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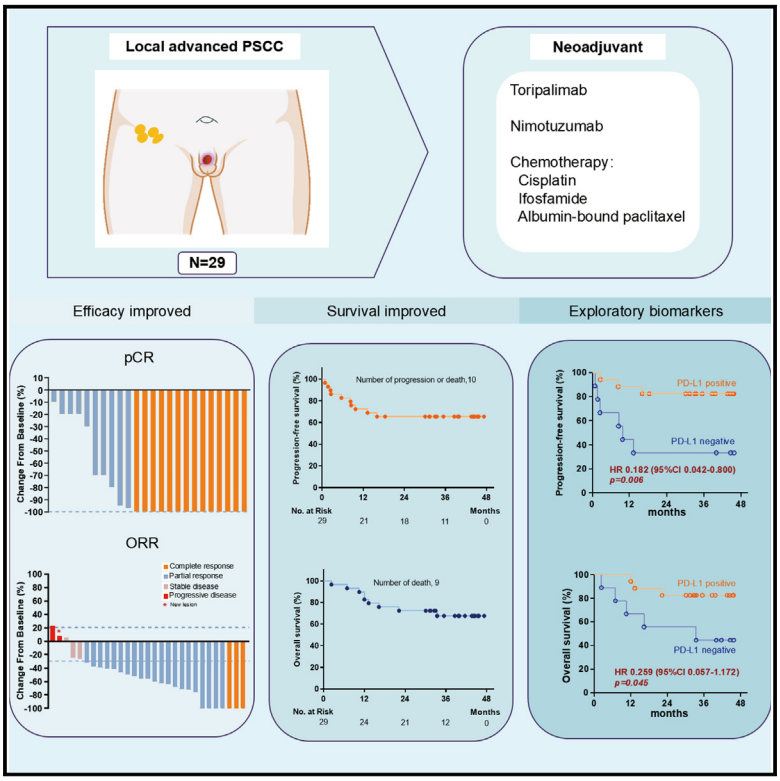
Report

Neoadjuvant toripalimab plus nimotuzumab combined with taxol-based chemotherapy in locally advanced penile squamous cell carcinoma

Graphical abstract



Highlights

- TNT regimen combines toripalimab, nimotuzumab, and taxol-based chemotherapy
- The TNT regimen increases the pCR rate and ORR in La-PSCC patients
- The TNT regimen improves PFS and OS in La-PSCC patients
- PD-L1, *TP53*, and CD8$^+$ T cells may serve as biomarkers for the synergistic effect

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In brief

An et al. demonstrate that neoadjuvant toripalimab (anti-PD-1) plus nimotuzumab (anti-EGFR) combined with taxol-based chemotherapy (TNT) has promising anti-tumor activity and acceptable toxicity in patients with locally advanced penile squamous cell carcinoma in a phase II trial. PD-L1 expression, *TP53* mutation status, and CD8$^+$ T cell density may serve as predictive biomarkers.

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Report

Neoadjuvant toripalimab plus nimotuzumab combined with taxol-based chemotherapy in locally advanced penile squamous cell carcinoma

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SUMMARY

The conventional neoadjuvant chemotherapy regimen for locally advanced penile squamous cell carcinoma (La-PSCC) has shown moderate response rates and survival benefits. This single-arm, phase II trial (NCT04475016) evaluated a neoadjuvant regimen of four cycles of toripalimab (anti-PD-1 antibody), nimotuzumab (anti-EGFR antibody), and taxol-based chemotherapy (TNT), followed by consolidative surgery. The primary endpoint was the pathological complete response (pCR) rate. Among 29 enrolled patients, 24 (82.8%) underwent consolidative surgery, with 14 (48.3%, 95% confidence interval [CI], 29.4–67.5%) achieving pCR. The objective response rate (ORR) was 82.8% (95% CI, 64.2–94.2). Median follow-up was 39.97 months, with two-year overall survival (OS) and progression-free survival (PFS) rates of 72.4% and 65.5%. Grade 3–4 treatment-related adverse events (TRAEs) occurred in 12 (41.4%) patients, with no treatment-related deaths. Biomarker analysis identified PD-L1 expression, TP53 mutation status, and CD8$^+$ T cell density as potential predictive markers. Therefore, neoadjuvant TNT shows promising anti-tumor activity and acceptable toxicity.

INTRODUCTION

Penile squamous cell carcinoma (PSCC) is a rare disease with a prevalence of 0.1–1 per 100,000 men in developed countries, but it constitutes up to 10% of malignancies in men in some regions of Africa, Asia, and South America.[1,2] Locally advanced penile squamous cell carcinoma (La-PSCC) has a dismal prognosis.[3–6] The 5-year survival rate of patients with pelvic lymph node

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metastasis is lower than 17%.[7,8] Neoadjuvant chemotherapy combined with consolidative surgery is the current standard of care for La-PSCC, which is recommended by the National Comprehensive Cancer Network and the European Association of Urology guidelines.[9,10] The neoadjuvant regimen of paclitaxel, ifosfamide, and platinum (TIP) only yielded an objective response rate (ORR) of 50.0%, a pathological complete response (pCR) rate of only 10%, a median time to progression of 8.1 months, and median overall survival (OS) of 17.1 months in a prospective phase II trial for N2-3M0 La-PSCC.[11] Nonetheless, 63% of patients suffered tumor recurrence or progression, and the median OS for second-line salvage therapy was only 5.6 months.[12] Therefore, there is an urgent need to identify and offer more effective systemic therapies to improve the survival of La-PSCC.

Owing to the high expression of human epidermal growth factor receptor (EGFR, >90% of cases) and programmed death-ligand 1 (PD-L1, expressed in 48%–62% of cases), coupled with pronounced immune cell infiltration, EGFR blockade and immunotherapy are considered compelling therapeutic avenues for PSCC.[13–17] However, single-agent EGFR inhibitors, such as panitumumab, nimotuzumab, and the oral agent dacomitinib, have demonstrated ORRs of approximately 30%.[18–20] The ORR of PD-1/PD-L1 blockades as monotherapy ranges from 0% to 16.7%, reflecting limited efficacy in PSCC.[21,22] The recent LACOG study reported an ORR of 39.4% for first-line pembrolizumab combined with chemotherapy in advanced PSCC, with a median progression-free survival (PFS) of 5.4 months and median OS of 9.6 months.[23] Another prospective study evaluating the anti-EGFR monoclonal antibody CDP1 in combination with TIP chemotherapy (paclitaxel, ifosfamide, cisplatin) demonstrated a promising ORR of 73.7%, though the median PFS was limited to just 6.9 months. Several trials have demonstrated promising outcomes and acceptable toxicity of immune checkpoint inhibitors (ICIs) combined with targeted therapy and chemotherapy in other malignancies.[24,25] Pre-clinical evidence also supported the synergistic effects of combining ICIs and EGFR inhibitors or chemotherapy.[26,27] Therefore, we hypothesized that a triple combination strategy of ICI, EGFR inhibitor, and chemotherapy might be effective in La-PSCC. Our retrospective case series (17 cases) primarily reported the durable benefit of first-line PD-1 inhibitor plus anti-EGFR antibody and cisplatin-based chemotherapy in high-risk cT1-4N3M0-1 La-PSCC, with 2-year PFS and OS rates of 68.4% and 62.9%, respectively.[28] Consequently, we initiated this prospective phase II trial to validate further the safety and efficacy of the triple combination regimen of toripalimab (anti-PD-1), nimotuzumab (anti-EGFR), and taxol-based chemotherapy (TNT) as neoadjuvant treatment in patients with high-risk La-PSCC.

RESULTS

Patient characteristics and treatment

Between August 12, 2020, and January 10, 2022, among the 54 patients evaluated for eligibility, 29 patients were enrolled in this study (Figure S1). The median age was 57 (31–71) years. All 29 patients were diagnosed with cTx-4N3M0 stage, in which ten patients had fixed inguinal lymph node metastasis, and 19 patients had imaging pelvic lymph node metastasis. Eight (27.6%) patients had human papilloma virus (HPV) type 16/18 infection. The detailed characteristics of these 29 patients are shown in Table 1.

Twenty-four patients (82.8%) completed four cycles of neoadjuvant TNT and underwent consolidative surgery (including bilateral or unilateral inguinal lymph node dissection or pelvic lymph node dissection). The other five patients discontinued TNT after 1 to 3 cycles due to disease progression ($n = 3$), grade 3 asthenia ($n = 1$), or withdrawal ($n = 1$) (Figure S1). The median time from the initiated date of the last cycle of neoadjuvant TNT to surgery was 28 days (range 20–39). All of the operated patients reached R0 resection. The median number of dissected inguinal and pelvic lymph nodes were 22 (range 1–45) and 23 (7–60), respectively.

Primary endpoint

The trial proceeded due to seven of the first ten patients achieving a pCR in the first stage. Of all enrolled patients, two and 12 achieved ypTisN0M0 and ypT0N0M0, respectively (Figure 1A). The pCR rate was 48.3% (14/29, 95% confidence interval [CI], 29.4–67.5) in all enrolled patients (Table S1). Representative radiologic images of three pCR patients are shown in Figures S2A and S2B.

Secondary endpoints

In all 29 patients, the ORR was 82.8% (24/29, 95% CI, 64.2–94.2%) after four cycles of neoadjuvant TNT, with the CR and PR rate as 10.3% (3/29, 95% CI, 2.2–27.4%) and 72.4%

Table 1. Baseline clinical characteristics of the 29 patients

Characteristic	Value
Age (years), median (range)	57 (31–71)
Smoking history, no. (%)	
No	11 (37.9)
Yes	18 (62.1)
ECOG performance-status score[a], no. (%)	
0	1 (3.4)
1	19 (65.6)
2	9 (31.0)
Clinical tumor stage[b], no. (%)	
Tx	12 (41.4)
T1-3	4 (13.8)
T4	13 (44.8)
Clinical lymph nodal stage[b], no. (%)	
N3	29 (100)
Inguinal lymph node metastasis[c]	10 (34.5)
Pelvic lymph node metastasis	19 (65.5)
Inguinal skin involvement, no. (%)	
Absent	11 (37.9)
Present	18 (62.1)
Extranodal extension, no. (%)	
Absent	7 (24.1)
Present	22 (75.9)
Previous local therapy, no. (%)	
Absent	6 (20.7)
Partial penectomy	19 (65.5)
Total penectomy	2 (6.9)
Unilateral ILND	7 (21.4)
Bilateral ILND	6 (20.7)
HPV status, no. (%)	
Negative	21 (72.4)
HPV 16/18 type	8 (27.6)
Histopathologic stage, no. (%)	
I	8 (27.6)
II	16 (55.2)
III	5 (17.2)

ECOG, Eastern Cooperative Oncology Group; ILND, inguinal lymph nodes dissection; HPV, human papilloma virus; AJCC, American Joint Committee on Cancer; PSCC, penile squamous cell carcinoma.
See also Figure S1.
[a]ECOG performance-status scores range from 0 to 5 with 0 indicating no symptoms and higher scores indicating greater disability.
[b]Clinical stage was assessed by the 8th edition of the AJCC TNM staging system for PSCC.
[c]Accompany with fixed inguinal lymph node metastasis or extranodal extension.

(21/29, 95% CI, 52.8–86.6%), respectively. Three of the 29 patients (3/29, 10.3%) had stable disease (SD), and two patients (2/29, 6.90%) suffered progressive disease (PD) during neoadjuvant TNT (Table S1E and Figure 1B).

As of June 30, 2024, the median follow-up time was 39.97 (95% CI, 35.695–44.245). Six (25.0%) of 24 operated patients suffered radiographic recurrence after surgery. Nine (9/29, 31.0%) patients died of disease progression (Figure 1C). Both median PFS and OS were not reached. Kaplan-Meier analysis yielded an estimated 2-year PFS rate of 65.5% (95% CI, 48.3–82.7) and a 2-year OS of 72.4% (95% CI, 56.1–88.7) (Figures 1D and 1E).

Subgroup analysis showed that patients who achieved pCR had significantly longer PFS (median NR [not reached] vs. 13.27 months, hazard ratio [HR] 0.105, 95% CI, 0.020–0.567, p = 0.01) and OS (median NR vs. NR, HR 0.148, 95% CI, 0.024–0.909, p = 0.048) than those without pCR (Figures S2C and S2D). In patients with radiologic clinical response (CR or PR), both PFS (median NR vs. 2.47 months, HR 0.067, 95% CI, 0.005–0.862, $p < 0.001$) and OS (median NR vs. 10.5 months, HR 0.063, 95% CI, 0.005–0.776, $p < 0.001$) were significantly longer than those of non-responders (SD or PD) (Figures S2E and S2F).

Safety

Any grade treatment-related adverse events (TRAEs) occurred in all patients (Table 2). The most common grade 1/2 TRAEs included alopecia (29/29), decreased appetite (27/29), and peripheral sensory neuropathy (23/29). Twelve (41.4%) patients experienced grade 3/4 TRAEs, and no treatment-related death occurred. The most common grade 3–4 TRAEs included neutropenia (7/29), anemia (3/29), febrile neutropenia (2/29), and rash (2/29). The most common potentially immune-related adverse events were grade 1/2 hypothyroidism (5/29), hyperthyroidism (3/29), and adrenal insufficiency (2/29). Three patients had a dose reduction due to grade 3/4 TRAEs, and one patient discontinued treatment because of grade 3 asthenia. Incidence of grade 3/4 TRAEs was higher in patients with ECOG-PS = 2 than those with PS < 2 (55.6% [5/9] vs. 35% [7/20]), with no statistical significance (p = 0.42) (Table S2).

Postoperative complications

The most common short-term (within 30 days after surgery) postoperative complications were lymphatic fistula (87.5%, grade 1 or 2) and lower extremity edema (29.2%, grade 1 or 2). No patients died due to postoperative complications. The main complications after 30 days of surgery included lower extremity edema (37.5%, grade 1 to 2) and scrotal edema (37.5%, grade 1 or 2) (Table S3).

Adjuvant therapy

In 24 operated patients, 18 received at least one cycle of adjuvant toripalimab therapy. The median cycle of adjuvant toripalimab was 4 (range 1–17). Only three patients completed one year (17 cycles) of adjuvant toripalimab. None of the patients received adjuvant radiotherapy.

Biomarker analyses

All 26 evaluated specimens presented microsatellite stability (MSS) status by immunohistochemistry (IHC) (Figures S3A–S3D). All patients were EGFR positive (Figure S3E). pCR was not significantly associated with baseline variables, including HPV status (Table S4). Patients with PD-L1 expression had a better treatment response (p = 0.034), with no significant associations found with other variables like HPV status (Figures 2A, Table S5, S3F, and S3G).

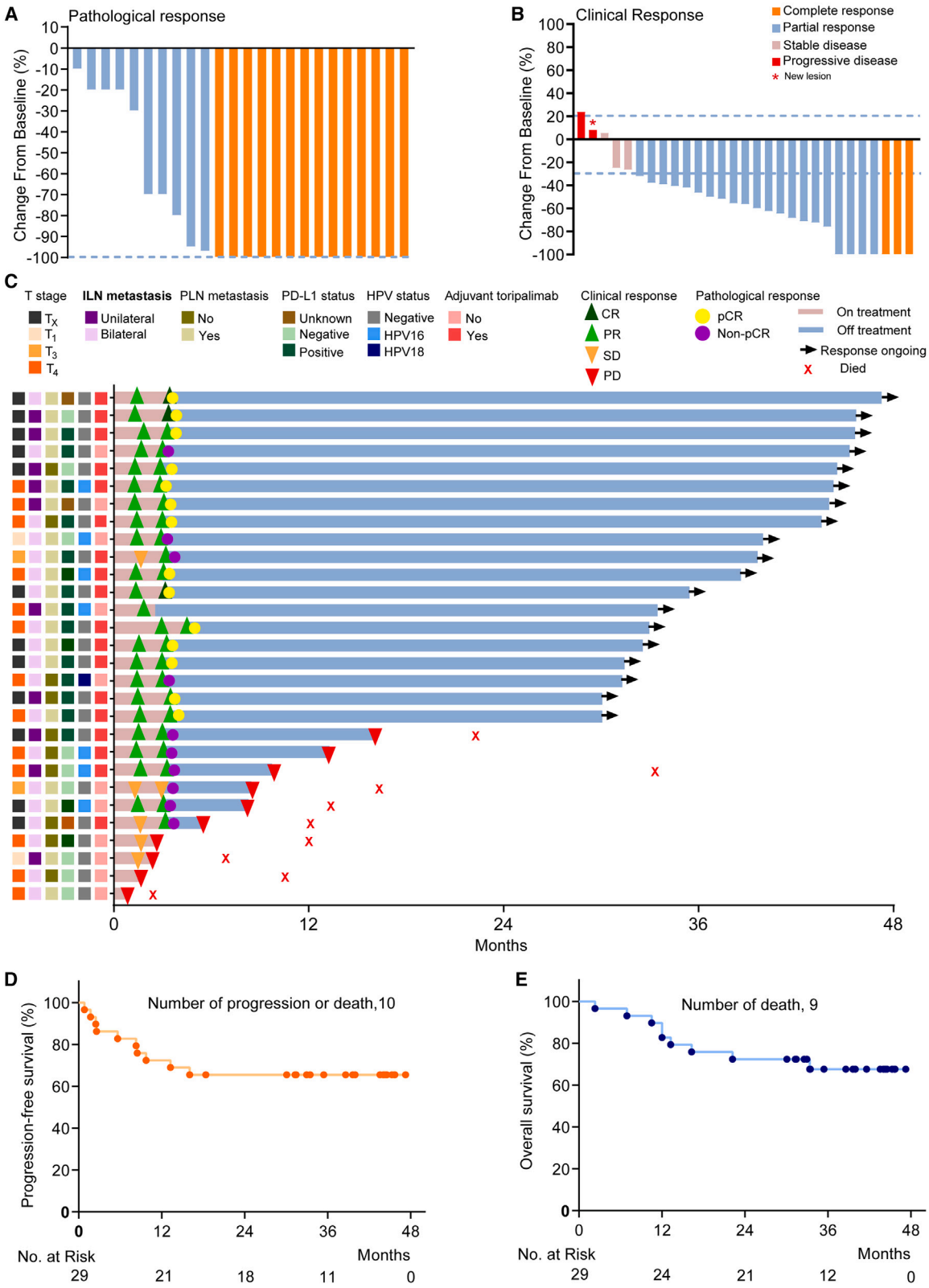


Figure 1. Anti-tumor response of TNT

(A) Pathological tumor regression in the operated population according to pathological assessment (n = 24). Dashed lines at -100% represent a pathological complete response.

(legend continued on next page)

Among 29 patients, 26 had evaluable PD-L1 status, and 17 tested positive for PD-L1 expression (65.3%). Responders presented higher PD-L1 expression proportion scores than non-responders ($p = 0.019$) (Figure 2B). Both PFS and OS benefit was observed in patients with PD-L1 positive expression (median PFS NR vs. 9.7 months, HR 0.182, 95% CI 0.042 to 0.800, $p = 0.006$; median OS NR vs. 33.37 months, HR 0.259, 95% CI 0.057 to 1.172, $p = 0.045$) (Figures 2C and 2D).

The genomic landscape of 28 tumor samples revealed mutations in 85.7% of cases (24/28), with *TP53* being the most frequently altered gene (43%), followed by *MUC4* (32%), *FAT1* (29%), *NOTCH1* (25%), and *DNAH14* (25%) (Figure 2E). Mutation patterns were predominantly missense mutations. Although differences in mutation frequencies between pCR and non-pCR groups (Figure 2F) were not statistically significant, *TP53* mutations were significantly more frequent in PD/SD cases compared to PR/CR cases (Figure 2G, $p < 0.05$). *TP53* wild-type (TP53-WT) patients showed a trend toward improved PFS compared to *TP53*-mutated (TP53-MUT) patients (HR 3.446, 95% CI: 0.889–13.37, $p = 0.061$, Figure 2H). In Figure 2I, TP53-WT patients demonstrated significantly better OS than TP53-MUT patients (HR 5.239, 95% CI: 1.241–22.12, $p = 0.022$). Tumor mutational burden (TMB) levels did not differ significantly between pCR and non-pCR groups ($p = 0.98$, Figure 2J) or between PR/CR and PD/SD groups ($p = 0.68$, Figure 2K).

Transcriptomic analysis (Figure 2L) revealed higher $CD8^+$ T cell levels in pCR compared to non-pCR patients ($p = 0.02$ and $p = 0.19$, Figures 2M and 2N) and in PR/CR compared to PD/SD patients ($p = 0.01$ and $p = 0.007$, Figures 2O and 2P). Total T cell abundance, cytotoxicity scores, and B cell levels were also significantly higher in PR/CR patients (all $p < 0.05$, Figure 2P). Immunohistochemical analysis further validated these findings, showing increased $CD8^+$ T cell density in responsive compared to non-responsive samples ($p = 0.04$, Figures 2Q and 2R). These results underscore the role of $CD8^+$ T cell infiltration in predicting therapeutic response.

DISCUSSION

In this phase II trial, we evaluated the efficacy and safety of neoadjuvant toripalimab and nimotuzumab combined with chemotherapy (TNT) in La-PSCC. TNT trial met its primary endpoint with a pCR rate of 48.3% in all enrolled patients. The pCR rates in patients with high-risk cT4/cN3M0 stage were better than those of standard chemotherapy, which reported a pCR rate of 10–13.6% for TIP chemotherapy in patients with N2-3M0 stage.[11] Our findings suggest that among patients with La-PSCC, TNT regimen showed a promising antitumor activity with acceptable toxicity.

pCR is a powerful predictive factor for the effectiveness of neoadjuvant treatment and prognosis of long-term survival in several malignancies, such as rectal cancer, breast cancer, and bladder cancer.[29–31] Previous studies showed that pCR rates were approximately 0–20% in PSCC patients treated with neoadjuvant chemotherapy.[11,32–36] Our study had a pCR rate of 48.3%, the highest reported so far, with only one patient with pCR experiencing disease recurrence. The ORR, including CR and PR, is also used to assess the impact of neoadjuvant chemotherapy, reflecting the sensitivity of chemotherapy and predicting patient outcomes. The ORR of 82.8% is also the highest among all La-PSCC reports to date.

The difference in numbers between patients reported to achieve complete pathological responses ($n = 14$) and patients reported to achieved clinical complete responses ($n = 3$) suggested that radiological assessments might underestimate the effectiveness of neoadjuvant therapies. Imaging focusing on changes in tumor size and potentially overlooking cellular death or necrosis may not accurately reflect tumor regression, especially in PSCC with the N3 stage. These bulky tumors often exhibit necrosis, posing a challenge in accurately assessing complete response through imaging alone.[37–39]

With a median follow-up time of 40 months, we observed that 2-year PFS and OS rates were 65.5% and 72.4%, respectively. These findings are consistent with our previous retrospective study (2-year PFS 68.4%) and also superior to historical data of neoadjuvant chemotherapy and first-line monotherapy with EGFR or ICI inhibitors.[19,22,28] Previous trials investigating TPF (cisplatin/5-FU/paclitaxel) and TIP in clinical N2-3 patients reported ORRs of 38%–60%, with median PFS ranging from 7.0 to 8.1 months and median OS between 10.1 and 17.1 months.[11,34,40] Necchi et al. evaluated the oral EGFR inhibitor dacomitinib as a first-line treatment in clinical N2-3 or M1 PSCC, reporting an ORR of 32.1% in the overall cohort, with a median PFS of 4.3 months and median OS of 20.0 months in the La-PSCC subgroup.[19] The PERICLES study of 32 patients with advanced PSCC reported an ORR of 16.7% and a median OS of 11.3 months for atezolizumab alone or in combination with radiotherapy.[22] In addition, the LACOG study reported a 39.4% ORR for first-line pembrolizumab plus chemotherapy in advanced PSCC, with median PFS and OS of 5.4 and 9.6 months, respectively.[23] A recent trial combining the anti-EGFR antibody CDP1 with TIP chemotherapy achieved a promising ORR of 73.7%, though median PFS was limited to 6.9 months. These findings are aligned with the BEATcc trial, where the addition of immunotherapy to a standard targeted therapy and chemotherapy regimen led to significant improvements in PFS and OS among cervical cancer patients.[41] Inhibiting the EGFR signaling pathway has direct antitumor effects and boosts immune response by activating natural killer cells and increasing immune cell infiltration.[42] These

(B) Radiologic tumor regression of the best percentage of the target lesions change from baseline in all enrolled patients according to RECIST v1.1. Dashed lines at 20% and -30% represent the RECIST v1.1 cut-off to define progressive disease and partial response, respectively ($n = 29$).
(C) The duration of response in all enrolled patients ($n = 29$). Bar indicates progression-free survival. The triangles with different colors show different clinical responses, according to the RECIST v1.1. The plots with different colors show different pathological responses after consolidative surgery. Response ongoing was defined as patients who had neither progressed nor died at the time of analysis.
(D and E) Kaplan-Meier curves for progression-free survival (D) and overall survival (E) in all enrolled patients ($n = 29$). PD, progressive disease; PR, partial response; RECIST, the response evaluation criteria in solid tumors; CR, complete response; SD, stable disease.
See also Figure S2 and Table S1.

Table 2. Adverse events of TNT

Adverse events, no (%)	Grade 1-2	Grade 3	Grade 4
Alopecia	29(100%)	0	0
Decreased appetite	27(93.1%)	0	0
Peripheral sensory neuropathy	23(79.3%)	1(3.4%)	0
Anemia	19(65.5%)	3(10.3%)	0
Nausea	19(65.5%)	0	0
Constipation	15(51.7%)	0	0
Pyrexia	11(37.9%)	0	0
Neutropenia	9(31.0%)	5(17.2%)	2(6.9%)
Dizziness	9(31.0%)	0	0
Pruritus	9(31.0%)	0	0
Asthenia	8(27.6%)	1(3.4%)	0
Skin pigmentation	8(27.6%)	0	0
Myalgia	8(27.6%)	0	0
Hypomagnaesemia	8(27.6%)	0	0
Arthralgia	7(24.1%)	0	0
Vomiting	7(24.1%)	0	0
Hyponatremia	7(24.1%)	0	0
Hypercholesterolemia	7(24.1%)	0	0
Hypertriglyceridemia	6(20.7%)	0	0
Hyperglycemia	6(20.7%)	0	0
Rash	6(20.7%)	2(6.9%)	0
Abdominal pain	6(20.7%)	0	0
Diarrhea	5(17.2%)	0	0
Hypothyroidism	5(17.2%)	0	0
Stomatitis	4(13.8%)	0	0
Increased aminotransferase	4(13.8%)	0	0
Increased creatinine	3(10.3%)	0	0
Hyperthyroidism	3(10.3%)	0	0
Thrombocytopenia	3(10.3%)	0	0
Insomnia	3(10.3%)	0	0
Adrenal insufficiency	2(6.9%)	0	0
Hypokalemia	2(6.9%)	0	0
Hyperbilirubinemia	1(3.4%)	0	0
Headache	1(3.4%)	0	0
Febrile neutropenia	0	2(6.9%)	0

See also Tables S2 and S3.

results highlight the potential efficacy of utilizing a combination of novel targeted agents to treat advanced PSCC.

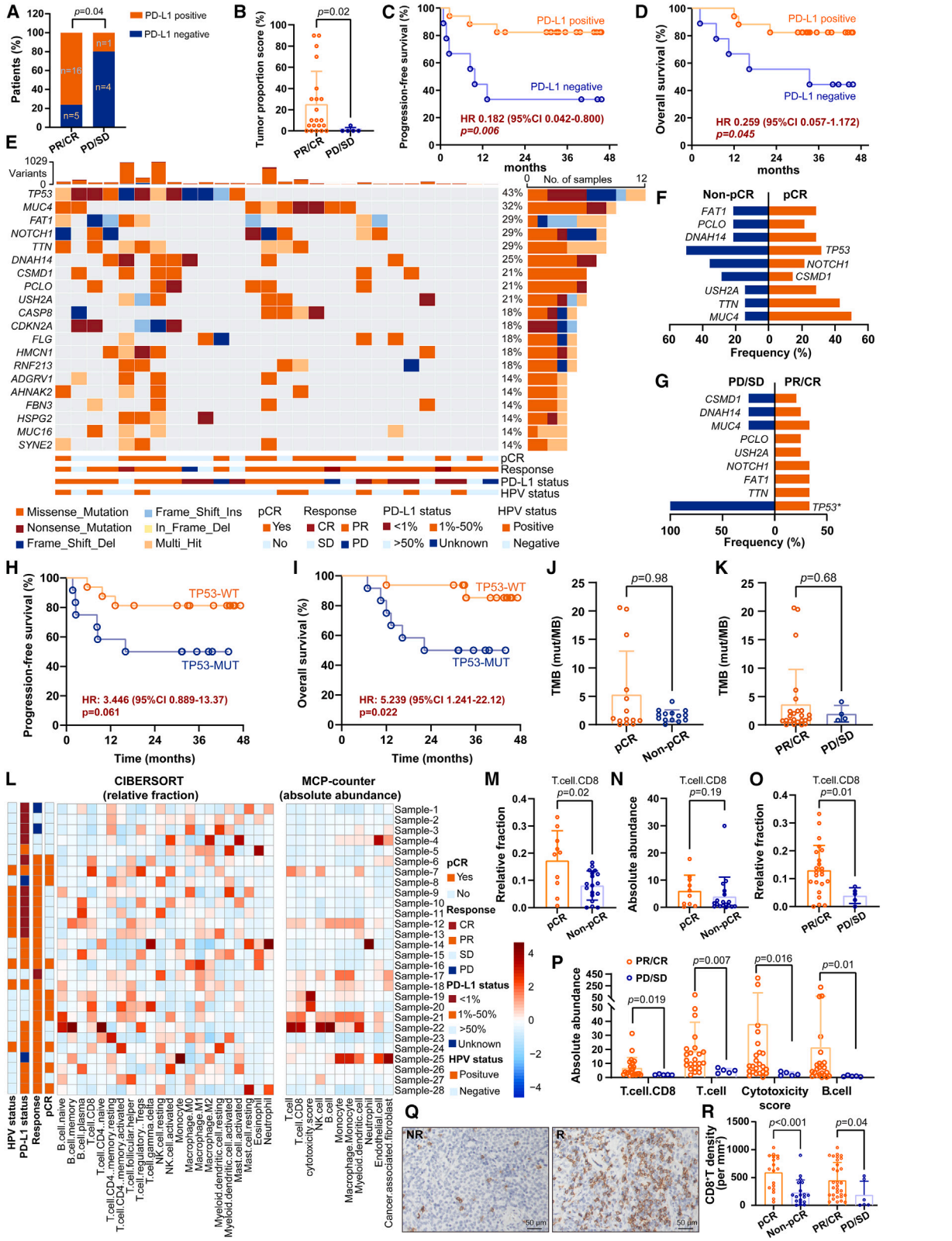
Overall, neoadjuvant TNT was well tolerated, with a toxicity profile consistent with TIP chemotherapy, toripalimab, and nimotuzumab monotherapy. Grade 1 to 2 TRAEs, such as alopecia and hematological toxicity, were higher than those reports of chemotherapy plus ICIs, potentially as a result of the combination of multiple medications.[43,44] In addition, peripheral sensory neuropathy (79.3%) of TNT was higher than that of the TIP regimen. Several studies showed that the prevalence of peripheral sensory neuropathy associated with albumin-bound paclitaxel is more significant than that observed with conventional paclitaxel, potentially attributable to the elevated dosing regimen of albumin-bound paclitaxel.[45]

We observed that the incidence of early or delayed postoperative complications, such as scrotal edema, hemorrhage, and seroma in our trial was higher than those of patients treated with traditional TIP chemotherapy, which potentially attributed to the high proportion of patients with N3 stage conducted pelvic plus inguinal lymph node dissection simultaneously. Moreover, the TNT regimen itself potentially increased the incidence of postoperative complications as well. Since there were only two patients who suffered grade 3 postoperative complications, we think that its impact on the quality of life of the patients is minute. Randomized control trials to confirm our results are needed. Moreover, in patients with CR, further research on avoiding surgery or reducing the scope of operation is warranted.

Consistent with previous studies regarding La-PSCC,[46] no patients were observed to have baseline proficient MSS in our trial. PD-L1 expression was an effective biomarker for predicting the response of ICIs.[47] We found that positive PD-L1 expression (TPS >1%) was associated with better treatment response and longer PFS/OS in La-PSCC, suggesting that exploring the best cut-off value of PD-L1 expression in a larger sample size is needed. Notably, all four patients with PD-L1 TPS >50% in this study were responders, with no disease progression observed after more than 32 months of follow-up. Moreover, it is necessary to explore more potential treatment options for patients without PD-L1 expression. The HERCULES trial suggests TMB as a potential biomarker for immunotherapy combined with chemotherapy in PSCC.[23] Similarly, in our study, all three patients with TMB >10 mutations/Mb achieved pCR, supporting TMB as a potential marker for penile cancer suitability of immunotherapy. Consistent with prior studies, TP53 has been identified as the most commonly mutated gene in PSCC[48,49] and is closely associated with survival outcomes.[50–52] In our study, TP53 mutations were significantly correlated with reduced therapeutic response and poorer survival outcomes following TNT treatment, highlighting its potential role as a predictive biomarker in PSCC. Previous studies indicated that a high infiltration rate of $CD8^+$ T cell was associated with improved PFS in patients with PSCC receiving immunotherapy,[22] and our results found higher intratumoral $CD8^+$ T infiltration in pretreatment samples of responders however this warrants further investigation. Beyond exploring combination therapies, future PSCC treatment should prioritize the development of novel targets. Whole-exome sequencing has revealed frequent mutations in *CDKN2A*, *NOTCH1*, and *PIK3CA*, indicating potential avenues for targeted interventions.[53] Additionally, a significant portion of metastatic PSCC cases may respond to treatments targeting the mTOR, DNA repair, and tyrosine kinase pathways.[54,55] These findings highlight the need for integrating new molecular targets into the treatment framework, advancing personalized and potentially more effective therapies for PSCC.

Limitations of the study

Firstly, this is a phase II study with a limited sample size among Chinese PSCC patients; hence, the generalizability of these findings in a broader, ethnically diverse patient population would be needed. The Global Society of Rare Genito-urinary Tumors (GSRGT) endeavors to rectify disparities in funding, occurrence rates, and knowledge allocation through global collaboration; such an idea is worthy of our study.[21] While a large phase III trial



(legend on next page)

could provide robust data for broader adoption, designing such studies for rare cancers like PSCC is challenging due to low incidence and limited patient recruitment. A multicenter phase II trial offers a practical alternative, enabling broader recruitment and delivering solid data on efficacy and safety.[56] This approach can establish the therapeutic potential and guide future randomized trials. Secondly, due to the lower prevalence of HPV infection in the Chinese population compared to Western populations, we did not observe a significant difference in treatment response between patients with and without HPV infection. Therefore, additional validation of these findings among a larger sample is necessary. Thirdly, the biomarker analysis using whole-exome and transcriptome sequencing was not pre-specified in the trial design, potentially introducing bias to exploratory post hoc findings. Finally, the long-term durable survival benefit of TNT remains to be observed.

Our findings indicate that TNT has promising benefits in the neoadjuvant settings of patients and offers an effective multi-agent therapeutic option in managing La-PSCC. A randomized phase II/III trial, likely through a large collaborative group and across a global patient population to validate our results is warranted in establishing this as an additional standard treatment in the management of PSCC.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hui Han (hanhui@sysucc.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The Whole-exome sequencing (WES) data and the RNA-seq raw data used in our study have been deposited in the SRA-NCBI (www.ncbi.nlm.nih.gov/sra), SRA accession: PRJNA1215548 (SRR32128796- SRR32128823 for WES and SRR32120503- SRR32120530 for RNA-seq). Any information required to reanalyze the data reported in this paper is available from the lead contact upon request. This study did not generate any new code.

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AUTHOR CONTRIBUTIONS

X.A., S.J.G., R.Y., T.X., L.B.X., Y.X.S., and H.H. contributed to the design of the study, data collection, statistical analysis, and manuscript drafting; X.A., S.J.G., Y.X.S., H.H., Z.L.Z., P.D., Y.H.L., K.Y., Z.Q.H., X.F.C., J.X.L., Y.H.L., P.Y.L., Z.Z.L., L.Q., W.F.X., Z.G.C., N.H.C., X.L., X.N.S., G.H.L., B.K.S., Q.X., Z.W.L., and FJ.Z. recruited patients and collected the data; H.L.M., C.X., Y.C.Z., J.B.L., M.T.C., Z.S.L., and T.Y.L. contributed to data analysis and interpretation; L.B.X., P.E.S., Y.X.S., and H.H. revised the manuscript; all authors have read and approved the article.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Study design and participants
  - Systematic treatment

Figure 2. Genomic, transcriptomic, and immunohistochemical profiling of penile squamous cell carcinoma (PSCC) and its association with treatment response and survival

(A) The distribution of PD-L1 expression between responders (CR/PR) and non-responders (SD/PD). PD-L1 positivity was defined as over 1% of tumor cells presenting positive stains. *p* values were calculated by the two-way Fisher's exact test.

(B) The comparison of the presence of tumor cell expression PD-L1 score between responders and non-responders. Data in bar graphs are shown as mean ± SD; with $n = 21$ in PR/CR and $n = 5$ in PD/SD. *p* values were calculated using the two-tailed Mann-Whitney U test.

(C and D) Kaplan–Meier curves of (C) progression-free survival and (D) overall survival group by PD-L1 expression ($n = 26$). *p* values were determined using the log-rank test.

(E) Genomic landscape of 28 tumor pretreatment samples from 28 patients showing mutation profiles and their association with clinical features, including pCR status, response type, PD-L1 status, and HPV status.

(F and G) Comparison of mutation frequencies between pCR and non-pCR groups (F) and PR/CR and PD/SD groups (G). Data in histogram are shown as patient proportion; with $n = 14$ in pCR and $n = 14$ in non-pCR; with $n = 24$ in PR/CR and $n = 4$ in PD/SD.

(H and I) Kaplan-Meier survival analysis of PFS (H) and OS (I) stratified by *TP53* mutation status. *p* values were determined using the log-rank test.

(J and K) Tumor mutational burden (TMB) analysis in relation to pCR status (J) and response type (K). Data in bar graphs are shown as mean ± SD; with $n = 14$ in pCR and $n = 14$ in non-pCR; with $n = 24$ in PR/CR and $n = 4$ in PD/SD.

(L) Immune cell profiling using CIBERSORT (relative fraction) and MCP-counter (absolute abundance) across tumor samples (28 pretreatment samples from 23 patients).

(M–P) Analysis of $CD8^+$ T cells and other significantly different cell populations, including their relative fractions and absolute abundances, using transcriptomic data (28 pretreatment samples from 23 patients) stratified by pCR status (I and K) and response type (J and L). Data in bar graphs are shown as mean ± SD; with $n = 10$ in pCR and $n = 18$ in non-pCR; with $n = 23$ in PR/CR and $n = 5$ in PD/SD.

(Q and R) Immunohistochemical analysis of $CD8^+$ T cell density, with representative staining images for responsive (CR/PR) and non-responsive (SD/PD) patients (37 pretreatment samples from 26 patients). Data in bar graphs are shown as mean ± SD; with $n = 18$ in pCR and $n = 19$ in non-pCR; with $n = 31$ in PR/CR and $n = 6$ in PD/SD. PD-L1, programmed death-ligand 1; NR, not reached; NE, not estimated; HR, hazard ratios; CI, confidence intervals; pCR, pathological complete response; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

See also Figure S3, Tables S4, and S5.

SUPPLEMENTAL INFORMATION

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REFERENCES

1. Olesen, T.B., Sand, F.L., Rasmussen, C.L., Albieri, V., Toft, B.G., Norrild, B., Munk, C., and Kjær, S.K. (2019). Prevalence of human papillomavirus DNA and p16(INK4a) in penile cancer and penile intraepithelial neoplasia: a systematic review and meta-analysis. Lancet Oncol. *20*, 145–158. https://doi.org/10.1016/s1470-2045(18)30682-x.
2. Douglawi, A., and Masterson, T.A. (2019). Penile cancer epidemiology and risk factors: a contemporary review. Curr. Opin. Urol. *29*, 145–149. https://doi.org/10.1097/mou.0000000000000581.
3. Leijte, J.A.P., Valdés Olmos, R.A., Nieweg, O.E., and Horenblas, S. (2008). Anatomical mapping of lymphatic drainage in penile carcinoma with SPECT-CT: implications for the extent of inguinal lymph node dissection. Eur. Urol. *54*, 885–890. https://doi.org/10.1016/j.eururo.2008.04.094.
4. Thomas, A., Necchi, A., Muneer, A., Tobias-Machado, M., Tran, A.T.H., Van Rompuy, A.S., Spiess, P.E., and Albersen, M. (2021). Penile cancer. Nat. Rev. Dis. Primers *7*, 11. https://doi.org/10.1038/s41572-021-00246-5.
5. Joshi, S.S., Handorf, E., Strauss, D., Correa, A.F., Kutikov, A., Chen, D.Y.T., Viterbo, R., Greenberg, R.E., Uzzo, R.G., Smaldone, M.C., and Geynisman, D.M. (2018). Treatment Trends and Outcomes for Patients With Lymph Node-Positive Cancer of the Penis. JAMA Oncol. *4*, 643–649. https://doi.org/10.1001/jamaoncol.2017.5608.
6. Chipollini, J., Necchi, A., and Spiess, P.E. (2018). Outcomes for Patients with Node-positive Penile Cancer: Impact of Perioperative Systemic Therapies and the Importance of Surgical Intervention. Eur. Urol. *74*, 241–242. https://doi.org/10.1016/j.eururo.2018.04.025.
7. Djajadiningrat, R.S., van Werkhoven, E., and Horenblas, S. (2015). Prophylactic pelvic lymph node dissection in patients with penile cancer. J. Urol. *193*, 1976–1980. https://doi.org/10.1016/j.juro.2014.12.019.
8. Pandey, D., Mahajan, V., and Kannan, R.R. (2006). Prognostic factors in node-positive carcinoma of the penis. J. Surg. Oncol. *93*, 133–138. https://doi.org/10.1002/jso.20414.
9. Clark, P.E., Spiess, P.E., Agarwal, N., Biagioli, M.C., Eisenberger, M.A., Greenberg, R.E., Herr, H.W., Inman, B.A., Kuban, D.A., Kuzel, T.M., et al. (2013). Penile cancer: Clinical Practice Guidelines in Oncology. J. Natl. Compr. Cancer Netw. *11*, 594–615. https://doi.org/10.6004/jnccn.2013.0075.
10. Hakenberg, O.W., Compérat, E.M., Minhas, S., Necchi, A., Protzel, C., and Watkin, N. (2015). EAU guidelines on penile cancer: 2014 update. Eur. Urol. *67*, 142–150. https://doi.org/10.1016/j.eururo.2014.10.017.
11. Pagliaro, L.C., Williams, D.L., Daliani, D., Williams, M.B., Osai, W., Kincaid, M., Wen, S., Thall, P.F., and Pettaway, C.A. (2010). Neoadjuvant paclitaxel, ifosfamide, and cisplatin chemotherapy for metastatic penile cancer: a phase II study. J. Clin. Oncol. *28*, 3851–3857. https://doi.org/10.1200/jco.2010.29.5477.
12. Wang, J., Pettaway, C.A., and Pagliaro, L.C. (2015). Treatment for Metastatic Penile Cancer After First-line Chemotherapy Failure: Analysis of Response and Survival Outcomes. Urology *85*, 1104–1110. https://doi.org/10.1016/j.urology.2014.12.049.
13. Mentrikoski, M.J., Stelow, E.B., Culp, S., Frierson, H.F., Jr., and Cathro, H.P. (2014). Histologic and immunohistochemical assessment of penile carcinomas in a North American population. Am. J. Surg. Pathol. *38*, 1340–1348. https://doi.org/10.1097/pas.0000000000000124.
14. Di Lorenzo, G., Buonerba, C., Gaudioso, G., Gigantino, V., Quarto, G., De Domenico, R., Caraglia, M., Di Trolio, R., Ascierto, P.A., De Placido, S., et al. (2013). EGFR mutational status in penile cancer. Expert Opin. Ther. Targets *17*, 501–505. https://doi.org/10.1517/14728222.2013.783571.
15. Ottenhof, S.R., Djajadiningrat, R.S., de Jong, J., Thygesen, H.H., Horenblas, S., and Jordanova, E.S. (2017). Expression of Programmed Death Ligand 1 in Penile Cancer is of Prognostic Value and Associated with HPV Status. J. Urol. *197*, 690–697. https://doi.org/10.1016/j.juro.2016.09.088.
16. Udager, A.M., Liu, T.Y., Skala, S.L., Magers, M.J., McDaniel, A.S., Spratt, D.E., Feng, F.Y., Siddiqui, J., Cao, X., Fields, K.L., et al. (2016). Frequent PD-L1 expression in primary and metastatic penile squamous cell carcinoma: potential opportunities for immunotherapeutic approaches. Ann. Oncol. *27*, 1706–1712. https://doi.org/10.1093/annonc/mdw216.
17. Davidsson, S., Carlsson, J., Giunchi, F., Harlow, A., Kirrander, P., Rider, J., Fiorentino, M., and Andrén, O. (2019). PD-L1 Expression in Men with Penile Cancer and its Association with Clinical Outcomes. Eur. Urol. Oncol. *2*, 214–221. https://doi.org/10.1016/j.euo.2018.07.010.
18. Huang, K.B., Liu, R.Y., Peng, Q.H., Li, Z.S., Jiang, L.J., Guo, S.J., Zhou, Q.H., Liu, T.Y., Deng, C.Z., Yao, K., et al. (2019). EGFR mono-antibody salvage therapy for locally advanced and distant metastatic penile cancer: Clinical outcomes and genetic analysis. Urol. Oncol. *37*, 71–77. https://doi.org/10.1016/j.urolonc.2018.10.016.
19. Necchi, A., Lo Vullo, S., Perrone, F., Raggi, D., Giannatempo, P., Calareso, G., Nicolai, N., Piva, L., Biasoni, D., Catanzaro, M., et al. (2018). 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. *121*, 348–356. https://doi.org/10.1111/bju.14013.
20. Necchi, A., Nicolai, N., Colecchia, M., Catanzaro, M., Torelli, T., Piva, L., and Salvioni, R. (2011). Proof of activity of anti-epidermal growth factor receptor-targeted therapy for relapsed squamous cell carcinoma of the penis. J. Clin. Oncol. *29*, e650–e652. https://doi.org/10.1200/jco.2011.34.8367.
21. El Zarif, T., Nassar, A.H., Pond, G.R., Zhuang, T.Z., Master, V., Nazha, B., Niglio, S., Simon, N., Hahn, A.W., Pettaway, C.A., et al. (2023). Safety and efficacy of immune checkpoint inhibitors in advanced penile cancer: report from the Global Society of Rare Genitourinary Tumors. J. Natl. Cancer Inst. *115*, 1605–1615. https://doi.org/10.1093/jnci/djad155.
22. de Vries, H.M., Rafael, T.S., Gil-Jimenez, A., de Feijter, J.M., Bekers, E., van der Laan, E., Lopez-Yurda, M., Hooijberg, E., Broeks, A., Peters, D., et al. (2023). Atezolizumab With or Without Radiotherapy for Advanced Squamous Cell Carcinoma of the Penis (The PERICLES Study): A Phase II Trial. J. Clin. Oncol. *41*, 4872–4880. https://doi.org/10.1200/jco.22.02894.
23. Maluf, F.C., Trindade, K., Preto, D.D.A., Monteiro, F.S.M., Luz, M., Beato, P.M., Bastos, D.A., Soares, J.P.H., Lopes dos Santos, V.M., Werneck De Carvalho, L.E., et al. (2024). A phase II trial of pembrolizumab plus platinum-based chemotherapy as first-line systemic therapy in advanced penile cancer. HERCULES (LACOG 0218) trial *42*, 5009. https://doi.org/10.1200/JCO.2024.42.16_suppl.5009.
24. Reck, M., Mok, T.S.K., Nishio, M., Jotte, R.M., Cappuzzo, F., Orlandi, F., Stroyakovskiy, D., Nogami, N., Rodríguez-Abreu, D., Moro-Sibilot, D., et al. (2019). Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. Lancet Respir. Med. *7*, 387–401. https://doi.org/10.1016/s2213-2600(19)30084-0.

25. Janjigian, Y.Y., Kawazoe, A., Yañez, P., Li, N., Lonardi, S., Kolesnik, O., Barajas, O., Bai, Y., Shen, L., Tang, Y., et al. (2021). The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. Nature *600*, 727–730. https://doi.org/10.1038/s41586-021-04161-3.

26. Vanneman, M., and Dranoff, G. (2012). Combining immunotherapy and targeted therapies in cancer treatment. Nat. Rev. Cancer *12*, 237–251. https://doi.org/10.1038/nrc3237.

27. Galon, J., and Bruni, D. (2019). Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat. Rev. Drug Discov. *18*, 197–218. https://doi.org/10.1038/s41573-018-0007-y.

28. Yan, R., Ma, H., Jiang, L., Guo, S., Shi, Y., Sheng, X., Zhang, Y., Spiess, P.E., Liu, T., Xue, T., et al. (2023). First-line PD-1 Inhibitor & EGFR Blockade & Platinum-based Chemotherapy (PEP) for Stage IV Penile Cancer. BJU Int. *131*, 198–207. https://doi.org/10.1111/bju.15828.

29. Ganguly, S., and Gogia, A. (2022). Pembrolizumab in Early Triple-Negative Breast Cancer. N. Engl. J. Med. *386*, 1771. https://doi.org/10.1056/NEJMc2203316.

30. Rose, T.L., Harrison, M.R., Deal, A.M., Ramalingam, S., Whang, Y.E., Brower, B., Dunn, M., Osterman, C.K., Heiling, H.M., Bjurlin, M.A., et al. (2021). Phase II Study of Gemcitabine and Split-Dose Cisplatin Plus Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in Patients With Muscle-Invasive Bladder Cancer. J. Clin. Oncol. *39*, 3140–3148. https://doi.org/10.1200/jco.21.01003.

31. Maas, M., Nelemans, P.J., Valentini, V., Das, P., Rödel, C., Kuo, L.J., Calvo, F.A., García-Aguilar, J., Glynne-Jones, R., Haustermans, K., et al. (2010). Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: a pooled analysis of individual patient data. Lancet Oncol. *11*, 835–844. https://doi.org/10.1016/s1470-2045(10)70172-8.

32. Leijte, J.A.P., Kerst, J.M., Bais, E., Antonini, N., and Horenblas, S. (2007). Neoadjuvant chemotherapy in advanced penile carcinoma. Eur. Urol. *52*, 488–494. https://doi.org/10.1016/j.eururo.2007.02.006.

33. Pizzocaro, G., Nicolai, N., and Milani, A. (2009). Taxanes in combination with cisplatin and fluorouracil for advanced penile cancer: preliminary results. Eur. Urol. *55*, 546–551. https://doi.org/10.1016/j.eururo.2008.07.014.

34. Djajadiningrat, R.S., Bergman, A.M., van Werkhoven, E., Vegt, E., and Horenblas, S. (2015). Neoadjuvant taxane-based combination chemotherapy in patients with advanced penile cancer. Clin. Genitourin. Cancer *13*, 44–49. https://doi.org/10.1016/j.clgc.2014.06.005.

35. Nicolai, N., Sangalli, L.M., Necchi, A., Giannatempo, P., Paganoni, A.M., Colecchia, M., Piva, L., Catanzaro, M.A., Biasoni, D., Stagni, S., et al. (2016). A Combination of Cisplatin and 5-Fluorouracil With a Taxane in Patients Who Underwent Lymph Node Dissection for Nodal Metastases From Squamous Cell Carcinoma of the Penis: Treatment Outcome and Survival Analyses in Neoadjuvant and Adjuvant Settings. Clin. Genitourin. Cancer *14*, 323–330. https://doi.org/10.1016/j.clgc.2015.07.009.

36. Azizi, M., Aydin, A.M., Hajiran, A., Lai, A., Kumar, A., Peyton, C.C., Minhas, S., Sonpavde, G.P., Chahoud, J., Pagliaro, L.C., et al. (2020). Systematic Review and Meta-Analysis-Is there a Benefit in Using Neoadjuvant Systemic Chemotherapy for Locally Advanced Penile Squamous Cell Carcinoma? J. Urol. *203*, 1147–1155. https://doi.org/10.1097/ju.0000000000000746.

37. Hanna, G.J., O'Neill, A., Shin, K.Y., Wong, K., Jo, V.Y., Quinn, C.T., Cutler, J.M., Flynn, M., Lizotte, P.H., Annino, D.J., Jr., et al. (2022). Neoadjuvant and Adjuvant Nivolumab and Lirilumab in Patients with Recurrent, Resectable Squamous Cell Carcinoma of the Head and Neck. Clin. Cancer Res. *28*, 468–478. https://doi.org/10.1158/1078-0432.Ccr-21-2635.

38. Leidner, R., Crittenden, M., Young, K., Xiao, H., Wu, Y., Couey, M.A., Patel, A.A., Cheng, A.C., Watters, A.L., Bifulco, C., et al. (2021). Neoadjuvant immunoradiotherapy results in high rate of complete pathological response and clinical to pathological downstaging in locally advanced head and neck squamous cell carcinoma. J. Immunother. Cancer *9*, e002485. https://doi.org/10.1136/jitc-2021-002485.

39. Ferrarotto, R., Amit, M., Nagarajan, P., Rubin, M.L., Yuan, Y., Bell, D., El-Naggar, A.K., Johnson, J.M., Morrison, W.H., Rosenthal, D.I., et al. (2021). Pilot Phase II Trial of Neoadjuvant Immunotherapy in Locoregionally Advanced, Resectable Cutaneous Squamous Cell Carcinoma of the Head and Neck. Clin. Cancer Res. *27*, 4557–4565. https://doi.org/10.1158/1078-0432.Ccr-21-0585.

40. Nicholson, S., Hall, E., Harland, S.J., Chester, J.D., Pickering, L., Barber, J., Elliott, T., Thomson, A., Burnett, S., Cruickshank, C., et al. (2013). Phase II trial of docetaxel, cisplatin and 5FU chemotherapy in locally advanced and metastatic penis cancer (CRUK/09/001). Br. J. Cancer *109*, 2554–2559. https://doi.org/10.1038/bjc.2013.620.

41. Oaknin, A., Gladieff, L., Martínez-García, J., Villacampa, G., Takekuma, M., De Giorgi, U., Lindemann, K., Woelber, L., Colombo, N., Duska, L., et al. (2024). Atezolizumab plus bevacizumab and chemotherapy for metastatic, persistent, or recurrent cervical cancer (BEATcc): a randomised, open-label, phase 3 trial. Lancet (London, England) *403*, 31–43. https://doi.org/10.1016/s0140-6736(23)02405-4.

42. Pozzi, C., Cuomo, A., Spadoni, I., Magni, E., Silvola, A., Conte, A., Sigismund, S., Ravenda, P.S., Bonaldi, T., Zampino, M.G., et al. (2016). The EGFR-specific antibody cetuximab combined with chemotherapy triggers immunogenic cell death. Nat. Med. *22*, 624–631. https://doi.org/10.1038/nm.4078.

43. Mittendorf, E.A., Zhang, H., Barrios, C.H., Saji, S., Jung, K.H., Hegg, R., Koehler, A., Sohn, J., Iwata, H., Telli, M.L., et al. (2020). Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. Lancet (London, England) *396*, 1090–1100. https://doi.org/10.1016/s0140-6736(20)31953-x.

44. Shu, C.A., Gainor, J.F., Awad, M.M., Chiuzan, C., Grigg, C.M., Pabani, A., Garofano, R.F., Stoopler, M.B., Cheng, S.K., White, A., et al. (2020). Neoadjuvant atezolizumab and chemotherapy in patients with resectable non-small-cell lung cancer: an open-label, multicentre, single-arm, phase 2 trial. Lancet Oncol. *21*, 786–795. https://doi.org/10.1016/s1470-2045(20)30140-6.

45. Gradishar, W.J., Tjulandin, S., Davidson, N., Shaw, H., Desai, N., Bhar, P., Hawkins, M., and O'Shaughnessy, J. (2005). Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J. Clin. Oncol. *23*, 7794–7803. https://doi.org/10.1200/jco.2005.04.937.

46. Stoehr, R., Wendler, O., Giedl, J., Gaisa, N.T., Richter, G., Campean, V., Burger, M., Wullich, B., Bertz, S., and Hartmann, A. (2019). No Evidence of Microsatellite Instability and Loss of Mismatch-Repair-Protein Expression in Squamous Cell Carcinoma of the Penis. Pathobiology : journal of immunopathology. Pathobiology *86*, 145–151. https://doi.org/10.1159/000495251.

47. Gavrielatou, N., Doumas, S., Economopoulou, P., Foukas, P.G., and Psyrri, A. (2020). Biomarkers for immunotherapy response in head and neck cancer. Cancer Treat Rev. *84*, 101977. https://doi.org/10.1016/j.ctrv.2020.101977.

48. Nazha, B., Zhuang, T., Wu, S., Brown, J.T., Magee, D., Carthon, B.C., Kucuk, O., Nabhan, C., Barata, P.C., Heath, E.I., et al. (2023). Comprehensive genomic profiling of penile squamous cell carcinoma and the impact of human papillomavirus status on immune-checkpoint inhibitor-related biomarkers. Cancer *129*, 3884–3893. https://doi.org/10.1002/cncr.34982.

49. Monteiro, F.S.M., Alencar Junior, A.M., da Trindade, K.M., Rebelatto, T.F., Maluf, F.C., Gazzola, A.A., Barrios, P.M., Bellmunt, J., de Jesus, R.G., Silva, G.E.B., et al. (2025). Molecular characterization of metastatic penile squamous cell carcinoma in developing countries and its impact on clinical outcomes: LACOG 2018 translational study. Oncologist *30*, oyae220. https://doi.org/10.1093/oncolo/oyae220.

50. Trias, I., Algaba, F., de Torres, I., Saco, A., Marimon, L., Peñuelas, N., Diez-Ahijado, L., Sisuashvili, L., Darecka, K., Morató, A., et al. (2024). p53 Immunohistochemistry Defines a Subset of Human Papillomavirus-Independent Penile Squamous Cell Carcinomas With Adverse Prognosis. Am. J. Surg. Pathol. *48*, 1439–1447. https://doi.org/10.1097/pas.0000000000002291.

51. Elst, L., Philips, G., Vandermaesen, K., Bassez, A., Lodi, F., Vreeburg, M.T.A., Brouwer, O.R., Schepers, R., Van Brussel, T., Mohanty, S.K., et al. (2024). Single-cell Atlas of Penile Cancer Reveals TP53 Mutations as a Driver of an Aggressive Phenotype, Irrespective of Human Papillomavirus Status, and Provides Clues for Treatment Personalization. Eur. Urol. *86*, 114–127. https://doi.org/10.1016/j.eururo.2024.03.038.

52. Chahoud, J., Yu, X., Necchi, A., and Spiess, P.E. (2024). TP53 Mutations Emerge as a Critical Biomarker in Penile Cancer, Superseding Human Papillomavirus Status. Eur. Urol. *86*, 128–129. https://doi.org/10.1016/j.eururo.2024.04.032.

53. Ali, S.M., Pal, S.K., Wang, K., Palma, N.A., Sanford, E., Bailey, M., He, J., Elvin, J.A., Chmielecki, J., Squillace, R., et al. (2016). Comprehensive Genomic Profiling of Advanced Penile Carcinoma Suggests a High Frequency of Clinically Relevant Genomic Alterations. Oncologist *21*, 33–39. https://doi.org/10.1634/theoncologist.2015-0241.

54. Jacob, J.M., Ferry, E.K., Gay, L.M., Elvin, J.A., Vergilio, J.A., Ramkissoon, S., Severson, E., Necchi, A., Killian, J.K., Ali, S.M., et al. (2019). Comparative Genomic Profiling of Refractory and Metastatic Penile and Nonpenile Cutaneous Squamous Cell Carcinoma: Implications for Selection of Systemic Therapy. J. Urol. *201*, 541–548. https://doi.org/10.1016/j.juro.2018.09.056.

55. Necchi, A., Spiess, P.E., Costa de Padua, T., Li, R., Grivas, P., Huang, R.S.P., Lin, D.I., Danziger, N., Ross, J.S., Jacob, J.M., et al. (2023). Genomic Profiles and Clinical Outcomes of Penile Squamous Cell Carcinoma With Elevated Tumor Mutational Burden. JAMA Netw. Open *6*, e2348002. https://doi.org/10.1001/jamanetworkopen.2023.48002.

56. Bandini, M., Ahmed, M., Basile, G., Watkin, N., Master, V., Zhu, Y., Prakash, G., Rodriguez, A., Ssebakumba, M.K., Leni, R., et al. (2022). A global approach to improving penile cancer care. Nat. Rev. Urol. *19*, 231–239. https://doi.org/10.1038/s41585-021-00557-y.

57. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., and DePristo, M.A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. *20*, 1297–1303. https://doi.org/10.1101/gr.107524.110.

58. Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. *38*, e164. https://doi.org/10.1093/nar/gkq603.

59. Newman, A.M., Liu, C.L., Green, M.R., Gentles, A.J., Feng, W., Xu, Y., Hoang, C.D., Diehn, M., and Alizadeh, A.A. (2015). Robust enumeration of cell subsets from tissue expression profiles. Nat. Methods *12*, 453–457. https://doi.org/10.1038/nmeth.3337.

60. Becht, E., Giraldo, N.A., Lacroix, L., Buttard, B., Elarouci, N., Petitprez, F., Selves, J., Laurent-Puig, P., Sautès-Fridman, C., Fridman, W.H., and de Reyniès, A. (2016). Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol. *17*, 218. https://doi.org/10.1186/s13059-016-1070-5.

61. Mayakonda, A., Lin, D.C., Assenov, Y., Plass, C., and Koeffler, H.P. (2018). Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Res. *28*, 1747–1756. https://doi.org/10.1101/gr.239244.118.

62. Yao, K., Tu, H., Li, Y.H., Qin, Z.K., Liu, Z.W., Zhou, F.J., and Han, H. (2010). Modified technique of radical inguinal lymphadenectomy for penile carcinoma: morbidity and outcome. J. Urol. *184*, 546–552. https://doi.org/10.1016/j.juro.2010.03.140.

63. Simon, R. (1989). Optimal two-stage designs for phase II clinical trials. Control. Clin. Trials *10*, 1–10. https://doi.org/10.1016/0197-2456(89)90015-9.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Toripalimab	Junshi Bioscience Co., Ltd.	JS001
Nimotuzumab	Biotech Pharmaceutical Co., Ltd.	h-R3
Anti-CD8A antibody	ZSGB-BIO	ZA-0508
Anti-CD4 antibody	ZSGB-BIO	ZA-0519
Anti-FOXP3 antibody	ZSGB-BIO	236A/E7
Anti-PD-L1 antibody	Junshi Bioscience Co., Ltd.	JS311
Anti-EGFR antibody	Abcam	ab52894
Anti- MLH1 antibody	Abcam	ab214441
Anti- MSH2 antibody	Abcam	ab228334
Anti- MSH6 antibody	Abcam	ab214454
Anti- PMS2 antibody	Abcam	ab110638
Biological samples		
Penile cancer biopsy samples	Sun Yat-sen University Cancer Center	N/A
Paraffin penile cancer sections	Sun Yat-sen University Cancer Center	N/A
Deposited data		
Raw RNA-seq data	This paper	SRA: SRR32120503- SRR32120530
Raw WES data	This paper	SRA: SRR32128796- SRR32128823
Critical commercial assays		
HPV detection kit	ACON Biotechnology Co., Ltd.	AK20120007
Software and algorithms		
R software 4.2.3	R Project	https://www.r-project.org
Graphpad Prism 9.0	GraphPad Software	www.graphpad.com
HALO software	Indicalab	https://www.indicalab.com/
Adobe Illustrator 2023	Adobe	https://www.adobe.com/
Mutect2-GATK	McKenna et al.[57]	https://gatk.broadinstitute.org/hc/en-us/articles/360037593851-Mutect2
ANNOVAR	Wang et al.[58]	https://annovar.openbioinformatics.org/en/latest/
CIBERSORT	Newman et al.[59]	http://timer.comp-genomics.org/
MCP-counter	Becht et al.[60]	http://timer.comp-genomics.org/
Maftools package	Mayakonda et al.[61]	https://github.com/PoisonAlien/Maftools
Other		
Clinical trial registration number	https://clinicaltrials.gov/	NCT04475016

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study design and participants

This was a prospective, single-center, single-arm, open-label, investigator-initiated, phase II trial (ClinicalTrials.gov number, NCT04475016) to determine the efficacy and safety of the TNT regimen in a neoadjuvant setting, using Simon's two-stage optimal design. The screened patients included those initially diagnosed in our center or referred from 18 medical centers in ten provinces of China. The major inclusion criteria were age 18-75, histologically confirmed La-PSCC (either cT4 or cN3 and M0, according to the staging criteria of the 8th American Joint Committee on Cancer [AJCC]) was confirmed by radiological imaging through enhanced computed tomography (CT) or magnetic resonance imaging (MRI), with at least one measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, an Eastern Cooperative Oncology Group (ECOG) performance-status (PS)

score ≤ two, an estimated survival time over 12 months; and adequate organ function. Patients who received previous systemic therapy were excluded. The full inclusion and exclusion criteria are provided in the protocol (Data S1).

This study was reviewed and approved by the Institutional Review Board of Sun Yat-sen University Cancer Center ethical committee (B2020-103-01). We conducted this study in accordance with the Declaration of Helsinki. All the patients have provided written informed consent. This trial was registered on ClinicalTrials.gov (NCT04475016).

Systematic treatment

Patients received toripalimab (anti-PD-1 monoclonal antibody, Junshi Biosciences Co., Ltd., Shanghai, China) 240mg intravenously on day 1, nimotuzumab (anti-EGFR monoclonal antibody, Biotech Pharmaceutical Co., Ltd, Beijing, China) 400mg intravenously on day 1, and chemotherapy (albumin-bound paclitaxel 260 mg/m^2 on day 1, cisplatin 25 mg/m^2 on day 1-3 and ifosfamide 1200 mg/m^2 on day 1-3) intravenously every three weeks, to a maximum of four cycles, or until dose-limiting toxicity or disease progression or patient withdrawal. Prophylactic granulocyte colony-stimulating factor (G-CSF) was allowed to be administrated after 24-48h of chemotherapy. The radiologic assessment was performed every six weeks and was independently reviewed by two blinded radiologists. Consolidative surgery was assessed and performed following neoadjuvant TNT for patients who were medically fit and had resectable residual lesions in the preoperative radiologic assessment as previously described.[62] The principle of surgery was R0 resection. Patients who were unresectable after neoadjuvant TNT were offered palliative therapy. One-year of adjuvant toripalimab (240 mg IV every 3 weeks) was recommended, with the decision primarily guided by physician (based on adverse tumor pathological characteristics and underlying suspicion of disease recurrence) and patient preference. The study profile is shown in Figure S1. All patients were scheduled follow-up visits every three months for the first year after surgery and were continued according to the study protocol. Additional details are provided in the study protocol (Data S1).

Consolidated surgery details

The alternative type of consolidative surgery includes penectomy (partial or total), unilateral or bilateral modified radical inguinal lymph node dissection (ILND), unilateral or bilateral pelvic lymph node dissection (PLND), and adjacent flap transfer. The surgery extent was based on the pre-TNT radiological imaging burden of the disease and the need for consolidative surgical resection to resect all sites of suspected disease following oncologic standards in the management of this disease, as detailed above.

The range of consolidative surgery included primary penile tumor (if there is one), inguinal lymph node, or pelvic lymph node, depending on the residual disease. If there was a residual primary penile tumor, partial penectomy or total penectomy plus urinary diversion was performed depending on the residual disease and the residual length of the penis.

The modified unilateral or bilateral inguinal lymphadenectomy to remove residual disease was based on the previous reports of our center; the boundaries of dissection were the apex of the femoral triangle distally, the sartorius muscle laterally, the adductor longus muscle medially, and the inguinal ligament superiorly. To ensure a negative resection margin, the saphenous vein, muscle fascia, or the skin invaded by the lymph node surface can be excised if necessary. The transfer of the adjacent skin flap or myocutaneous flap was performed to cover the exposed vasculature and provide rapid wound healing without tension.

The scope of pelvic lymphadenectomy included the genitofemoral nerve outside, the ureter inside, the common iliac vessels proximally, the inside of the bladder distally, and the Cloquet node distal to the femoral canal. Unilateral or bilateral pelvic lymph node dissection depends on the location of inguinal and pelvic lymph node metastases, and open or laparoscopic surgery depends on the decision of the surgeon. Additional details are provided in the study protocol (Data S1)

METHOD DETAILS

Outcomes

The primary endpoint was the pCR rate, defined as the percentage of patients achieving pCR in all enrolled patients. pCR was defined as no residual invasive tumor cell in the resected tumor sites (ypTis/T0N0M0). The pCR was assessed using the 8th edition AJCC TNM cancer staging system by two pathologists independently, and another (the 3rd) urological pathologist reviewed all specimens blindly to the results. Secondary end points included ORR (defined as the percentage of patients who had the best response of complete response [CR] or partial response [PR]), PFS (defined as the time from the initiation of neoadjuvant TNT until disease progression [included local and/or distant progression] or death from any cause), OS (defined as the time from the initiation of neoadjuvant TNT until death from any cause) and safety. Clinical responses were evaluated through enhanced CT or MRI according to the RECIST v1.1 every six weeks. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE version 4.0). Postoperative complications were graded through the modified Clavien-Dindo system.

HPV detection and immunohistochemistry

Human papillomavirus (HPV) status in fresh-frozen tumor samples was assessed at a central laboratory using fluorescence quantitative polymerase chain reaction assay. Formalin-fixed, paraffin-embedded tumor samples obtained from 26 of 29 patients (89.6%) were available to evaluate the expression of PD-L1 (JS311, Junshi Bioscience), EGFR (ab52894, Abcam), mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2) by immunohistochemical (IHC) assay. PD-L1 positive status was defined as the presence of ≥1% of tumor cells with membrane or cytoplasm staining. Positive expression of EGFR was defined as >5% of positive-stained cells in the tumor. Mismatch repair (MMR) status was determined according to the expression of MLH1, MSH1, MSH6, and PMS2 by IHC assay.

The deficient mismatch repair (dMMR) status was defined as the absent expression of more than one MMR protein which indicated microsatellite instability-high (MSI-H), and proficient mismatch repair (pMMR) tumors had normal MMR protein expression, which indicated microsatellite stability (MSS) or microsatellite instability-low (MSI-L). All of the specimens were analyzed and counted by 2 masked independent pathologists (J.T.J and Y.C.Z) without knowledge of any clinical information. $CD8^+$ T cell infiltration were validated by at IHC assay from 37 samples from 26 patients. $CD8^+$ T cell density was quantified using HALO software (Indica Labs).

Whole-exome sequencing (WES) analysis

Tumor samples and matched normal tissues were obtained from 28 patients with penile squamous cell carcinoma (PSCC). DNA was extracted and quantified. Libraries were prepared using the Agilent SureSelect XT Human All Exon V6 kit and sequenced on an Illumina platform. Raw sequencing data were processed using the GATK pipeline. Briefly, reads were aligned to the human reference genome (hg38) using Burrows Wheeler Aligner (BWA) software. Duplicates were marked using Picard Tools, and base quality recalibration was performed using Genome Analysis Toolkit (GATK v4.2). Somatic single-nucleotide variants (SNVs) and insertions/deletions (indels) were identified using Mutect2, with tumor-only mode enabled for unmatched samples. High-confidence variants were annotated using ANNOVAR and filtered based on allele frequency, read depth, and functional impact. Mutation frequencies were calculated for each gene, and the top mutated genes were visualized. TMB was computed by normalizing the total number of eligible somatic mutations to the size of the captured exonic region, approximately 38 megabases for the Agilent SureSelect Human All Exon V6 kit. The results were expressed as mutations per megabase (mut/Mb), and the maftools package (v2.14.0) in R was utilized for calculation and visualization.

Transcriptomic analysis and immune cell profiling

Total RNA was extracted using the RNeasy Mini Kit (Qiagen) and sequenced on an Illumina NovaSeq 6000 platform. Reads were aligned to the hg38 genome using STAR, and gene expression was normalized to TPM values. Immune cell profiling was conducted using CIBERSORT (relative abundance) and MCP-counter (absolute abundance) through the TIMER 2.0 online platform.

QUANTIFICATION AND STATISTICAL ANALYSIS

Simon's two-stage optimal design was used with the null hypothesis of a 10% pCR rate, which was the historical pCR rate of the standard neoadjuvant TIP regimen in La-PSCC.[11,63] This design would yield a one-sided alpha level of 5% and a power of 80% if the true pCR rate is 30%. In the first stage, ten patients were accrued. If there were > 1 patient who achieved pCR, the study would proceed to the second stage with an additional 19 patients. The study result was considered positive if more than five of the 29 patients achieved pCR. PFS duration was censored for patients meeting any of the following criteria: (1) initiation of new anticancer therapy prior to documented disease progression or death; (2) death or disease progression following two consecutive missed disease assessment visits; or (3) patients alive without documented disease progression at the study's conclusion. OS duration was censored at the last day of follow up for patients who were still alive. Kaplan-Meier method was used to estimate the median PFS and OS or the survival rates at fixed time points. Log-rank test was used to conduct subgroup analysis. A Cox proportional hazards model was performed to calculate the hazard ratios (HR) and 95% confidence intervals (CI). The proportional hazards assumption was tested using Schoenfeld's residuals. Chi-square test or Fisher's exact test was used to assess the association of variables with pCR or treatment response. The two-sided p-value of 0.05 or less was considered to indicate statistical significance. Additional details are provided in the study protocol (Data S1, p 40 - 42)

ADDITIONAL RESOURCES

Clinical trial registration number: NCT04475016 (https://clinicaltrials.gov/ct2/show/NCT04475016).