z, Wang M. Exosomal lncRNA UCA1 From Cancer-Associated Fibroblasts Enhances Chemoresistance in Vulvar Squamous Cell Carcinoma Cells. *J Obstet Gynaecol Res* (2021) 47(1):73–87. doi: 10.1111/jog.14418
73. Macedo J, Silva E, Nogueira L, Coelho R, Silva J, Santos A, et al. Genomic Profiling Reveals the Pivotal Role of hrHPV Driving Copy Number and Gene Expression Alterations, Including mRNA Downregulation of TP53 and RB1 in Penile Cancer. *Mol Carcinog* (2020) 59(6):604–17. doi: 10.1002/mc.23185
74. Feng Y, Dramani Maman STD, Zhu X, Liu X, Bongolo CC, Liang C, et al. Clinical Value and Potential Mechanisms of LINC00221 in Hepatocellular Carcinoma Based on Integrated Analysis. *Epigen* (2021) 13(4):299–317. doi: 10.2217/epi-2020-0363
75. Tang H, Han X, Li M, Li T, Hao Y. Linc00221 Modulates Cisplatin Resistance in Non-Small-Cell Lung Cancer *via* Sponging miR-519a. *Biochimie* (2019) 162:134–43. doi: 10.1016/j.biochi.2019.04.019
76. Kakimoto Y, Tanaka M, Kamiguchi H, Ochiai E, Osawa M. MicroRNA Stability in FFPE Tissue Samples: Dependence on GC Content. *PloS One* (2016) 11(9):e0163125. doi: 10.1371/journal.pone.0163125

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Trends in Incidence, Mortality, and Survival of Penile Cancer in the United States: A Population-Based Study

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Purpose: The aim of this study is to investigate the trends in incidence and mortality, and explore any change in survival of penile cancer in the United States.

Methods: We obtained data from the Surveillance, Epidemiology, and End Results (SEER) database (2000–2018) utilizing the SEER Stat software. The joinpoint regression was used to analyze the secular trend of incidence and incidence-based mortality (IBM) stratified by age, race, and summary stage. The 5-year relative survival rate was also calculated.

Result: The age-adjusted rates of penile cancer patients were 0.38 (0.37–0.39) and 0.21 (0.2–0.21) for overall incidence and IBM, respectively. The 5-year relative survival rates were 67.7%, 66.99%, and 65.67% for the calendar periods of 2000–2004, 2005–2009, and 2010–2014, respectively. No significant changes in incidence by era were observed from 2000 to 2018 [annual percentage change (APC) = 0.5%, $p = 0.064$]. The IBM rate of penile cancer showed an initial significant increase from 2000 to 2002 (APC = 78.6%, 95% CI, −1.7–224.6) followed by a deceleration rate of 4.6% (95% CI, 3.9–5.3) during 2002 to 2018. No significant improvement in 5-year relative survival was observed. The trends by age, race, and summary stage in incidence and IBM were significantly different.

Conclusion: This study, using population-level data from the SEER database, showed an increasing trend in IBM and no significant improvement in the 5-year relative survival rate. Meanwhile, the incidence of penile cancer exhibited a relatively stable trend during the study period. These results might be due to the lack of significant progress in the treatment and management of penile cancer patients in the United States in recent decades. More efforts, like increasing awareness among the general population and doctors, and centralized management, might be needed in the future to improve the survival of this rare disease.

Keywords: penile cancer, incidence, mortality, survival, SEER, epidemiology, trend

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INTRODUCTION

Penile cancer is a relatively rare neoplasm in Western countries, presenting an incidence rate of less than 1 per 100,000 men (1). However, a prominent geographic variation in incidence can be observed, and it may be due to different socioeconomic status, hygiene, religious, and cultural conditions (2, 3). For example, incidence rates in some developing countries like India (up to 2.3 per 100,000) and Eastern and Southern Africa (up to 2.7 per 100,000) were significantly higher than 1 per 100,000 men (1, 4). Brazil is one of the countries with the highest penile cancer incidence rates, which reached up to 3.3 per 100,000 based on record. A relatively higher mortality and a gradually increasing trend with an annual percent change (APC) of 1.4% are also reported in these countries during 1996 to 2010 (1, 5). Relative survival rates also showed a geographic variation between countries. For instance, the relative 5-year survival rate in Norway is 80%, while this value is only 62% in Finland from 1999 to 2003 (6). Risk factors confirmed to be associated with penile cancer include human papillomavirus (HPV) infection, smoking, circumcision status, and lower socioeconomic status (7–9). Although the exact pathogenesis is still unclear, some studies suggested that inflammation may play an essential role in tumor development or progression because tumors may likely arise from sites of penile infection and chronic irritation (10, 11).

Significant differences in incidence and mortality rate trends of penile cancer existed among different countries. For example, the trend in the incidence of penile cancer has been presented as increasing in Denmark during 1978–2008. However, in the United States, the trend of penile cancer incidence showed a significant decrease with a rate of 0.84, 0.69, and 0.58 per 100,000 for 1973 –1982, 1982–1992, and 1993–2002, respectively (12).

The trend of incidence rate and mortality rate of a disease can reflect the prevention, treatment, and management level of the disease, thereby deepening the understanding of disease and making recommendations for disease guidelines. As a developed country, America's advanced medical technology and disease management strategy are often explored and used for reference by other countries. To our knowledge, there has been a lack of studies describing the trend of incidence, mortality, and survival of penile cancer in the United States over the past 10 years. In addition, a comparative study to explore the association between incidence, mortality, and survival rate has not been performed. This analysis, based on the Surveillance, Epidemiology, and End Results (SEER) database (2000–2018), aims to explore the up-to-date epidemiology of penile cancer in the United States. The trends in incidence, mortality, and survival of penile cancer by age, race, and summary stage are investigated according to the up-to-date information of epidemiology.

MATERIALS AND METHODS

Data Sources and Study Population

We obtained penile cancer patients from the SEER Program of the National Cancer Institute (ID: 20420-Nov2020). Patients diagnosed with penile cancer as their first malignancy according to the list of *Site Recode the International Classification of Diseases for Oncology, Third Edition (ICD-O-3)* and cases who were coded with penis were enrolled in our study.

Incident cases were obtained from the database of incidence–SEER 18 registries of the US National Cancer Institute from 2000 to 2018, which collected data on cancer incidence and mortality involving approximately 26.4% of the U.S. population.

Incidence-based mortality (IBM) cases were obtained from the database of IBM–SEER 18 registries of the US National Cancer Institute from 2000 to 2018.

Survival cases were obtained from the database of incidence–SEER 18 registries of the U.S. National Cancer Institute from 2000 to 2014. We failed to acquire more data considering the 5-year relative survival rate was not recorded after 2014 in the SEER database. The study period was averagely divided into three time periods (2000–2004, 2005–2009, and 2010–2014) to observe prominent survival rate disparities.

Outcomes and Descriptions

Three primary outcomes were calculated in this study: incidence, IBM, and 5-year relative survival rate. Incidence and IBM rates were adjusted to the 2000 U.S. standard population and calculated by 100,000 person-years. We calculated IBM rates as the number of all-cause death cases diagnosed with penile cancer among cases diagnosed over person-time at risk among people in areas of the SEER. In the registries of population-based SEER cancer, the incidence of individuals was linked to their mortality outcomes. It could calculate mortality rates by variables like the stage at diagnosis. This special mortality measure was defined as IBM (13, 14). Relative survival estimates were defined as the ratio of the observed survival of penile cancer patients and the expected survival of the underlying general population (15).

Then, we analyzed the annual percentage change (APC) of incidence and IBM rates stratified by age (15–44, 45–54, 55–64, 65–74, and 75+), race [White, Black, American/Indian/Alaska/Native (AIAN), and Asian/Pacific Islander (API)], and summary stage (localized, regional, and distant). Localized stage referred to an invasive neoplasm confined entirely to the penis (mainly including $T_{1\text{-}4}N_0M_0$) and tumor staged as regional was defined as extending to surrounding organs, tissues, or regional lymph nodes (mainly including $T_4N_0M_0$ or $T_{1\text{-}4}N_{1\text{-}3}M_0$). Distant disease referred to the tumor that had spread to remote sites of the body (mainly including M_1).

Statistical Analysis

SEER_Stat version 8.3.2 was used to calculate incidence, mortality rates, and 5-year relative survival rate of penile cancer. Then, joinpoint regression was performed to identify the best-fitting log-linear regression model, which appropriately demonstrated the incidence and mortality rate trend by era. The National Cancer Institute's Joinpoint Regression Program, Version 4.6.0.0, was utilized to calculate the APC and 95% confidence intervals (95% CIs) (16). The Joinpoint Regression software utilized *t*-tests to confirm whether statistical differences existed between APC and zero, and $p < 0.05$ was considered statistically significant. All statistical results were two-sided.

Notably, we excluded the data not recorded from the joinpoint regression because no cases were reported at a certain year.

RESULTS

Patient Characteristics

Finally, 6,397 patients diagnosed with penile cancer, who were from 18 SEER registries from 2000 to 2018, were enrolled in our study. **Table 1** demonstrates the characteristics of patients for incidence and IBM analysis. For all cases, the most common age group was 75+ years [2,132 (33.33%)], and White cases accounted for the most significant proportion in the study population [5,017 (83.55%)]. Compared to patients with other stages, patients with localized stage were more commonly seen [3,032 (47.4%)]. Of the eligible patients, 3,348 patients with penile cancer died during the study period. Of all the deaths, 1,399 (45.85%) patients were observed to be aged 75+ years, and 2,572 (84.3%) cases were White patients. A total of 1,495 (49%) patients who were recorded as dead were diagnosed with localized stage.

Overall Incidence and Mortality Rates and Trends Over Time

Of all study populations, the age-adjusted rates of penile cancer patients were 0.38 (0.37–0.39) and 0.21 (0.2–0.21) for incidence and IBM rate, respectively (**Table 1**). The incidence of penile cancer had no significant change from 2000 to 2018 (APC = 0.5%, 95% CI = −1.1–2.0; $p = 0.064$) (**Figure 1A** and **Table 2**). The IBM rate of penile cancer showed an initial significant increase from 2000 to 2002 (APC = 78.6%, 95% CI, −1.7–224.6) followed by a deceleration rate of 4.6% (95% CI, 3.9–5.3) during 2002 to 2018 (**Figure 1B** and **Table 2**).

Incidence and Mortality Rates and Trends by Age, Race, and Summary Stage

The penile cancer incidence rates were highest among cases aged over 75 years (2.31, 95% CI, 2.22–2.42), White patients (0.402, 95% CI, 0.391–0.413), and patients diagnosed with localized stage (0.18, 95% CI, 0.18–0.19) (**Table 1**). Similarly, the IBM rates of penile cancer were highest among patients aged 75+ years (0.094, 95% CI, 0.089–0.099), AIAN patients (0.259, 95% CI, 0.172–0.37), and patients with localized stage (0.098, 95% CI, 0.093–0.104) (**Table 1**).

The incidence rates among penile cancer patients in the age group of 45–54 and 75+ years exhibited a slight increase with an APC of 1.9% (95% CI, 0.1–3.7, $p = 0.043$) and 1.2% (95% CI, 0.5–1.9, $p = 0.002$), respectively, for the period of 2000–2018 (**Figures 2B, E** and **Table 2**). We did not obtain statistically significant trends in incidence rates in other age groups. For IBM rate analysis by age, patients diagnosed at ages 45–54, 55–64, and 75+ years exhibited an increasing trend at the rate of 7.1% (95% CI, 3.4–10.8, $p = 0.001$), 1.8% (95% CI, 0.6–3.2, $p = 0.008$), and 4.8% (95% CI, 2.6–7.0, $p = 0.001$), respectively, in 2000 to 2017 (**Figures 2G, H, J** and **Table 2**). In addition, for those aged 65–74 years, the trend of IBM presented a rapid initial increase (APC = 99.1%, 95% CI, −0.2–297.1) and then showed a deceleration for 2002–2017 (APC = 4.3%, 95% CI, 2.8–5.8, $p < 0.001$). (**Figure 2I** and **Table 2**).

A slightly increased incidence trend was observed from 2000 to 2017 with an APC of 0.7% (95% CI, 0.01–1.5, $p = 0.044$) among

TABLE 1 | Penile cancer incidence (2000–2018) and incidence-based mortality (2000–2018): the SEER-18 registry database.

Characteristic	Incidence Cases No. (%)	Rate (95% CI)	Incidence-based mortality Deaths No. (%)	Rate (95% CI)
All patients (2000–2018)	6,397 (100)	0.38 (0.37–0.39)	3,348 (100)	0.21 (0.2–0.21)
Age, years				
Overall (2000–2018)	6,397 (100)		3,051 (100)	
15–44	478 (7.47)	0.08 (0.07–0.08)	122 (4)	0.009 (0.007–0.01)
45–54	779 (12.18)	0.34 (0.32–0.37)	245 (8.03)	0.014 (0.013–0.016)
55–64	1,358 (21.23)	0.77 (0.73–0.81)	514 (16.85)	0.029 (0.027–0.032)
65–74	1,650 (25.79)	1.51 (1.44–1.59)	771 (25.27)	0.054 (0.05–0.058)
75+	2,132 (33.33)	2.31 (2.22–2.42)	1,399 (45.85)	0.094 (0.089–0.099)
Race[1]				
Overall (2000–2017)	6,005 (100)		3,051 (100)	
White	5,017 (83.55)	0.402 (0.391–0.413)	2,572 (84.3)	0.205 (0.198–0.214)
Black	597 (9.94)	0.401 (0.368–0.435)	328 (10.75)	0.241 (0.215–0.269)
AIAN	56 (0.92)	0.4 (0.296–0.525)	31 (1.02)	0.259 (0.172–0.37)
API	272 (4.53)	0.198 (0.175–0.223)	117 (3.83)	0.093 (0.077–0.112)
Summary stage[2]				
Overall (2000–2018)	6,397 (100)		3,051 (100)	
Localized	3,032 (47.4)	0.18 (0.18–0.19)	1,495 (49)	0.098 (0.093–0.104)
Regional	1,564 (24.45)	0.09 (0.09–0.1)	964 (31.6)	0.063 (0.063–0.067)
Distant	296 (4.63)	0.02 (0.02–0.02)	259 (8.49)	0.017 (0.015–0.019)

1: The limited number of patients whose race was unknown was excluded from further evaluation in the incidence and incidence-based mortality (IBM) (n = 63 and n = 3, respectively) analyses. Therefore, the percentages of patients of different races in the incidence and IBM analyses do not add up to 100%.

2: The limited number of patients whose summary was unknown was excluded from further evaluation in the incidence and incidence-based mortality (IBM) (n = 1505 and n = 332, respectively) analyses. Therefore, the percentages of patients of different races in the incidence and IBM analyses do not add up to 100%.

CI, confidence interval; SEER, Surveillance, Epidemiology, and End Results database. AIAN, American/Indian/Alaska/Native; API, Asian/Pacific Islander; NA, not applicable.

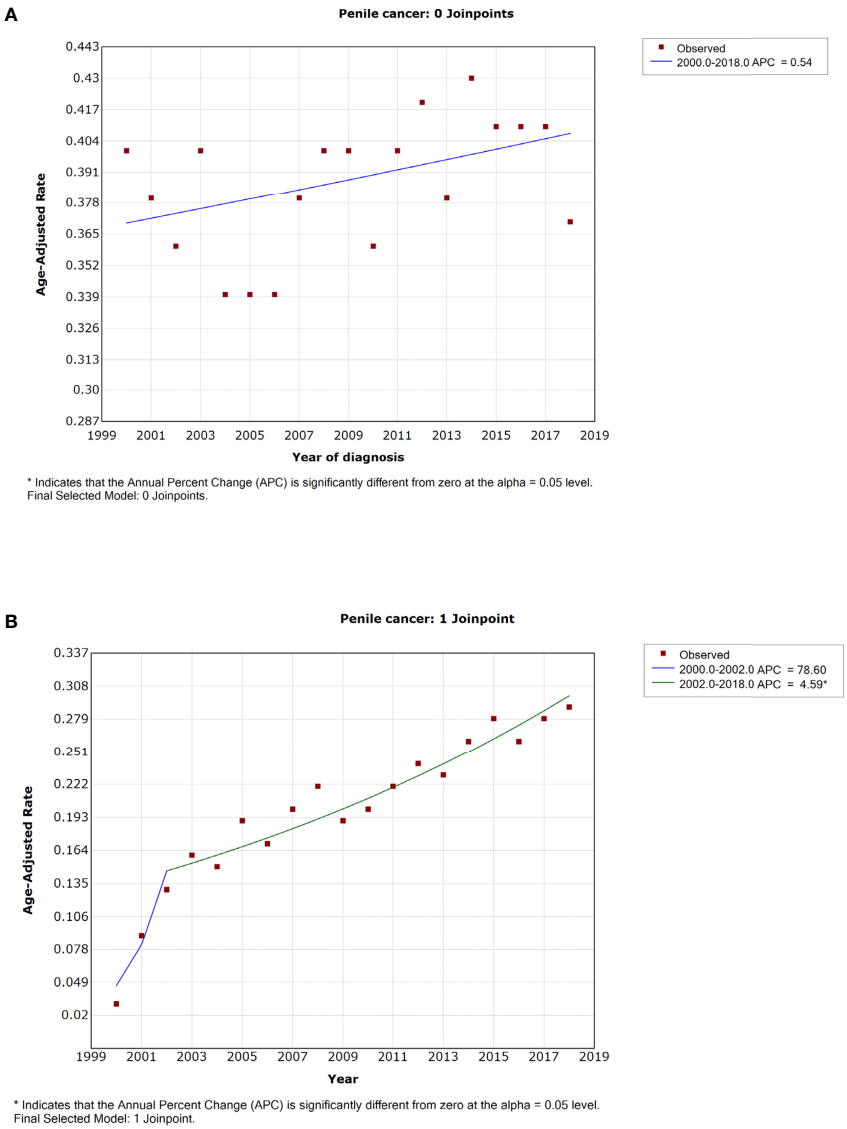

FIGURE 1 | The overall trends in incidence **(A)** and incidence-based mortality **(B)** of penile cancer.

White penile cancer patients (**Figure 3A** and **Table 2**). No noticeable change in incidence was observed in other races. Of Black and API patients, the trend of IBM rates demonstrated a slowly rising trend with an APC of 4.6% (95% CI, 3.6–5.5, $p < 0.001$) and 4.9% (95% CI, 0.6–9.4, $p = 0.029$), respectively (**Figures 3F, H** and **Table 2**). The IBM rate in White patients increased sharply at the initial time of 2000 to 2002 (APC = 96.2%, 95% CI 24.0–210.4, $p = 0.007$), and the increasing trend had slowed down in 2002 (APC = 4.6%, 95% CI 3.6–5.5, $p < 0.001$). (**Figure 3E** and **Table 2**).

No significant changes were observed in the incidence trend by summary stage from 2000 to 2015. We failed to obtain a best-fitting line and APC for patients with distant stage due to the relatively low incidence and the lack of regular variation (**Figure 4F**). Of patients diagnosed with localized stage, the trend of IBM rates showed an initial prominent increase during 2000 to 2005 (APC = 29.6%, 95% CI = 11.2–51.1, $p = 0.003$), followed by a deceleration thereafter (APC = 2.7%, 95% CI, 0.6–4.9, $p = 0.015$) (**Figure 4A** and **Table 2**). For patients with regional stage, a continuous increasing IBM rate was observed from 2002 to 2015 (APC = 4.7%, 95% CI, 3.0–6.5; $p < 0.001$), but a steep decline in the trend of IBM rates was exhibited after 2015 (APC = −32.5%, 95% CI, −49.9–−9.2, $p = 0.014$) (**Figure 4B** and **Table 2**).

Trend of the 5-Year Relative Survival Rate of Penile Cancer

The overall 5-year relative survival rates showed a slight decrease with a rate of 67.7% (SE = 1.76%), 66.99% (SE = 1.7%), and 65.67% (SE = 1.66%) for the time periods 2000–2004, 2005–2009, and 2010–2014, respectively (**Table 3**). However, this change was not

TABLE 2 | Trends in the incidence rates and incidence-based mortality of penile cancer (2000–2018): the SEER-18 registry database.

Characteristic	Incidence			Incidence-based mortality		
	Year	APC (95% CI)	*p*-value	Year	APC (95% CI)	*p*-value
All patients	2000–2018	0.5 (–1.1–2.0)	0.064	2000–2002	78.6 (–1.7–224.6)	0.052
				2002–2018	4.6 (3.9–5.3)	<0.001※
Age, years						
15–44	2000–2018	–0.5 (–2.0–1.1)	0.506	2000–2017	1.5 (–0.01–3.1)	0.054
45–54	2000–2018	1.9 (0.1–3.7)	0.043※	2000–2017	7.1 (3.4–10.8)	0.001※
55–64	2000–2018	0.9 (–1.7–3.5)	0.491	2000–2017	1.8 (0.6–3.2)	0.008※
65–74	2000–2018	–0.2 (–1.1–0.8)	0.741	2000–2002	99.1 (–0.2–297.1)	0.050
				2002–2017	4.3 (2.8–5.8)	<0.001※
75+	2000–2018	1.2 (0.5–1.9)	0.002※	2000–2017	4.8 (2.6–7.0)	<0.001※
Race						
White	2000–2017	0.7 (0.01–1.5)	0.044※	2000–2002	96.2 (24.0–210.4)	0.007※
				2002–2017	4.6 (3.6–5.5)	<0.001※
Black	2000–2017	0.4 (–0.8–1.6)	0.472	2000–2017	3.6 (0.6–6.6)	0.021※
AIAN	2000–2017	2.7 (–2.5–8.1)	0.289	2000–2017	1.7 (–0.5–4.0)	0.118
API	2000–2017	–0.7 (–3.1–1.7)	0.546	2000–2017	4.9 (0.6–9.4)	0.029※
Summary stage						
Localized	2000–2015	0.5 (–0.5–1.5)	0.313	2000–2005	29.6 (11.2–51.1)	0.003※
				2005–2017	2.7 (0.6–4.9)	0.015※
Regional	2000–2015	0.5 (–0.9–1.9)	0.459	2000–2002	94.8 (–1.0–283.5)	0.053
				2002–2015	4.7 (3.0–6.5)	<0.001※
				2015–2017	–32.5 (–49.9––9.2)	0.014※
Distant	NA	NA	NA	2000–2017	1.8 (–1.0–4.6)	0.203

AIAN, American/Indian/Alaska/Native; API, Asian/Pacific Islander; NA, not applicable.
※: Statistical significance.

statistically significant (p = 0.12). The descending trend in relative survival rates was observed in White and Black patients, and it was relatively prominent in Black patients (change −8.08%, $p < 0.001$) (**Table 3**). For patients diagnosed with localized stage, the 5-year relative survival rates exhibited an increasing trend with a rate of 76.6% (SE = 2.2%), 79.57% (SE = 2.11%), and 81.55% (SE = 2.1%) for time periods 2000–2004, 2005–2009, and 2010–2014, respectively (change 4.95%, $p < 0.001$) (**Table 3**). For API, the 5-year relative survival rates increased from 72.62% in 2000–2004 to 91.52% in 2005–2009, and then dropped to 65.72% in 2010–2014. Similar trends were observed in patients diagnosed with regional stage and diagnosed at 15–44, 45–54, and 75+ years. However, none of these trends was regular and easily to explain.

DISCUSSION

This study comprehensively explored the trend of incidence, IBM, and 5-year relative survival rate of penile cancer in the United States during 2000–2018, and further examined the trend by stratifying age, race, and tumor stage. There were no significant changes in the trend of incidence of penile cancer from 2000 to 2018. However, we

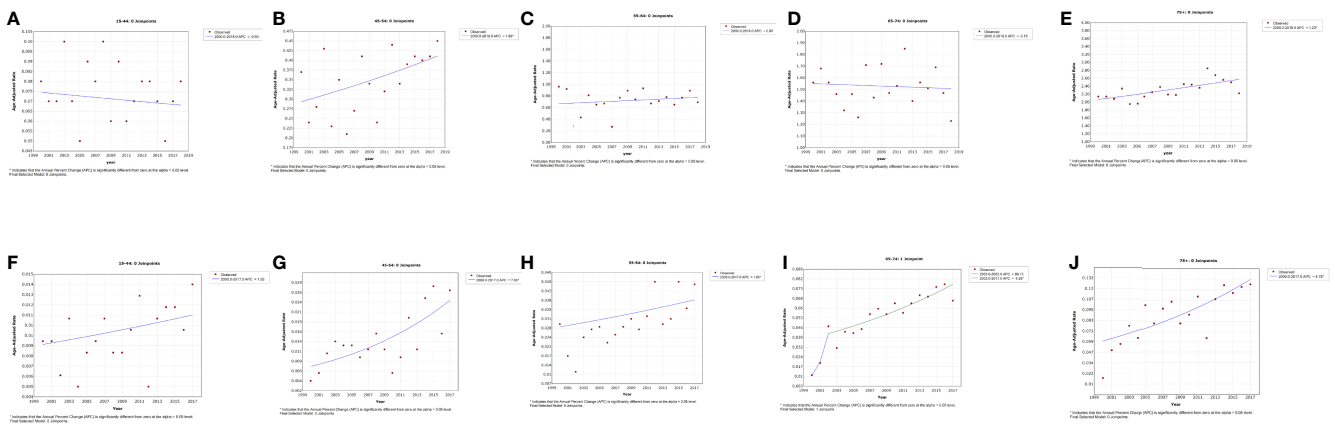

FIGURE 2 | Trends in the annual incidence **(A–E)** and incidence-based mortality **(F–J)** of penile cancer in patients stratified by age at diagnosis.

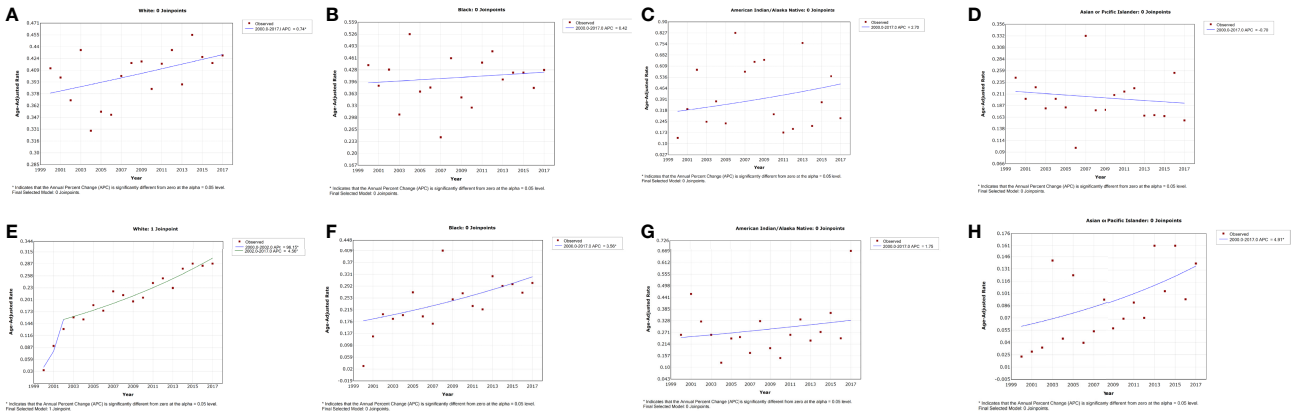

FIGURE 3 | Trends in the annual incidence **(A–D)** and incidence-based mortality **(E–H)** of penile cancer in patients stratified by race.

found that the IBM rate of penile cancer significantly increased and that there was no significant improvement in the 5-year relative survival rate over the study period.

The incidence rate of penile cancer, at 0.38 per 100,000 over 2000 to 2018, was relatively lower than the result from a previous study based on the SEER database (12). They found an incidence rate of 0.84, 0.69, and 0.58 per 100,000 for the calendar periods 1973–1982, 1982–1992, and 1993–2002, respectively, and the data were collected from 9 SEER registries, which cover approximately 9.4% of the U.S. population. Compared to the previous incidence, the incidence in this study had still decreased. However, considering the geographical variation, we should seriously explain this discrepancy considering that our data came from 18 registries.

There is a relatively big difference between the trend in the incidence rate of different countries. For instance, the trend in the incidence of penile cancer was increasing for Denmark over 1978–2008 and England over 1979–2009 (17, 18), whereas this tendency was inverse in Finland during 1971–1995 and the United States during 1973–2002 (19, 20). In this study, we found a stable trend in the incidence of penile cancer in the United States during 2000–2018, although we obtained a slight upward best-fitting line ($p > 0.05$, **Figure 1A**). Although we observed a slight increase in incidence rate for patients aged 45–54 and 75+ years and White

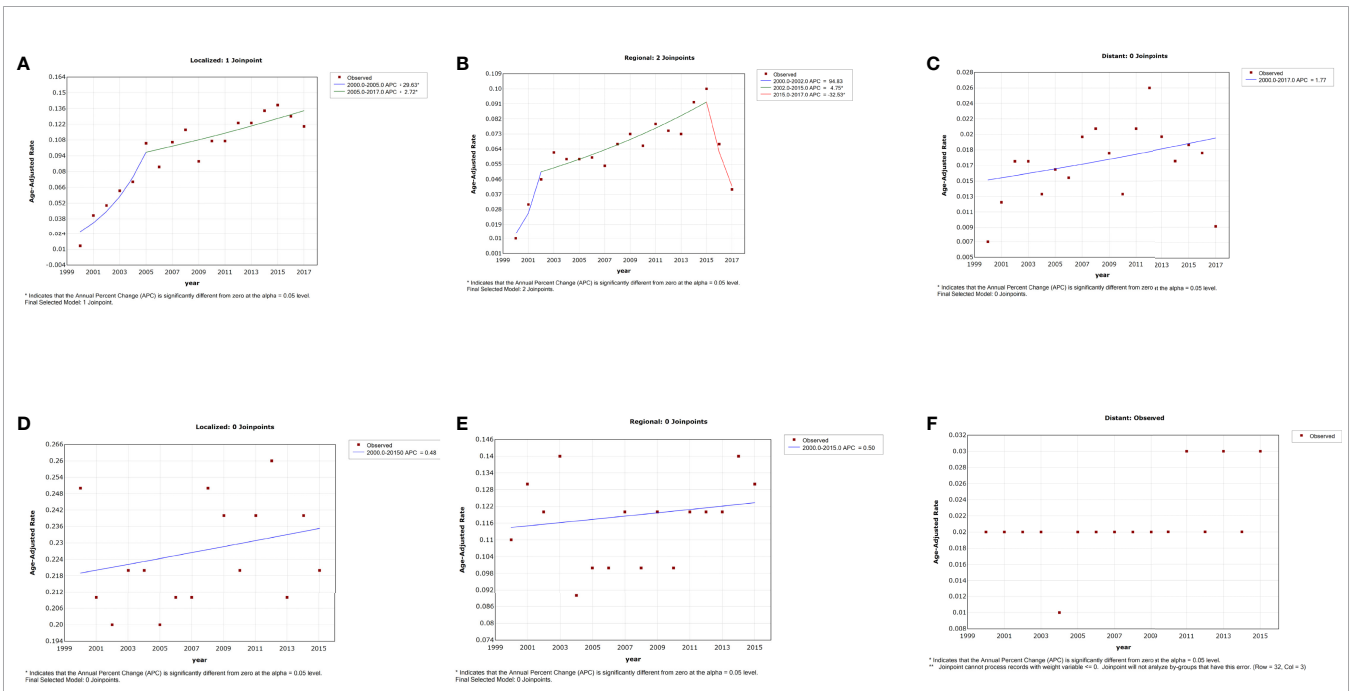

FIGURE 4 | Trends in the annual incidence **(D–F)** and incidence-based mortality **(A–C)** of penile cancer in patients stratified by summary stage.

TABLE 3 | Five-year relative survival rate of penile cancer patients by race, stage, and age.

Characteristic	Year diagnosed						Change※			
	2000–2004			2005–2009			2010–2014			
	N	5-year rate	SE	*N*	5-year rate	SE	*N*	5-year rate	SE	
Overall	1,175	67.7%	1.76%	1,187	66.99%	1.7%	1,379	65.67%	1.66%	–2.03%
Race										
White	996	67.18%	1.91%	1,007	66%	1.86%	1,133	65.63%	1.85%	–1.55%
Black	122	67.66%	5.64%	105	65.88%	5.61%	140	59.58%	4.9%	–8.08%
API	46	72.62%	7.68%	52	91.52%	4.61%	71	65.72%	6.37%	–6.87%
Stage										
Localized	687	76.6%	2.2%	703	79.57%	2.11%	800	81.55%	2.1%	4.95%
Regional	371	59.74%	3.21%	367	62.03%	3.11%	422	52.69%	2.91%	–7.05%
Distant	59	17.29%	5.51%	61	14.75%	4.83%	96	15.56%	4.03%	–1.73%
Age										
15–44	145	77.61%	3.75%	121	78.51%	4%	123	73.34%	4.44%	–4.27%
45–54	201	69.93%	3.46%	175	81.31%	3.22%	184	69.42%	3.69%	–0.54%
55–64	305	72.7%	2.9%	278	69.58%	3.07%	342	69.74%	2.86%	–2.96%
65–74	353	67.62%	3.09%	291	65.82%	3.36%	351	71.11%	3.16%	3.49%
75+	391	56.83%	3.92%	322	63.77%	4.2%	379	54.86%	4.17%	–1.97%

Change※: in the 5-year relative survival between 2000–2004 and 2010–2014, in % units.
5-year rate: 5-year relative survival rate.
N, number of patients; SE, standard error; API, Asian/Pacific Islander.

patients, the extent of this change was quite small. Previous studies usually explain the decreasing incidence rate with improved sanitation, declining smoking rate, and newborn male circumcision (21–23). For example, several data-based studies suggested that the rate of male circumcision ranged from 42% to 80% in the United States, and the procedure is commonly performed during the newborn period (24). The available evidence proved that male circumcision had special benefits in preventing urinary tract infection, HIV infection, the transmission of some sexually transmitted infections, and penile cancer (25). A relatively higher rate of male circumcision was considered a protective factor for penile cancer, and it might be a crucial reason for the stable incidence in the United States. Another notable reason was chronic inflammation, which was considered as a significant pathogenic pathway of penile cancer (25–28). A relatively perfect healthcare system and universal sex education might account for the lower rate of chronic inflammation than those in developing countries. These results showed that the prevention of penile cancer in the United States had a good performance.

There were relatively few studies focusing on the trend of penile cancer mortality. A retrospective study, whose data were from the Netherlands during 1989–2006, suggested a slight decrease in mortality (11, 29). Similarly, a decrease in mortality was also observed in England for 1979–2009 (30). Nevertheless, we found a prominent increase in the IBM rate in the United States for the period of 2000–2018. Interestingly, a rapid increase of IBM was observed at the initial period of 2000–2002 (APC = 78.6%), but it failed to obtain a statistically significant *p*-value due to the relatively short study period. Similarly, of patients aged 65–74 years, White cases, and patients with regional stage, we also observed a sharp increase in IBM in the initial period of 2000–2002 (**Figures 2I**, **3A**, **4B**). In addition, we also did not observe any improvement in the 5-year relative survival rate of penile cancer in the United States. The phenomenon of no significant improvement in the 5-year survival rate and increased mortality of penile cancer might be due to the lack of significant progress in the treatment and management of penile cancer (31).

A likely explanation for these results was difficult to make. A recent study suggested that penile sparing surgery had been increasingly adopted, and no prominent differences in survival were observed between patients undergoing sparing and complete surgery (32). This improved surgical approach might lead to a better quality of life. Still, its contribution to high-risk patients, especially those with positive lymph nodes and distant metastasis, was not remarkable. In the past two decades, the most significant progress in the treatment of penile cancer was treating primary lesions, modified lymphadenectomy, and identifying and treating occult regional lymph node metastasis with the help of sentinel lymph node biopsy (SNB) (33, 34). About 80% of patients with one or two lymph nodes involved can be cured by lymphadenectomy. Even patients with pelvic lymph node involvement can still be cured by surgery.

The main goal of SNB was to reduce mortality and improve survival in clinical lymph node-negative (cN0) patients. Reported data showed that about 20% to 25% of the cN0 penile cancer patients had occult lymph node metastases at diagnosis, and early surgical resection of these occult lymph nodes could obtain better survival than those with clinically apparent nodes (35). The introduction of SNB might thus have improved survival, especially those with occult lymph nodes. An unpublished study from the Netherlands does show that cancer-specific survival in cN0 patients had improved since the introduction of SNB. However, we did not observe an improvement in 5-year relative survival, and even an increase in mortality in patients with penile cancer was obtained in this study. This result might account for the relatively low referral

rate to hospitals specializing in the treatment of penile cancer, or the improvement of penile cancer treatment had not been fully implemented in hospitals.

For the management of penile cancer, several European countries have centralized management of penile cancer. The interval between diagnosis and treatment was significantly shortened, and compliance with the guidelines for patients with penile cancer was improved through this method (36, 37). Notably, the major delay in diagnosis of penile cancer was the time between the first symptom and diagnostic confirmation considering that patients and doctors might misinterpret the symptoms of penile cancer as condyloma, benign phimosis, or benign skin disease. This centralized management strategy could shorten this interval. In addition, this strategy was proved to work in improving survival and reducing mortality in the long run. Verhoeven et al. compared the 5-year relative survival rate of penile cancer patients between Europe and the United States over 1985–2007, and they found an increase from 65% to 70% and a decrease from 72% to 63% in the 5-year relative survival rate for Europe and the United States, respectively (38). For Norway and Denmark, the 5-year relative survival increased from 61% to 80% and 63% to 74%, respectively (6). However, the United States had not fully adopted this centralized management system, which might be an important explanation for the condition.

Another possible explanation for this result was that the main population of penile cancer patients was aging. For example, previous studies showed that the most common age of penile cancer patients was between 50 and 70 years (29, 39). However, patients aged 75+ years were the main population age group in our study. A higher proportion of elderly patients might lead to higher mortality and poor survival in penile cancer patients.

This is the first study that comprehensively explored the epidemiology of a rare disease from incidence, IBM, and 5-year relative survival for the period of 2000 to 2018 in the United States. The condition of penile cancer patients seemed to not have a noticeable improvement and progression considering the increasing IBM and the lack of significant change in the 5-year relative survival rate. Multiple comprehensive factors like changes in treatment and demographics, increase in exposure to HPV, and variation of cancer should be considered when interpreting results (22, 23, 40).

Several limitations should be noted when interpreting the results of this study. First, we selected a data list that collected epidemic information of approximately 26.4% of the U.S. population. Meanwhile, a relatively shorter study period was also chosen compared to previous similar studies. In addition, except for the low case numbers resulting in high standard errors of incidence, IBM, and survival estimates, essential pieces of information such as HPV infection, smoking, diagnosis, and follow-up treatment were not obtained in the SEER database. Finally, similar to the limitations of most epidemiological studies, our study has revealed a phenomenon in a period but cannot provide a definite explanation for the condition (6, 23, 38, 41). Therefore, more evidence was needed to explain these results.

CONCLUSION

The current study, using population-level data from the SEER database, provides valuable data on penile cancer. It shows an increasing trend in IBM and no significant improvement in the 5-year relative survival rate among penile cancer patients for the period of 2000 to 2018 in the United States. Meanwhile, the incidence of penile cancer exhibited a relatively stable trend during the study period. These results indicate the lack of significant progress in the treatment and management of penile cancer patients in the United States in recent decades. More efforts, like increasing awareness among the general population and doctors, and centralized management, may be needed in the future to improve the survival of this rare disease.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The data from SEER is publicly available and de-identified. This study was approved by the institution of the First Affiliated Hospital of Nanchang University. No informed consent was needed.

AUTHOR CONTRIBUTIONS

XD, YL and XZ contributed equally to this work. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

1. Bray F, Ferlay J, Laversanne M, Brewster DH, Gombe Mbalawa C, Kohler B, et al. Cancer Incidence in Five Continents: Inclusion Criteria, Highlights From Volume X and the Global Status of Cancer Registration. *Int J Cancer* (2015) 137(9):2060–71. doi: 10.1002/ijc.29670
2. Hernandez BY, Barnholtz-Sloan J, German RR, Giuliano A, Goodman MT, King JB, et al. Burden of Invasive Squamous Cell Carcinoma of the Penis in

the United States, 1998-2003. *Cancer* (2008) 113(10 Suppl):2883–91. doi: 10.1002/cncr.23743
3. Maiche AG. Epidemiological Aspects of Cancer of the Penis in Finland. *Eur J Cancer Prev* (1992) 1(2):153–8. doi: 10.1097/00008469-199202000-00008
4. Misra S, Chaturvedi A, Misra NC. Penile Carcinoma: A Challenge for the Developing World. *Lancet Oncol* (2004) 5(4):240–7. doi: 10.1016/S1470-2045(04)01427-5
5. de Souza DL, Curado MP, Bernal MM, Jerez-Roig J, Boffetta P. Mortality Trends and Prediction of HPV-Related Cancers in Brazil. *Eur J Cancer Prev* (2013) 22(4):380–7. doi: 10.1097/CEJ.0b013e32835b6a43
6. Bray F, Klint A, Gislum M, Hakulinen T, Engholm G, Tryggvadottir L, et al. Trends in Survival of Patients Diagnosed With Male Genital Cancers in the Nordic Countries 1964-2003 Followed Up Until the End of 2006. *Acta Oncol* (2010) 49(5):644–54. doi: 10.3109/02841860903575315
7. Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG, et al. Detection and Typing of Human Papillomavirus DNA in Penile Carcinoma: Evidence for Multiple Independent Pathways of Penile Carcinogenesis. *Am J Pathol* (2001) 159(4):1211–8. doi: 10.1016/S0002-9440(10)62506-0
8. Heideman DA, Waterboer T, Pawlita M, Delis-van Diemen P, Nindl I, Leijte JA, et al. Human Papillomavirus-16 is the Predominant Type Etiologically Involved in Penile Squamous Cell Carcinoma. *J Clin Oncol* (2007) 25(29):4550–6. doi: 10.1200/JCO.2007.12.3182
9. Bleeker MC, Heideman DA, Snijders PJ, Horenblas S, Dillner J, Meijer CJ. Penile Cancer: Epidemiology, Pathogenesis and Prevention. *World J Urol* (2009) 27(2):141–50. doi: 10.1007/s00345-008-0302-z
10. Dillner J, von Krogh G, Horenblas S, Meijer CJ. Etiology of Squamous Cell Carcinoma of the Penis. *Scandinavian J Urol Nephrol Supplementum* (2000) 205):189–93. doi: 10.1080/00365590050509913
11. Graafland NM, Verhoeven RH, Coebergh JW, Horenblas S. Incidence Trends and Survival of Penile Squamous Cell Carcinoma in the Netherlands. *Int J Cancer* (2011) 128(2):426–32. doi: 10.1002/ijc.25355
12. Barnholtz-Sloan JS, Maldonado JL, Pow-sang J, Giuliano AR. Incidence Trends in Primary Malignant Penile Cancer. *Urol Oncol* (2007) 25(5):361–7. doi: 10.1016/j.urolonc.2006.08.029
13. Chu KC, Miller BA, Feuer EJ, Hankey BF. A Method for Partitioning Cancer Mortality Trends by Factors Associated With Diagnosis: An Application to Female Breast Cancer. *J Clin Epidemiol* (1994) 47(12):1451–61. doi: 10.1016/0895-4356(94)90089-2
14. Guo F, Kuo YF, Shih YCT, Giordano SH, Berenson AB. Trends in Breast Cancer Mortality by Stage at Diagnosis Among Young Women in the United States. *Cancer* (2018) 124(17):3500–9. doi: 10.1002/cncr.31638
15. Ederer F, Axtell LM, Cutler SJ. The Relative Survival Rate: A Statistical Methodology. *Natl Cancer Institute Monograph* (1961) 6:101–21.
16. *Joinpoint Regression Program, Version 4.6.0.0 -April 2018; Statistical Methodology and Applications Branch, Surveillance Research Program*. National Cancer Institute.
17. Baldur-Felskov B, Hannibal CG, Munk C, Kjaer SK. Increased Incidence of Penile Cancer and High-Grade Penile Intraepithelial Neoplasia in Denmark 1978-2008: A Nationwide Population-Based Study. *Cancer Causes Control* (2012) 23(2):273–80. doi: 10.1007/s10552-011-9876-7
18. Daubisse-Marliac L, Colonna M, Tretarre B, Defossez G, Molinie F, Jehannin-Ligier K, et al. Long-Term Trends in Incidence and Survival of Penile Cancer in France. *Cancer Epidemiol* (2017) 50(Pt A):125–31. doi: 10.1016/j.canep.2017.08.014
19. Pukkala E, Weiderpass E. Socio-Economic Differences in Incidence Rates of Cancers of the Male Genital Organs in Finland, 1971-95. *Int J Cancer* (2002) 102(6):643–8. doi: 10.1002/ijc.10749
20. Backes DM, Kurman RJ, Pimenta JM, Smith JS. Systematic Review of Human Papillomavirus Prevalence in Invasive Penile Cancer. *Cancer Causes Control* (2009) 20(4):449–57. doi: 10.1007/s10552-008-9276-9
21. Larke NL, Thomas SL, dos Santos Silva I, Weiss HA. Male Circumcision and Penile Cancer: A Systematic Review and Meta-Analysis. *Cancer Causes Control* (2011) 22(8):1097–110. doi: 10.1007/s10552-011-9785-9
22. Schoffer O, Neumann A, Stabenow R, Schulein S, Bohm WD, Gonsior A, et al. Penile Cancer - Incidence, Mortality, and Survival in Saxony, Germany. *Urol Oncol* (2019) 37(4):295 e1– e8. doi: 10.1016/j.urolonc.2018.12.003
23. Hansen BT, Orumaa M, Lie AK, Brennhovd B, Nygard M. Trends in Incidence, Mortality and Survival of Penile Squamous Cell Carcinoma in Norway 1956-2015. *Int J Cancer* (2018) 142(8):1586–93. doi: 10.1002/ijc.31194
24. American Academy of Pediatrics Task Force on C. Male Circumcision. *Pediatrics* (2012) 130(3):e756–85. doi: 10.1542/peds.2012-1990
25. Hakenberg OW, Drager DL, Erbersdobler A, Naumann CM, Junemann KP, Protzel C. The Diagnosis and Treatment of Penile Cancer. *Dtsch Arztebl Int* (2018) 115(39):646–52. doi: 10.3238/arztebl.2018.0646
26. Attalla K, Paulucci DJ, Blum K, Anastos H, Moses KA, Badani KK, et al. Demographic and Socioeconomic Predictors of Treatment Delays, Pathologic Stage, and Survival Among Patients With Penile Cancer: A Report From the National Cancer Database. *Urol Oncol* (2018) 36(1):14 e7– e24. doi: 10.1016/j.urolonc.2017.09.014
27. Hakenberg OW, Comperat EM, Minhas S, Necchi A, Protzel C, Watkin N. EAU Guidelines on Penile Cancer: 2014 Update. *Eur Urol* (2015) 67(1):142–50. doi: 10.1016/j.eururo.2014.10.017
28. Marchioni M, Berardinelli F, De Nunzio C, Spiess P, Porpiglia F, Schips L, et al. New Insight in Penile Cancer. *Minerva Urol Nefrol* (2018) 70(6):559–69. doi: 10.23736/S0393-2249.18.03215-0
29. Qi F, Wei X, Zheng Y, Ren X, Li X, Zhao E. Incidence Trends and Survival Outcomes of Penile Squamous Cell Carcinoma: Evidence From the Surveillance, Epidemiology and End Results Population-Based Data. *Ann Transl Med* (2020) 8(21):1428. doi: 10.21037/atm-20-1802
30. Arya M, Li R, Pegler K, Sangar V, Kelly JD, Minhas S, et al. Long-Term Trends in Incidence, Survival and Mortality of Primary Penile Cancer in England. *Cancer Causes Control* (2013) 24(12):2169–76. doi: 10.1007/s10552-013-0293-y
31. Verhoeven RH, Janssen-Heijnen ML, Saum KU, Zanetti R, Caldarella A, Holleczek B, et al. Population-Based Survival of Penile Cancer Patients in Europe and the United States of America: No Improvement Since 1990. *Eur J Cancer* (2013) 49(6):1414–21. doi: 10.1016/j.ejca.2012.10.029
32. Kamel MH, Bissada N, Warford R, Farias J, Davis R. Organ Sparing Surgery for Penile Cancer: A Systematic Review. *J Urol* (2017) 198(4):770–9. doi: 10.1016/j.juro.2017.01.088
33. McDougal WS. Advances in the Treatment of Carcinoma of the Penis. *Urology* (2005) 66(Suppl 5):114–7. doi: 10.1016/j.urology.2005.06.007
34. Kroon BK, Horenblas S, Nieweg OE. Contemporary Management of Penile Squamous Cell Carcinoma. *J Surg Oncol* (2005) 89(1):43–50. doi: 10.1002/jso.20170
35. Kroon BK, Horenblas S, Lont AP, Tanis PJ, Gallee MP, Nieweg OE. Patients With Penile Carcinoma Benefit From Immediate Resection of Clinically Occult Lymph Node Metastases. *J Urol* (2005) 173(3):816–9. doi: 10.1097/01.ju.0000154565.37397.4d
36. Jakobsen JK, Jensen JB. DaPeCa-2: Implementation of Fast-Track Clinical Pathways for Penile Cancer Shortens Waiting Time and Accelerates the Diagnostic Process – A Comparative Before-And-After Study in a Tertiary Referral Centre in Denmark. *Scandinavian J Urol* (2015) 50(1):80–7. doi: 10.3109/21681805.2015.1077472
37. Kirrander P, Sherif A, Friedrich B, Lambe M, Hakansson U. Steering Committee of the Swedish National Penile Cancer R. Swedish National Penile Cancer Register: Incidence, Tumour Characteristics, Management and Survival. *BJU Int* (2016) 117(2):287–92. doi: 10.1111/bju.12993
38. Koh WJ, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Chan J, et al. Uterine Neoplasms, Version 1.2014. *J Natl Compr Canc Netw* (2014) 12(2):248–80. doi: 10.6004/jnccn.2014.0025
39. Clark PE, Spiess PE, Agarwal N, Biagioli MC, Eisenberger MA, Greenberg RE, et al. Penile Cancer: Clinical Practice Guidelines in Oncology. *J Natl Compr Cancer Network* (2013) 11(5):594–615. doi: 10.6004/jnccn.2013.0075
40. Rippentrop JM, Joslyn SA, Konety BR. Squamous Cell Carcinoma of the Penis: Evaluation of Data From the Surveillance, Epidemiology, and End Results Program. *Cancer* (2004) 101(6):1357–63. doi: 10.1002/cncr.20519
41. Ornellas AA, Kinchin EW, Nobrega BL, Wisnescky A, Koifman N, Quirino R. Surgical Treatment of Invasive Squamous Cell Carcinoma of the Penis: Brazilian National Cancer Institute Long-Term Experience. *J Surg Oncol* (2008) 97(6):487–95. doi: 10.1002/jso.20980

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REVIEW
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Emerging Therapies in Penile Cancer

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Advances in the treatment of rare tumors like penile cancer were always hampered by the lack of deep comprehension of the molecular biology and genomic and epigenomic alterations involved in carcinogenesis and tumor progression, as well as by the difficulty in recruitment of patients for prospective clinical trials. Despite the high rates of cure in early localized penile cancers with surgery or other local procedures, locally advanced and metastatic tumors require systemic treatment, with chemotherapy being the current standard, but with high toxicity and no proven real impact on survival. Recent important findings of frequent genomic alterations and mutation signatures in penile cancer have motivated several trials in new modalities of systemic treatments, especially immunotherapy. This review aims to present the most recent advances and the prospect of new modalities of systemic therapies with ongoing studies in penile cancer.

Keywords: penile cancer, human papilloma virus, chemotherapy, immune therapy, targeted therapy

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INTRODUCTION

Penile cancer is a rare disease with a total number of cases estimated at 36,068 globally in 2020 (0.92 cases/100,000 people) (1). However, these tumors have a higher incidence in developing countries, reaching up to 6.1 cases/100,000 people (2). The most frequent histology, responsible for almost the totality of cases, is squamous cell carcinoma (SCC). Overall survival (OS) in early disease without nodal involvement is 96% in 10 years with curative surgery (3), while 5-year median OS of patients with regional node disease and distant metastatic disease are, respectively, 50% and 12% (4).

Cytotoxic chemotherapy plays a key role in the systemic treatment and consists mainly of platinum and taxane combination regimens based on the results of small phase II trials, with typical chemotherapeutic toxicities and modest survival outcomes, both in advanced disease treatment (5, 6) and in the neoadjuvant setting (7, 8). There are no prospective randomized trials that address this issue. In the adjuvant and neoadjuvant scenario, the real role and better sequence of multimodal treatment with radiotherapy, surgery, and chemotherapy in patients with operable nodal involvement are still under investigation in the ongoing phase III International Penile Advanced Cancer trial (InPACT) study (NCT02305654).

There is an urgent need for more efficient and less toxic new modalities of systemic treatment for advanced penile SCC based on the current knowledge of its molecular pathogenesis, including targeted therapy, immunotherapy, and new classes of drugs and combinations regimens that can meet this demand. This review displays the current therapies available and the perspective of novel therapies under investigation.

CURRENT STANDARD OF SYSTEMIC TREATMENT: CYTOTOXIC CHEMOTHERAPY

Cytotoxic chemotherapy, based on different combinations that include platinum, 5-FU, taxanes, and ifosfamide remains the mainstay of systemic treatment. For patients with locally advanced disease (T3N+, T4, or N2/N3) overall response rate (ORR) with neoadjuvant chemotherapy varies from 50% to 60% (7, 8). The most recommended combination is paclitaxel, ifosfamide, and cisplatin (TIP). In a phase II trial, 30 patients with N2 or N3 disease were treated with neoadjuvant TIP and pathologic complete response occurred in 10%. Surgery was performed in 73.3% of patients and the median OS was 17.1 months (7). A different drug combination containing paclitaxel, 5-FU, and cisplatin (TPF) was evaluated in a phase II trial that included 26 patients with a successful surgery rate of 53% and median OS of 10.1 months (8). However, there are no phase III trial results to date that supports the use of neoadjuvant chemotherapy, and the rate of grade 3 toxicity of neoadjuvant chemotherapy containing taxanes is 49% (9). The InPACT (NCT02305654) is the first phase III trial of neoadjuvant chemotherapy in penile cancer and its results are expected in July 2022. This trial evaluates the role of neoadjuvant chemotherapy with or without radiation before surgery and the role of prophylactic pelvic lymph node dissection in those receiving adjuvant chemoradiation for high-risk inguinal node-positive disease. Regarding systemic treatment, there are three arms comparing no neoadjuvant treatment (arm A) vs. neoadjuvant TIP (arm B) vs. neoadjuvant chemoradiotherapy (radiation therapy + cisplatin).

In distant metastatic disease in patients with good performance status, TIP or TPF are frequently the first choice of treatment, although TIP was only evaluated in the neoadjuvant setting. The ORR with TPF was 38.5% and median OS of 7 months, but with grade 3 toxicities in 65% of patients (6). A less toxic two-drug regimen with cisplatin and 5-FU had an ORR of 32% and a median OS of 8 months (10). All the above results are from phase II trials, since there is no phase III trial in first or subsequent lines of treatment of metastatic penile SCC. No major advances have been made in recent years in this field. The most recent study with a different cytotoxic agent, vinflunine, showed a 27.3% ORR and 8.4 months OS (11). A phase II trial with gemcitabine and cisplatin, a widely used regimen in other advanced SCC, was completed, but the results were not published until this date (NCT00210041). In second-line treatment, a small phase II trial demonstrated an ORR of 20% and 5 months of median OS with paclitaxel in monotherapy (12).

GENOMIC LANDSCAPE

In the last few years, with the advances in next-generation sequencing (NGS) technologies, most of the genomic landscape of penile SCC became known (**Figure 1**), although the molecular signaling pathways and its role in carcinogenesis and tumor progression are yet to be better understood. Few studies have been reported from low-income countries, where the highest incidences of penile SCC are registered, especially in South America and Africa, and this can hamper a broader comprehension of the molecular pathogenesis of this disease. Some of the most relevant studies were analyzed in a very recent systematic review (13), where the most frequent somatic mutations found were TP53 (in up to 48%), CDKN2A, NOTCH1, PIK3CA, FAT1, CASP8, and FBXW7, and the most common copy number variations included gains in MYC (8q24) and amplification on EGFR (in up to 70% of cases). Amplifications or gains at HPV integration sites were identified in high frequencies (85 – 100%) in a single Brazilian study (14). The mutational burden was generally low and was found to be higher in HPV negative than in HPV positive associated penile SCC (15) and an even lower mutational burden was present in HPV positive malignancies with high viral load (16). HPV positive tumors were also associated with a lack of TP53 and CDKN2A mutations (15).

The most altered signaling pathways in penile SCC were NOTCH, RTK-RAS, and Hippo pathway (which frequently involves PIK3CA and EGFR alterations) in one recent study, which accounted for over 50% of tumors, and the frequently altered genes in these pathways were expressed in immunohistochemistry assay. RAS and Hippo pathways are potentially targetable with EGFR and mTOR inhibitors. Two mutation signatures were also identified in this study: the APOBEC-related signature, with a higher tumor mutational burden (TMB) with great potential to benefit from immunotherapy with checkpoint inhibitors, and the defective DNA repair system signature, which involves mutations in BRCA1, BRCA2, ARID1A, ATR, CHEK2, PARP1, FANCA, PALB2, and RAD51, a favorable scenario for treatment with immunotherapy and PARP inhibitors. The enrichment of NOTCH pathway alterations and the mutation signatures found in penile SCC in this trial were similar to head and neck SCC (17).

A study that performed comparative genomic profiling of refractory and metastatic penile and nonpenile cutaneous SCCs found a distinctive genomic pattern in penile SCC cases, including alterations in the mTOR pathway (NF1 and PTEN), in the DNA repair pathway (BRCA2 and ATM), and tyrosine kinase (EGFR, FGFR3, and ERBB2), all of them actionable therapeutic targets (18).

TARGETED AND ANTI-ANGIOGENIC AGENT THERAPY

The EGFR family is important in penile SCC biology. One of the largest series, with 112 patients, showed that 44% had high expression of EGFR by immunohistochemistry, despite histologic subtype, histologic grade, or HPV status (19), and since KRAS mutations (which confers resistance to EGFR blockage in cancer treatment) are rare in these tumors (20),

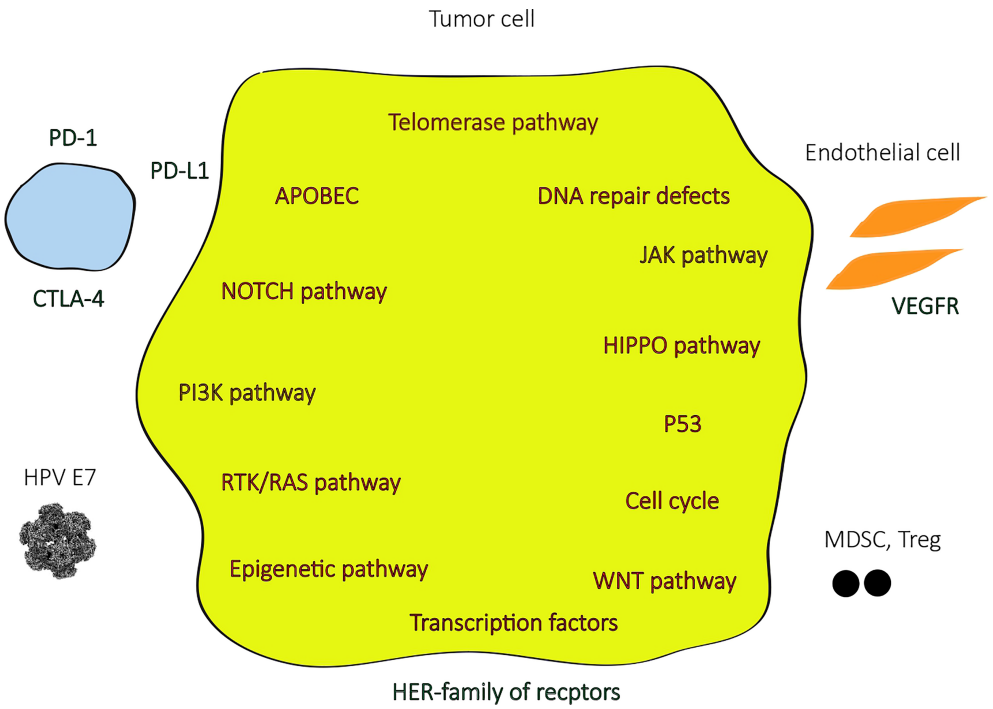

FIGURE 1 | Genomic alterations, mutation signatures, and tumor microenvironment on penile SCC.

EGFR inhibitors have a potential role as systemic treatment. In a retrospective study, of 28 patients that received anti-EGFR monoclonal antibodies (cetuximab and panitumumab), alone or in combination with chemotherapy, 50% had a response to treatment and the median PFS was 3 months (21). One phase II trial with 11 advanced penile SCC pretreated patients that received panitumumab as a salvage therapy reached complete response in two patients and partial response in one, all of them with skin or lymph node metastasis, with a 1.9 months PFS and 9.5 months OS. Patients with visceral metastasis had no response. Grade 3 toxicity occurred in four patients (22). The NCCN lists monoclonal antibody EGFR inhibitors cetuximab or panitumumab as potential options based on modest datasets of retrospective and prospective studies demonstrating evidence of activity. The pan-HER (EGFR/HER1, HER2, and HER4) inhibitor dacomitinib produced a complete response in one and partial responses in eight of 28 patients (ORR 32.1%) in a single-arm phase II trial. The 12-month progression-free survival (PFS) was 26.2% and 12-month OS was 54.9% (23). The PENILANE trial (NCT02014831), a phase II with the association of cetuximab + TIP chemotherapy, active from 2013 to 2016, was withdrawn by the industry sponsor.

Vascular endothelial growth factor-A (VEGF-A) is the activating ligand of the VEGF receptor (VEGF-R), which plays a major role in cancer angiogenesis, and was overexpressed in 53.7% of penile SCC in a retrospective study (24). A small series of anti-angiogenic tyrosine kinase inhibitors sorafenib or sunitinib in six pretreated advanced penile SCC patients did not show exciting results. One partial response and four stable diseases were observed. Three patients showed pain response and had an improvement in quality of life (25). The phase II PAZOPEN-SOGUG trial (NCT02279576) that was evaluating the use of pazopanib with low doses of paclitaxel in advanced penile cancer was not completed due to its low recruitment.

IMMUNE THERAPY

Immune Checkpoint Inhibitors

Many HPV related cancers, with the similar histologic, epidemiologic, and therapeutic background to penile SCC, like head and neck, cervical, and anal carcinomas, have demonstrated good responses to immunotherapy with checkpoint inhibitors (26), due to its immunologic profile probably related to a higher mutational load and a high expression of PD-L1 (27). PD-L1 is expressed in 32.1% to 51.4% of penile cancer cells and 62.4% of tumor immune infiltrating cells and this biomarker is related to poor survival (28, 29).

Recently presented results of PERICLES phase II trial (NCT03686332), which included 32 patients with advanced penile cancer treated with atezolizumab, an anti-PD-L1 monoclonal antibody, alone or associated with local radiotherapy, showed a 30% oobjective response rate among 10 evaluable patients for response by RECIST 1.1 (including two complete responses), but the trial did not reach the expected PFS, its primary endpoint. Immunotherapy-related grade 3 or 4 adverse events occurred in 9.4% of patients (30). Avelumab, another anti-PD-L1 antibody already approved for the treatment of advanced urothelial cancer,

is also under investigation in penile cancer as maintenance (NCT03774901) or second-line therapy (NCT03391479) after chemotherapy. Pembrolizumab, an anti-PD1 antibody already approved in a variety of advanced solid tumors, has shown durable responses (until 38 months) in case reports of penile cancer (31, 32), and the results of a prospective trial with this drug as monotherapy (NCT02721732) is expected (**Table 1**). This is a drug with a large experience in clinical practice and wide availability. Pembrolizumab is already US FDA approved for the agnostic treatment of high microsatellite instability (MSI-H) and high tumor mutational burden (TMB) ≥10 mutations/megabase in advanced solid tumors. However, the frequency of MSI-H penile SCC is very low (17) to translate the use of this drug commonly in practice following this criterion.

TABLE 1 | Ongoing clinical trials in advanced penile cancer.

Status	Prior therapy required?	Tumors	Agent	Phase	Primary endpoint	n	ID
Single agent immune checkpoint inhibitors							
Active, not recruiting	yes	Rare tumors	Pembrolizumab	2	Non-progression rateIncidence of adverse events	202	NCT02721732
Recruiting	no	Advanced solid tumors	INCB099318	1	Number of treatment emergent adverse events	100	NCT04272034
Active, not recruiting	no	Penile Cancer	INCMGA0012 (Retifanlimab)	2	ORR	18	NCT04231981 (ORPHEUS)
Recruiting	no	Male genital tumors	LPD	2	pCR, ORR	127	NCT04718584
Recruiting	yes	Penile carcinoma	Avelumab (maintainance)	2	PFS	32	NCT03774901 (PULSE)
Recruiting	no	Penile carcinoma	Avelumab +/- radiotherapy	2	PFS	32	NCT03686332 (PERICLES)
Recruiting	yes	Penile carcinoma	Avelumab	2	ORR	24	NCT03391479
Recruiting	yes	Advanced solid tumors	XmAb20717	1	Safety and tolerability	154	NCT03517488 (DUET-2)
Combinations of immune checkpoint inhibitors							
Recruiting	no	Rare genitourinary tumors	Nivolumab + Ipilimumab	2	ORR	100	NCT03333616
Recruiting	yes	Rare tumors	Nivolumab + Ipilimumab	2	ORR	818	NCT02834013
Recruiting	yes	Advanced solid tumors	XmAb 22841 + Pembrolizumab	1	Safety and tolerability	242	NCT03849469 (DUET-4)
Immune checkpoint inhibitors + chemotherapy							
Recruiting	neoadjuvant	Penile Cancer	TIP + Nimotuzumab + Triprilimab	2	pCR	29	NCT04475016
Recruiting	no	Penile Carcinoma	Pembrolizumab + Cisplatin/ Carboplatin + 5-FU	2	ORR	33	NCT04224740 (HERCULES)
Immune checkpoint inhibitors + anti-angiogenicg agents							
Recruiting	no	Rare genitourinary tumors	Nivolumab + Ipilimumab + Cabozantinib	2	ORR	224	NCT03866382
Active, not recruiting	yes	Genitourinary tumors	Nivolumab + Cabozantinib +/- Ipilimumab	1	Recommended phase II dose Incidence of adverse events	152	NCT02496208
Active, not recruiting	no	Rare solid tumors	Avelumab + Bevacizumab	2		137	NCT03074513
Immune checkpoint inhibitors + epigenetic modifying agents							
Recruiting	no	Advanced mucosal cancer	Pembrolizumab + Vorinostat	2	ORR	111	NCT04357873 (PEVOsq)
Recruiting	no	Virus-associated cancers	Avelumab + valproic acid	2	ORR	39	NCT03357757 (LATENT)
HPV-directed therapies +/- combinations							
Recruiting	yes	HPV-associated Squamous cell carcinomas	HB-201 and HB-202 (Arenavirus vectors)	1/2	Dose escalation Dose expansion	200	NCT04180215
Active, not recruiting	yes	HPV-associated cancers	DNA plasmids therapeutic vaccine MEDI0457 + Durvalumab	2	ORR	77	NCT03439085
Active, not recruiting	yes	Head and neck, cervical and penile squamous cell carcinomas	HPV anti-CD40 RNA vaccine	1/2	Safety and tolerability	44	NCT03418480 (HARE-40)
Completed	no	HPV-indeuced cancers	P16_37-63 peptide vaccination + cisplatin based chemotherapy	1	Immune response	11	NCT02526316 (VICORYX-2)
Active, not recruiting	no	HPV associated cancers	HPV specific T cells + Nivolumab	1	Safety and tolerability	32	NCT02379520 (HESTIA) NCT00019110
Drug conjugate							
Recruiting	yes	Advanced solid malignancies	PEN-866	1/2	Safety and tolerability ORR	340	NCT03221400

The combined therapy with two classes of checkpoint inhibitors, anti-PD-1/PD-L1 and anti-CTLA4 antibodies, can improve the response to immunotherapy, as the blockage of B7-CTLA-4 pathway leads to increased activation of CD8+ cells in the lymph nodes as well as increased infiltration of activated CD8+ T cells into the tumor, which enhances the antitumor immunity induced by anti-PD-1/PD-L1 drugs (33). The combination of nivolumab plus ipilimumab has demonstrated higher efficacy than monotherapy in advanced melanoma (34), lung cancer (35), renal cancer (36), hepatocellular carcinoma (37), and MSI-H colorectal cancer (38). A multi-cohort phase II trial investigated the combination of nivolumab and ipilimumab in 56 patients with advanced rare genitourinary cancers. Despite the 16% ORR in the entire cohort, there were, unfortunately, no objective responses among the five penile cancer patients, and only two stable diseases. Grade 3 or higher toxicity was observed in 39% of patients (39). Nivolumab plus Ipilimumab is being tested in penile cancer in one ongoing trial (NCT02834013) and this checkpoint inhibitors combination is in association with cabozantinib in two other ongoing trials (NCT03866382, NCT02496208) (**Table 1**).

Immune Checkpoint Inhibitors Combined With Cytotoxic Chemotherapy

It is known that even with a minor response, cytotoxic chemotherapy is associated with tumor cell death and antigen shedding, which can be taken up by monocytes, macrophages, and dendritic cells and presented to T cells, initiating an antitumor immune response (40). Chemotherapy can also have an inhibitory effect on regulatory cells and myeloid suppressive cells (41). Immunotherapy with checkpoint inhibitors can enhance the response to chemotherapy by blocking the "silencing" signals of the immune response.

An association of pembrolizumab with cisplatin/carboplatin and 5-FU in inoperable and metastatic penile SCC is being evaluated on phase II LACOG 0218 trial (NCT04224740), which deserves special attention, as it is one of the few prospective studies underway in developing countries that have areas of higher incidence of this neoplasia (**Table 1**).

The single-center and single-arm phase II B2020-103-01 trial (NCT04475016) is evaluating the combination of TIP with nimotuzumab and triprilimab as a neoadjuvant treatment in locally advanced penile cancer (**Table 1**). Nimotuzumab is an intermediate affinity anti-EGFR antibody that inhibits cell proliferation and angiogenesis, activates natural killer cells, stimulates dendritic cell maturation, induces cytotoxic T cells, and restores MHC-I expression on tumor cells, hindering one of the EGFR immune-escape ways. In patients with locally advanced SCC of the head and neck, nimotuzumab in combination with low-dose cisplatin and radiotherapy was superior to cisplatin and radiotherapy in progression-free survival, disease-free survival, and locoregional tumor control (42). Triprilimab (JS001) is a recombinant humanized IgG4 anti-PD-1antibody that has demonstrated clinical activity in heavily pretreated nasopharyngeal cancer (43).

Immune Checkpoint Inhibitors Combined With Anti-Angiogenic Agents

The association of checkpoint inhibitors and anti-angiogenic drugs is a well-known strategy that impacts on overall response rate and survival in another hypervascularized advanced urological tumors such as renal cell cancer (44, 45). Results of the phase I trial and expansion cohorts of Nivolumab, Ipilimumab, and Cabozantinib, a multitarget tyrosine kinase inhibitor (NCT02496208), presented at ASCO GU 2021, demonstrated an ORR of 44% in the penile SCC group of nine patients. The grade 3 or 4 treatment-related adverse events to the whole population of the study was 80% with the three-drug combination (46). Two phase II trials currently ongoing address this therapeutic approach, all of them basket trials including patients with penile SCC. One of them is also evaluating the combination of Nivolumab and Ipilimumab with Cabozantinib (NCT03866382), and the other one, the association of Atezolizumab and Bevacizumab, an anti-VEGF antibody (NCT03074513) (**Table 1**).

Immune Checkpoint Inhibitors Combined With Epigenetic Modifying Agents

Although activity with immunotherapy is expected in penile SCC, similar to SCCs originating in other organs, there is a subset of tumors that presents with primary or secondary resistance to checkpoint inhibitors. One of these mechanisms of resistance is related to epigenetic processes that involve antitumor immunity pathways by affecting the antigenic presentation machinery and/or expression of the tumor antigen recognized by the immune system. The frequency of mutations in epigenetic modulator genes was found to be as high as 47% in SCCs (47). The Histone Deacetylases (HDAC) are a class of enzymes that play a crucial role of epigenetic modifications related to T cell differentiation and effector functions (48). The use of HDAC inhibitors can restore antigen presentation through an increase of TAP-1 and TAP-2, which allows the formation of the MHC I-peptide complex (49) and also increases PD-L1 expression (50). Vorinostat is an HDAC inhibitor that has shown a higher ORR when combined with pembrolizumab versus pembrolizumab alone (48% versus 25%, $P = 0.026$) in advanced PD-L1 > 1% NSCLC in the preliminary results of a phase II trial in 47 patients, with patients in the combination arm experiencing more fatigue, anorexia, and nausea, but with grade 3 or higher adverse events in only one out of 23 patients (51), while Etinostat, another HDAC inhibitor, associated with pembrolizumab, produced a 19% ORR in patients with metastatic melanoma pretreated with anti-PD-1/PD-L1 drugs (52). The combination of vorinostat and pembrolizumab is under investigation in a phase II basket trial of metastatic SCCs, including penile tumors (NCT04357873) (**Table 1**).

Other agents can lead to epigenetic modifications that enhance responses to therapy. Valproic acid has been demonstrated to enhance cisplatin-induced DNA damage through the downregulation of Excision Repair Cross-Complementing 1 (ERCC1), which is critical in DNA repair, and by increasing cisplatin influx and decreasing cisplatin export from human head and neck SCC cancer cells and decreases cetuximab-induced nuclear translocation of EGFR, a mechanism known to render chemotherapy resistance (53). Valproic acid also has an immunoregulatory activity through inhibition of

histone deacetylases by decreasing the proportion of polymorphonuclear myeloid-derived suppressor cells (MDSCs) and attenuating the immunosuppressive function of these cells in patients with cancer. It was also found that valproic acid downregulates the expression of PD-L1 on MDSCs attenuating the suppressive effect of PD-1 on CD8+ T cells and promoting CD8+ T cells' function (54). The ongoing phase II trial LATENT (NCT03357757) combines avelumab with valproic acid in the treatment of advanced viral-associated cancer (including penile SCC) (**Table 1**).

NOVEL THERAPEUTIC TARGETS

Heat shock proteins (HSPs) are molecular chaperones that function to maintain protein homeostasis through the proper folding and activation of client proteins in the cell and are characterized by their ability to become overexpressed under conditions of stress. HSP90 is one of the best understood of these proteins. Cancer cells are able to selectively modulate HSP90 activity through favorable complexes to satisfy the cells' requirement to survive (55). A previous phase I study with a small-molecule inhibitor that targets HSP90 (PU-H71) showed objective responses in lymphomas and solid tumors, including 20.8% of tumor regression in a penile SCC patient (56).

PEN-866 is a miniature drug conjugate that targets and binds to activated tumor HSP90 protein and releases an SN-38 (an active metabolite of irinotecan) cytotoxic payload. This drug was well tolerated and demonstrated preliminary evidence of antitumor activity in a previous study (57). An ongoing phase I/IIa trial is investigating the role of PEN-866 in previously treated advanced solid malignancies, including penile SCC (NCT03221400) (**Table 1**).

M7824 is an innovative first-in-class bifunctional fusion protein composed of a human IgG1 monoclonal antibody against PD-L1 fused with two extracellular domains of TGF-bRII (a TGF-b "trap") (58) that have demonstrated signs of efficacy in a phase I trial, with one complete response and partial responses in other cervical and anal cancer patients, that are HPV related tumors with histologic similarities to penile SCC. A phase I trial of M7824 in 16 patients with HPV associated malignancies showed a safety profile and a 37.5% ORR. The ORR in 11 HPV+ patients was 45.5% (59). There is a completed phase II trial with M7824 in the same subset of patients (NCT03427411), but the results were not published to date (**Table 1**).

A phase I trial with a small-molecule PD-L1 blocker, INCB099318, an oral drug, is ongoing and includes many advanced solid tumors, among which are penile SCCs (NCT04272034) (**Table 1**). This is an innovative administration of immunotherapy. Preliminary results of a phase I trial with a similar drug, INCB086550, reported in 2021, showed a similar toxicity profile to those seen with antibody immune checkpoint inhibitors, with the exception of a higher incidence of peripheral neuropathy (60).

XmAb22841 is a bispecific antibody that simultaneously targets immune checkpoint receptors CTLA-4 and LAG-3 that has a bispecific Fc domain to the two antigen-binding domains that confers long circulating half-life and stability and have been engineered to eliminate Fc gamma receptor (FcγR) binding, and can prevent the inhibitory action of some FcγR, avoiding resistance and improving the response to checkpoint inhibitors antibodies (61). The effect of this new drug is being studied in advanced solid tumors, including penile SCC, associated with pembrolizumab in the phase I trial DUET-4 (NCT03849469). Another bisepecific antibody, XmAb20717, which simultaneously targets PD-1 and CTLA-4, is also under investigation in a phase I trial (NCT03517488) for multiple types of advanced solid tumors, and preliminary results of 110 patients with a median of four previous systemic therapies (including immunotherapy checkpoint inhibitors in 64.5%) showed an ORR of 13% with very similar adverse events to anti-PD-1/PD-L1 antibodies (62) (**Table 1**).

HPV-DIRECTED THERAPIES

Human papillomavirus (HPV) is strongly implicated in penile SCC carcinogenesis, although exact pathways are not completely understood, and is an important area of interest regarding tumor prevention and treatment of this neoplasia, as well as in other HPV-related neoplasia such as cervical cancer, where it is better established. Approximately 20% to 50% of penile cancer is driven by HPV infection (63). The largest analyzed sample relies on a systematic review of 1266 invasive penile SCC patients in North America and reported that up to 48.7% of penile SCC harbors HPV DNA (64). Differently, we can find a higher proportion of HPV positive tumors in populations with a higher incidence as in northeast Brazil, where a study with 55 patients found that 89.1% of samples were positive for HPV DNA (65). The majority of the HPV infection in penile SCC is represented by the high-risk subtypes 16 and 18 (30.8% and 6.6%, respectively) (66). HPV positive tumors have a better prognosis than HPV negative tumors and PD-L1 expression is higher in HPV negative than in HPV positive penile SCC (49.4 vs. 32.7%, respectively, $p = 0.03$) (67). Preclinical studies in head and neck SCC suggest that the use of the HPV vaccine can upregulate PD-1 acting as a synergistic therapy with PD-1 checkpoint inhibitors to enhance antitumor efficacy (68).

Patients with cervical intraepithelial neoplasia 2/3 were treated with a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins in a phase IIb trial and had a significantly higher histologic regression when compared to placebo (48.2% vs. 30%, respectively, $p = 0.034$) demonstrating that it is possible to block the progression to malignant tumors using an anti-viral immunotherapy (69). However, in HPV16-positive advanced or recurrent gynecological carcinoma, an HPV16 synthetic long peptide vaccine produced an immune response, but no tumor regression (70), suggesting that the action of vaccine-activated T cells on invasive tumors is blocked by a tumor-induced immunosuppressive microenvironment.

The association of an HPV vaccine and a checkpoint inhibitor was evaluated in a single-arm phase II trial that enrolled 24 patients with incurable HPV-16-positive cancer, most of them

with oropharyngeal cancer, treated with ISA101, a synthetic long-peptide HPV-16 vaccine, and nivolumab. The ORR was 33%, median OS of 17.5 months and five patients had durable responses. Grade 3 toxicity was observed in two patients (71).

Ongoing trials of HPV vaccines, which include penile cancer patients, are a phase I/II trial of an HPV Anti-CD40 RNA Vaccine (HARE-40) (NCT03418480); a phase I trial of vaccine with human papillomavirus 16 E7 peptide and synthetic human papillomavirus 16 E6 peptide (NCT00019110); a phase I trial with a P16_37-63 peptide vaccine combined or not with ISA 51 VG (an emulsion with immunomoadjuvant activity that enhancing the cytotoxic T-lymphocyte response against antigens in vaccines) and associated with cisplatin-based chemotherapy (NCT02526316); phase II trial combining Durvalumab (an anti-PD-L1 antibody) with the DNA Plasmid-encoding Interleukin-12/HPV DNA Plasmids Therapeutic Vaccine MEDI0457 (NCT03439085); a phase I/II trial of treatment of HPV16+ cancers with arenavirus vectors HB-201 and HB-202, that expresses the same non-oncogenic HPV16 E7E6 fusion protein and induces tumor-specific T-cell responses (NCT04180215) (**Table 1**). In this last trial, in a preliminary analysis, two of 11 evaluable patients treated with HB-201 had a partial response and six had stable disease, with a duration of response of 4.8 months. All six evaluable patients that received HB-201/HB-202 had stable disease and serious adverse events related to treatment occurred in 24% of patients (72).

Adoptive T-cell therapy (ACT) is also a promising cancer treatment modality that is showing encouraging results in clinical trials. Infusion of tumor-infiltrating T cells preceded by a lymphocyte-depleting conditioning regimen and followed by systemic high-dose aldesleukin was performed in 29 patients with metastatic HPV related cancers (18 cervical and 11 non-cervical). Objective tumor responses occurred in 28% of patients in the cervical cancer cohort and 18% of patients in the noncervical cancer cohort. Two of the responses in cervical cancer were complete and are ongoing 67 and 53 months after treatment. Responses in the noncervical cancer cohort were in anal cancer and oropharyngeal cancer. There were no acute infusion-related toxicities and no autoimmune adverse events (73).

Successful expansion of tumor-reactive tumor-infiltrating lymphocytes (TIL) from lymph nodal metastasis of penile cancer patients, with 46.8% of CD8+ T cells and 45.4% from expanded TIL secreting IFN-γ in response to autologous tumor, supports the development of ACT strategies using TIL for the treatment of advanced and recurrent penile cancer (74).

Patients with penile cancer are currently included in the eligibility criteria of the HESTIA trial, a phase I trial using HPV-specific T cells collected from the blood of patients with HPV cancers associated with nivolumab (NCT02379520) (**Table 1**).

CONCLUSIONS

Despite its rarity, advanced penile cancer is an important health issue, considering the poorer prognosis compared to early disease which is curable with surgery alone, and the absence of a highly efficient standard systemic treatment. Cytotoxic chemotherapy remains the mainstay of treatment, even though it is based on small phase II trials, due to the lack of trials with a higher level of evidence. Toxicity with chemotherapy combination regimens is high to the point that about half of patients experience a grade 3 adverse event.

A better knowledge of the genomic landscape and immune microenvironment of penile SCC demonstrated similarities with head and neck SCC and allowed the development of clinical trials with different modalities of systemic treatment. Alterations in NOTCH, RTK-RAS, Hippo, mTOR, and DNA repair pathways offer actionable targets with potential for new treatments. High T cell infiltration and expression and PD-L1 in a large part of these tumors led to trials with a variety of immune checkpoint inhibitors, alone or in combination with other immunotherapies, cytotoxic drugs, or targeted therapies, with favorable preliminary results for some of them. Positivity for HPV infection is also propitious to HPV-directed therapies, like vaccines and adoptive T-cell therapy, since they have been demonstrated to have good preliminary results with other HPV-associated cancers. However, most of these studies are basket trials and include a wide range of rare tumors with similar molecular alterations, for the extreme difficulty to recruit patients precludes the execution of large prospective trials in penile cancer exclusively.

The better way to increase accrual and consequently improve clinical outcomes resides in global collaborative studies, including centers located in proportionally higher incidence areas. Additionally, a paradigm of decentralized accrual of patients and global retrospective studies may be necessary to make advances, which will require an extremely collaborative effort with multiple stakeholders involved. Scientific collaboration is also the key to a deeper knowledge of the different genomic and epigenomic alterations in HPV positive and negative tumors, in addition to the development and sharing of penile SCC cell lines and animal models in order to boost a more profound comprehension of the tumor biology and more accurate planning of future trials.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660
2. Coelho RWP, Pinho JD, Moreno JS, Garbis D, do Nascimento AMT, Larges JS, et al. Penile Cancer in Maranhao, Northeast Brazil: The Highest Incidence Globally? *BMC Urol* (2018) 18(1):50. doi: 10.1186/s12894-018-0365-0
3. Ornellas AA, Kinchin EW, Nóbrega BL, Wisnescky A, Koifman N, Quirino R. Surgical Treatment of Invasive Squamous Cell Carcinoma of the Penis:

Brazilian National Cancer Institute Long-Term Experience. *J Surg Oncol* (2008) 97(6):487–95. doi: 10.1002/jso.20980
4. Qi F, Wei X, Zheng Y, Ren X, Li X, Zhao E. Incidence Trends and Survival Outcomes of Penile Squamous Cell Carcinoma: Evidence From the Surveillance, Epidemiology and End Results Population-Based Data. *Ann Transl Med* (2020) 8(21):1428. doi: 10.21037/atm-20-1802
5. Nicholson S, Hall E, Harland SJ, Chester JD, Pickering L, Barber J, et al. Phase II Trial of Docetaxel, Cisplatin and 5FU Chemotherapy in Locally Advanced and Metastatic Penis Cancer (CRUK/09/001). *Br J Cancer* (2013) 109 (10):2554–9. doi: 10.1038/bjc.2013.620
6. Zhang S, Zhu Y, Ye D. Phase II Study of Docetaxel, Cisplatin, and Fluorouracil in Patients With Distantly Metastatic Penile Cancer as First-Line Chemotherapy. *Oncotarget* (2015) 6(31):32212–9. doi: 10.18632/oncotarget.4802
7. Pagliaro LC, Williams DL, Daliani D, Williams MB, Osai W, Kincaid M, et al. Neoadjuvant Paclitaxel, Ifosfamide, and Cisplatin Chemotherapy for Metastatic Penile Cancer: A Phase II Study. *J Clin Oncol* (2010) 28 (24):3851–7. doi: 10.1200/JCO.2010.29.5477
8. Djajadiningrat RS, Bergman AM, van Werkhoven E, Vegt E, Horenblas S. Neoadjuvant Taxane-Based Combination Chemotherapy in Patients With Advanced Penile Cancer. *Clin Genitourin Cancer* (2015) 13(1):44–9. doi: 10.1016/j.clgc.2014.06.005
9. Azizi M, Aydin AM, Hajiran A, Lai A, Kumar A, Peyton CC, 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):1147–55. doi: 10.1097/JU.0000000000000746
10. Di Lorenzo G, Buonerba C, Federico P, Perdona S, Aieta M, Rescigno P, et al. Cisplatin and 5-Fluorouracil in Inoperable, Stage IV Squamous Cell Carcinoma of the Penis. *BJU Int* (2012) 110(11 Pt B):E661–6. doi: 10.1111/j.1464-410X.2012.11453.x
11. Nicholson S, Tovey H, Elliott T, Burnett SM, Cruickshank C, Bahl A, et al. VinCaP: A Phase II Trial of Vinflunine in Locally Advanced and Metastatic Squamous Carcinoma of the Penis. *Br J Cancer* (2022) 126(1):34–41. doi: 10.1038/s41416-021-01574-9
12. Di Lorenzo G, Federico P, Buonerba C, Longo N, Carteni G, Autorino R, et al. Paclitaxel in Pretreated Metastatic Penile Cancer: Final Results of a Phase 2 Study. *Eur Urol* (2011) 60(6):1280–4. doi: 10.1016/j.eururo.2011.08.028
13. Ribera-Cortada I, Guerrero-Pineda J, Trias I, Veloza L, Garcia A, Marimon L, et al. Pathogenesis of Penile Squamous Cell Carcinoma: Molecular Update and Systematic Review. *Int J Mol Sci* (2021) 23(1):251. doi: 10.3390/ijms23010251
14. Macedo J, Silva E, Nogueira L, Coelho R, da Silva J, Dos Santos A, et al. Genomic Profiling Reveals the Pivotal Role of hrHPV Driving Copy Number and Gene Expression Alterations, Including mRNA Downregulation of TP53 and RB1 in Penile Cancer. *Mol Carcinog* (2020) 59(6):604–17. doi: 10.1002/mc.23185
15. McDaniel AS, Hovelson DH, Cani AK, Liu CJ, Zhai Y, Zhang Y, et al. Genomic Profiling of Penile Squamous Cell Carcinoma Reveals New Opportunities for Targeted Therapy. *Cancer Res* (2015) 75(24):5219–27. doi: 10.1158/0008-5472.CAN-15-1004
16. Feber A, Worth DC, Chakravarthy A, de Winter P, Shah K, Arya M, et al. CSN1 Somatic Mutations in Penile Squamous Cell Carcinoma. *Cancer Res* (2016) 76(16):4720–7. doi: 10.1158/0008-5472.CAN-15-3134
17. Chahoud J, Gleber-Netto FO, McCormick BZ, Rao P, Lu X, Guo M, 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):2560–70. doi: 10.1158/1078-0432.CCR-20-4004
18. Jacob JM, Ferry EK, Gay LM, Elvin JA, Vergilio JA, Ramkissoon S, 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):541–8. doi: 10.1016/j.juro.2018.09.056
19. Chaux A, Munari E, Katz B, Sharma R, Lecksell K, Cubilla AL, et al. The Epidermal Growth Factor Receptor is Frequently Overexpressed in Penile Squamous Cell Carcinomas: A Tissue Microarray and Digital Image Analysis Study of 112 Cases. *Hum Pathol* (2013) 44(12):2690–5. doi: 10.1016/j.humpath.2013.07.012
20. Gou HF, Li X, Qiu M, Cheng K, Li LH, Dong H, et al. Epidermal Growth Factor Receptor (EGFR)-RAS Signaling Pathway in Penile Squamous Cell Carcinoma. *PloS One* (2013) 8(4):e62175. doi: 10.1371/journal.pone.0062175
21. Di Lorenzo G, Buonerba C, Ferro M, Calderoni G, Bozza G, Federico P, et al. The Epidermal Growth Factor Receptors as Biological Targets in Penile Cancer. *Expert Opin Biol Ther* (2015) 15(4):473–6. doi: 10.1517/14712598.2015.993377
22. Necchi A, Giannatempo P, Lo Vullo S, Raggi D, Nicolai N, Colecchia M, et al. Panitumumab Treatment for Advanced Penile Squamous Cell Carcinoma When Surgery and Chemotherapy Have Failed. *Clin Genitourin Cancer* (2016) 14(3):231–6. doi: 10.1016/j.clgc.2015.08.001
23. Necchi A, Lo Vullo S, Perrone F, Raggi D, Giannatempo P, Calareso G, 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):348–56. doi: 10.1111/bju.14013
24. Li D, Han Z, Liu J, Zhang X, Ren J, Yan L, et al. Upregulation of Nucleus HDGF Predicts Poor Prognostic Outcome in Patients With Penile Squamous Cell Carcinoma Bypass VEGF-A and Ki-67. *Med Oncol* (2013) 30(4):702. doi: 10.1007/s12032-013-0702-9
25. Zhu Y, Li H, Yao XD, Zhang SL, Zhang HL, Shi GH, et al. Feasibility and Activity of Sorafenib and Sunitinib in Advanced Penile Cancer: A Preliminary Report. *Urol Int* (2010) 85(3):334–40. doi: 10.1159/000315432
26. Kanaan H, Kourie HR, Awada AH. Are Virus-Induced Cancers More Sensitive to Checkpoint Inhibitors? *Future Oncol* (2016) 12(23):2665–8. doi: 10.2217/fon-2016-0283
27. Udager AM, Liu TY, Skala SL, Magers MJ, McDaniel AS, Spratt DE, et al. Frequent PD-L1 Expression in Primary and Metastatic Penile Squamous Cell Carcinoma: Potential Opportunities for Immunotherapeutic Approaches. *Ann Oncol* (2016) 27(9):1706–12. doi: 10.1093/annonc/mdw216
28. Davidsson S, Carlsson J, Giunchi F, Harlow A, Kirrander P, Rider J, et al. PD-L1 Expression in Men With Penile Cancer and its Association With Clinical Outcomes. *Eur Urol Oncol* (2019) 2(2):214–21. doi: 10.1016/j.euo.2018.07.010
29. De Bacco MW, Carvalhal GF, MacGregor B, Marçal JMB, Wagner MB, Sonpavde GP, et al. PD-L1 and P16 Expression in Penile Squamous Cell Carcinoma From an Endemic Region. *Clin Genitourin Cancer* (2020) 18(3): e254-e9. doi: 10.1016/j.clgc.2019.10.014
30. de Vries HM, Feijter JD, Bekers E, Lopez-Yurda M, Pos FJ, Horenblas S, et al. Clinical Results of PERICLES: A Phase II Trial Investigating Atezolizumab +/- Radiotherapy for Advanced Squamous Cell Carcinoma of the Penis. *J Clin Oncol* (2022) 40(6_suppl):3–3. doi: 10.1200/JCO.2022.40.6_suppl.003
31. Hahn AW, Chahoud J, Campbell MT, Karp DD, Wang J, Stephen B, et al. Pembrolizumab for Advanced Penile Cancer: A Case Series From a Phase II Basket Trial. *Invest New Drugs* (2021) 39(5):1405–10. doi: 10.1007/s10637-021-01100-x
32. Chahoud J, WPt S, PE S, Walko C, Dhillon J, KL G, et al. Case Report: Two Cases of Chemotherapy Refractory Metastatic Penile Squamous Cell Carcinoma With Extreme Durable Response to Pembrolizumab. *Front Oncol* (2020) 10:615298. doi: 10.3389/fonc.2020.615298
33. Kudo M. Scientific Rationale for Combination Immunotherapy of Hepatocellular Carcinoma With Anti-PD-1/PD-L1 and Anti-CTLA-4 Antibodies. *Liver Cancer* (2019) 8(6):413–26. doi: 10.1159/000503254
34. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, et al. Five-Year Survival With Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med* (2019) 381(16):1535–46. doi: 10.1056/NEJMoa1910836
35. Hellmann MD, Paz-Ares L, Bernabe Caro R, Zurawski B, Kim SW, Carcereny Costa E, et al. Nivolumab Plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* (2019) 381(21):2020–31. doi: 10.1056/NEJMoa1910231
36. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab Plus Ipilimumab Versus Sunitinib in Advanced Renal-Cell Carcinoma. *N Engl J Med* (2018) 378(14):1277–90. doi: 10.1056/NEJMoa1712126
37. Yau T, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, et al. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol* (2020) 6(11): e204564. doi: 10.1001/jamaoncol.2020.4564

38. Lenz HJ, Van Cutsem E, Luisa Limon M, Wong KYM, Hendlisz A, Aglietta M, et al. First-Line Nivolumab Plus Low-Dose Ipilimumab for Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: The Phase II CheckMate 142 Study. *J Clin Oncol* (2022) 40(2):161–70. doi: 10.1200/JCO.21.01015
39. McGregor BA, Campbell MT, Xie W, Farah S, Bilen MA, Sonpavde G, et al. Phase II Study of Nivolumab and Ipilimumab for Advanced Rare Genitourinary Cancers. *J Clin Oncol* (2020) 38(15_suppl):5018. doi: 10.1200/JCO.2020.38.15_suppl.5018
40. Lake RA, Robinson BW. Immunotherapy and Chemotherapy–a Practical Partnership. *Nat Rev Cancer* (2005) 5(5):397–405. doi: 10.1038/nrc1613
41. Dalgleish AG. Rationale for Combining Immunotherapy With Chemotherapy. *Immunotherapy* (2015) 7(3):309–16. doi: 10.2217/imt.14.111
42. Crombet Ramos T, Mestre Fernández B, Mazorra Herrera Z, Iznaga Escobar NE. Nimotuzumab for Patients With Inoperable Cancer of the Head and Neck. *Front Oncol* (2020) 10:817. doi: 10.3389/fonc.2020.00817
43. Xu R, Wang FH, Feng FJ, Li Q, Xu N, Hu XC, et al. Recombinant Humanized Anti-PD-1 Monoclonal Antibody (JS001) in Patients With Refractory/Metastatic Nasopharyngeal Carcinoma: Preliminary Results of an Open-Label Phase II Clinical Study. *Ann Oncol* (2018) 29:viii409. doi: 10.1093/annonc/mdy288.023
44. Choueiri TK, Powles T, Burotto M, Escudier B, Bourlon MT, Zurawski B, et al. Nivolumab Plus Cabozantinib Versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med* (2021) 384(9):829–41. doi: 10.1056/NEJMoa2026982
45. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab Plus Axitinib Versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med* (2019) 380(12):1116–27. doi: 10.1056/NEJMoa1816714
46. Apolo AB, DdM G, SA N, RM N, LM C, SM S, et al. Final Results From a Phase I Trial and Expansion Cohorts of Cabozantinib and Nivolumab (CaboNivo) Alone or With Ipilimumab (CaboNivoIpi) for Metastatic Genitourinary Tumors. *J Clin Oncol* (2021) 39(6_suppl):3–3. doi: 10.1200/JCO.2021.39.6_suppl.3
47. Scholl S, Popovic M, de la Rochefordiere A, Girard E, Dureau S, Mandic A, et al. Clinical and Genetic Landscape of Treatment Naive Cervical Cancer: Alterations in PIK3CA and in Epigenetic Modulators Associated With Sub-Optimal Outcome. *EBioMedicine* (2019) 43:253–60. doi: 10.1016/j.ebiom.2019.03.069
48. Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, et al. Epigenetic Stability of Exhausted T Cells Limits Durability of Reinvigoration by PD-1 Blockade. *Science* (2016) 354(6316):1160–5. doi: 10.1126/science.aaf2807
49. Arenas-Ramirez N, Sahin D, Boyman O. Epigenetic Mechanisms of Tumor Resistance to Immunotherapy. *Cell Mol Life Sci* (2018) 75(22):4163–76. doi: 10.1007/s00018-018-2908-7
50. Woods DM, Sodré AL, Villagra A, Sarnaik A, Sotomayor EM, Weber J. HDAC Inhibition Upregulates PD-1 Ligands in Melanoma and Augments Immunotherapy With PD-1 Blockade. *Cancer Immunol Res* (2015) 3(12):1375–85. doi: 10.1158/2326-6066.CIR-15-0077-T
51. Saltos AN, Tanvetyanon T, Creelan BC, Shafique MR, Antonia SJ, Haura EB, et al. Phase II Randomized Trial of First-Line Pembrolizumab and Vorinostat in Patients With Metastatic NSCLC (mNSCLC). *J Clin Oncol* (2020) 38(15_suppl):9567. doi: 10.1200/JCO.2020.38.15_suppl.9567
52. Sullivan RJ, Moschos SJ, Johnson ML, Opyrchal M, Ordentlich P, Brouwer S, et al. Abstract CT072: Efficacy and Safety of Entinostat (ENT) and Pembrolizumab (PEMBRO) in Patients With Melanoma Previously Treated With Anti-PD1 Therapy. *Cancer Res* (2019) 79(13_Supplement):CT072–CT. doi: 10.1158/1538-7445.AM2019-CT072
53. Iannelli F, Zotti AI, Roca MS, Grumetti L, Lombardi R, Moccia T, et al. Valproic Acid Synergizes With Cisplatin and Cetuximab *In Vitro* and *In Vivo* in Head and Neck Cancer by Targeting the Mechanisms of Resistance. *Front Cell Dev Biol* (2020) 8:732. doi: 10.3389/fcell.2020.00732
54. Xie Z, Ago Y, Okada N, Tachibana M. Valproic Acid Attenuates Immunosuppressive Function of Myeloid-Derived Suppressor Cells. *J Pharmacol Sci* (2018) 137(4):359–65. doi: 10.1016/j.jphs.2018.06.014
55. Shrestha L, Bolaender A, Patel HJ, Taldone T. Heat Shock Protein (HSP) Drug Discovery and Development: Targeting Heat Shock Proteins in Disease. *Curr Top Med Chem* (2016) 16(25):2753–64. doi: 10.2174/1568026616666160413141911
56. Gerecitano JF, Modi S, Rampal R, Drilon AE, Fury MG, Gounder MM, et al. Phase I Trial of the HSP-90 Inhibitor PU-H71. *J Clin Oncol* (2015) 33(15_suppl):2537. doi: 10.1200/jco.2015.33.15_suppl.2537
57. Falchook GS, Bendell JC, Ulahannan SV, Sen S, Vilimas R, Kriksciukaite K, et al. Pen-866, a Miniature Drug Conjugate of a Heat Shock Protein 90 (HSP90) Ligand Linked to SN38 for Patients With Advanced Solid Malignancies: Phase I and Expansion Cohort Results. *J Clin Oncol* (2020) 38(Suppl 15):3515. doi: 10.1200/JCO.2020.38.15_suppl.3515
58. Strauss J, Heery CR, Schlom J, Madan RA, Cao L, Kang Z, et al. Phase I Trial of M7824 (MSB0011359C), a Bifunctional Fusion Protein Targeting PD-L1 and Tgfβ, in Advanced Solid Tumors. *Clin Cancer Res* (2018) 24(6):1287–95. doi: 10.1158/1078-0432.CCR-17-2653
59. Strauss J, Gatti-Mays ME, Redman J, Madan RA, Lamping E , Manu M, et al. Safety and Activity of M7824, a Bifunctional Fusion Protein Targeting PD-L1 and TGF-β, in Patients With HPV Associated Cancers. *J Clin Oncol* (2018) 36(15_suppl):3007. doi: 10.1200/JCO.2018.36.15_suppl.3007
60. Cutsem EV, Prenen H, Delafontaine B, Spencer K, Mitchell T, Burris H, et al. 529 Phase 1 Study of INCB086550, an Oral PD-L1 Inhibitor, in Immune-Checkpoint Naive Patients With Advanced Solid Tumors. *J Immunother Cancer* (2021) 9(Suppl 2):A559–A60. doi: 10.1136/jitc-2021-SITC2021.529
61. Dahal LN, Roghanian A, Beers SA, Cragg MS. Fcγr Requirements Leading to Successful Immunotherapy. *Immunol Rev* (2015) 268(1):104–22. doi: 10.1111/imr.12342
62. Shum E, Reilley M, Najjar Y, Daud A, Thompson J, Baranda J, et al. 523 Preliminary Clinical Experience With XmAb20717, a PD-1 X CTLA-4 Bispecific Antibody, in Patients With Advanced Solid Tumors. *J Immunother Cancer* (2021) 9(Suppl 2):A553–A. doi: 10.1136/jitc-2021-SITC2021.523
63. Miralles-Guri C, Bruni L, Cubilla AL, Castellsagué X, Bosch FX, de Sanjosé S. Human Papillomavirus Prevalence and Type Distribution in Penile Carcinoma. *J Clin Pathol* (2009) 62(10):870–8. doi: 10.1136/jcp.2008.063149
64. Sand FL, Rasmussen CL, Frederiksen MH, Andersen KK, Kjaer SK. Prognostic Significance of HPV and P16 Status in Men Diagnosed With Penile Cancer: A Systematic Review and Meta-Analysis. *Cancer Epidemiol Biomarkers Prev* (2018) 27(10):1123–32. doi: 10.1158/1055-9965.EPI-18-0322
65. Martins VA, Pinho JD, Teixeira Júnior AAL, Nogueira LR, Silva FF, Maulen VE, et al. P16INK4a Expression in Patients With Penile Cancer. *PloS One* (2018) 13(10):e0205350. doi: 10.1371/journal.pone.0205350
66. Backes DM, Kurman RJ, Pimenta JM, Smith JS. Systematic Review of Human Papillomavirus Prevalence in Invasive Penile Cancer. *Cancer Causes Control* (2009) 20(4):449–57. doi: 10.1007/s10552-008-9276-9
67. Ottenhof SR, Djajadiningrat RS, Thygesen HH, Jakobs PJ, Jóźwiak K, Heeren AM, et al. The Prognostic Value of Immune Factors in the Tumor Microenvironment of Penile Squamous Cell Carcinoma. *Front Immunol* (2018) 9:1253. doi: 10.3389/fimmu.2018.01253
68. Badoual C, Hans S, Merillon N, Van Ryswick C, Ravel P, Benhamouda N, et al. PD-1-Expressing Tumor-Infiltrating T Cells are a Favorable Prognostic Biomarker in HPV-Associated Head and Neck Cancer. *Cancer Res* (2013) 73(1):128–38. doi: 10.1158/0008-5472.CAN-12-2606
69. Trimble CL, Morrow MP, Kraynyak KA, Shen X, Dallas M, Yan J, et al. Safety, Efficacy, and Immunogenicity of VGX-3100, a Therapeutic Synthetic DNA Vaccine Targeting Human Papillomavirus 16 and 18 E6 and E7 Proteins for Cervical Intraepithelial Neoplasia 2/3: A Randomised, Double-Blind, Placebo-Controlled Phase 2b Trial. *Lancet* (2015) 386(10008):2078–88. doi: 10.1016/S0140-6736(15)00239-1
70. van Poelgeest MI, Welters MJ, van Esch EM, Stynenbosch LF, Kerpershoek G, van Persijn van Meerten EL, et al. HPV16 Synthetic Long Peptide (HPV16-SLP) Vaccination Therapy of Patients With Advanced or Recurrent HPV16-Induced Gynecological Carcinoma, a Phase II Trial. *J Transl Med* (2013) 11:88. doi: 10.1186/1479-5876-11-88
71. Massarelli E, William W, Johnson F, Kies M, Ferrarotto R, Guo M, et al. Combining Immune Checkpoint Blockade and Tumor-Specific Vaccine for Patients With Incurable Human Papillomavirus 16-Related Cancer: A Phase 2 Clinical Trial. *JAMA Oncol* (2019) 5(1):67–73. doi: 10.1001/jamaoncol.2018.4051
72. Ho AL, Posner MR, Niu J, Fu S, Leidner RS, Pearson AT, et al. First Report of the Safety/Tolerability and Preliminary Antitumor Activity of HB-201 and HB-202, an Arenavirus-Based Cancer Immunotherapy, in Patients With

HPV16+ Cancers. *J Clin Oncol* (2021) 39(Suppl 15):2502. doi: 10.1200/JCO.2021.39.15_suppl.2502
73. Stevanović S, Helman SR, Wunderlich JR, Langhan MM, Doran SL, Kwong MLM, et al. A Phase II Study of Tumor-Infiltrating Lymphocyte Therapy for Human Papillomavirus-Associated Epithelial Cancers. *Clin Cancer Res* (2019) 25(5):1486–93. doi: 10.1158/1078-0432.CCR-18-2722
74. Aydin AM, Hall M, Bunch BL, Branthoover H, Sannasardo Z, Mackay A, et al. Expansion of Tumor-Infiltrating Lymphocytes (TIL) From Penile Cancer Patients. *Int Immunopharmacol* (2021) 94:107481. doi: 10.1016/j.intimp.2021.107481

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