Penile cancer: Updates in systemic therapy

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Abstract Penile cancer is a rare genitourinary malignancy with a greater incidence in parts of Asia, South America, and Africa. Outcomes are very poor in patients with advanced disease and in those who do not respond to first-line multimodal therapy. Among systemic therapy options, platinum-based chemotherapy is used in the first-line; however, approximately half of patients do not benefit. Response rates to systemic therapy as subsequent line treatment are historically dismal. There is also a paucity of prognostic and predictive tools within the context of penile cancer. As such, there remains an urgent need to expand systemic treatment options for patients with advanced penile cancer. The purpose of this review is to summarize the existing evidence for standard-of-care lines of systemic treatment, examine the potential of novel lines of systemic therapy, and provide an update as to the status of these new therapies within the context of penile cancer.

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1. Introduction

In 2020, the worldwide cancer incidence of penile cancer was 0.8 per 100,000, with 56.3% of cases being reported in Asia [1]. As such, while rare as compared to other genitourinary malignancies, penile cancer poses a greater risk to individuals in developing countries [2]. Human papilloma virus (HPV), phimosis, lack of circumcision, and smoking are additional risk factors that substantively impact the chances of developing penile cancer [3–7].

While dependent on the size, location, stage, and grade of the tumor, penile cancer lesions that are confined to the penis can frequently be managed successfully with either organ-sparing or non-organ-sparing surgical approaches combined with active surveillance, often resulting in low recurrence rates [8,9]. However, in the setting of locally advanced bilateral inguinal, unilateral/bilateral pelvic lymph nodes, and/or extranodal extension demonstrate poor 5-year overall survival (OS) rates of 10%–20% [10]. Some predictors of lymph node metastases have been shown to include higher stage, higher grade, lymphovascular invasion, and perineural invasion [11,12]. Thus,
current National Comprehensive Cancer Network (NCCN) and European Association of Urology (EAU) guidelines recommend a multimodal first-line approach involving neoadjuvant chemotherapy (NAC) followed by lymph node dissection for the treatment of bulky nodal disease [13,14]. As will be discussed later in this review, these guidelines recommending the use of NAC are largely based on a single prospective study and a limited number of retrospective reports [15–17]. Similarly, adjuvant chemotherapy for locally advanced disease is recommended based on a small number of retrospective studies demonstrating its utility in patients with positive pelvic nodes [18–20]. In the setting of relapsed disease following first-line systemic treatment, there is an even greater lack of consensus guidelines based on higher-level evidence, contributing to the dismal survival outcomes in these patients. In fact, median OS has previously been reported to be less than 6 months in patients who received a variety of salvage therapies after progressing on NAC [21].

Thus, there have been considerable efforts in recent years to further validate the utility of NAC, identify prognostic indicators of response to chemotherapy, and perhaps most importantly, explore novel systemic treatment options for penile cancer including various targeted therapies and immune-based therapies. Due to the fact that recurrence rates for locally advanced, relapsed, and metastatic disease remain high for penile cancer, there exists an unmet need to both conduct studies that may provide higher-level evidence for established lines of systemic therapy as well as investigate novel treatment modalities through randomized-controlled trials. However, the rarity and disproportionate impact of penile cancer in underdeveloped nations that lack the resources to run international collaborations hinder these efforts.

In this review, we briefly summarize the evidence supporting current guidelines on the use of systemic therapy in penile cancer, review prognostic indicators that may be predictive of treatment response, and examine ongoing efforts to identify novel systemic treatment options.

2. Chemotherapy in locally advanced penile cancer

2.1. NAC

As noted previously, NAC is recommended as first-line treatment for patients with bulky nodal disease per NCCN and EAU guidelines [13,14]. The standard regimen is four cycles of paclitaxel, ifosfamide, and cisplatin (TIP), based on a single-arm, non-randomized phase II trial in 30 patients by Pagliaro and colleagues [15]. In this prospective study, NAC was significantly associated with an improvement in OS and time to progression among responders versus non-responders, and 30% of the cohort remained free of disease recurrence following a median follow-up period of 34 months. Pathologically, 50% of patients responded to NAC and 10% of patients showed a pathological complete response. While a small number of retrospective and prospective studies have examined response rates and survival benefit following NAC (Table 1), it is difficult to directly compare their findings with the trial by Pagliaro and colleagues [15] due to the heterogeneity between studies, including the type, dosage, and frequency of chemotherapy regimens used. A recent meta-analysis attempted to consolidate the findings of these studies, while also grouping studies based on their use of taxane-platinum (TP) or non-taxane platinum (NTP) regimens followed by subgroup analysis [22]. The pooled analysis reported an objective response rate (ORR) of 53%, with the subgroup analysis showing comparable response rates of 49% versus 55% for TP versus NTP regimens, respectively. Of note, this meta-analysis also included the study by Pagliaro and colleagues [15].

With respect to delivering chemotherapy in the peri-operative setting, it is critical that any adverse effects of NAC be managed effectively in an effort to operate safely on the patient without added risks as a result of NAC and minimize post-operative complications. In this regard, the phase II prospective trial by Pagliaro and colleagues [15] reported post-surgical complication rates that are comparable to rates observed in the absence of peri-operative chemotherapy [23,24].

This trial also demonstrated the overall safety of the TIP regimen, with Grade 3 infections comprising the most frequent adverse event (AE) and no Grade 5 AEs in patients who received NAC. Although the meta-analysis by Azizi and colleagues [22] found no significant difference between Grade 3 or greater AEs between NTP and TP regimens, the authors noted that the types of toxicities resulting from bleomycin-containing regimens are more difficult to manage than those associated with TP regimens. Additionally, a phase II trial of bleomycin, methotrexate, and cisplatin by Haas and colleagues [25] showed an inferior response rate of 32.5% as compared to TIP and treatment-related death in 13.9% of patients. Among TP regimens, a limited number of retrospective and prospective studies have investigated the safety and efficacy of docetaxel, cisplatin, and fluorouracil (TPF) [19,26,27]. A phase II study of TPF by Nicholson and colleagues [26] in 2013 reported an inferior ORR of 38.5% as compared to TIP, while documenting at least one Grade 3 or Grade 4 AE in 65.5% of patients. In light of these findings, the authors concluded that TPF should not be investigated further as NAC for penile cancer. Taken together, these retrospective and prospective studies of various chemotherapy regimens provide the historical context for the current guidelines that solely favor the use of TIP as NAC due to the greater toxicities and inferior efficacy associated with other regimens.

With respect to prognostic indicators of improved survival following NAC, few reports have been published, perhaps due to the rarity of penile cancer and insufficient patient numbers to power these studies. A retrospective analysis of 61 patients with majority clinical N2-N3 disease—of which 54 received NAC in the form of TIP and 52/54 patients underwent lymphadenectomy following NAC—sought to identify prognostic indicators of favorable outcomes [28]. In this regard, response to chemotherapy and pN0-N1 disease at the time of surgery were both factors associated with improved OS upon univariate analysis. In a multicenter analysis of 140 patients that received first-line systemic therapy, of which 59 received NAC, an ECOG
performance status of ≥1 was a poor prognostic indicator for both OS and progression-free survival [29].

At the present time, there is a lack of useful prognostic indicators following first-line platinum-based NAC [30]. Such prognostic indicators would enable clinicians to better select patients that are at high risk of recurrence for adjuvant therapy following surgery, such as radiotherapy or other systemic treatments. Additionally, as is the current

Table 1  Summary of studies on NAC in penile cancer.

| Study                      | Study type; total number of patients | Chemotherapy agent(s); number of patients | Responses and safety | Survival (median follow-up time, months) |
|----------------------------|--------------------------------------|-------------------------------------------|----------------------|----------------------------------------|
| Bermejo et al., 2007 [125] | Retrospective; n=10                  | • TIP; n=5, • BMP; n=5, • PC; n=2          | • 4 CR (TIP [n=4]), 1 PR (PC [n=1]), and 5 SD (BMP [n=3], TIP [n=1], PC [n=1]) observed | 40% 5-year OS (62 months) |
| Zou et al., 2014 [29]      | Retrospective; n=24                  | • Bleomycin; n=3, • 5-FU + CIS; n=1, • PBM; n=10, • CIS + CPT-11; n=7 | • Grade 3 toxicities observed in 3/10 patients, 2 CR and 10 PR observed | 32% 5-year OS (23 months) |
| Theodore et al., 2008 [126]| Prospective, phase II; n=7           | • Bleomycin; n=3, • VBM; n=5, • 5-FU + CIS; n=1 | • 2 CR and 10 PR observed | 32% 5-year OS (23 months) |
| Chiang et al., 2014 [128]  | Retrospective; n=12                  | • MTX + CIS + 5-FU + mitomycin C + bleomycin; n=12 | • ORR of 83.3% observed | NA |
| Nicholson et al., 2013 [26]| Prospective, phase II; n=29          | • TPF; n=29                                | • Grade 3 toxicities observed in 1/6 patients | NA |
| Pagliaro et al., 2010 [15] | Prospective, phase II; n=30          | • TIP; n=30                                 | • 3 CR, 12 PR, and 9 SD observed | Median OS of 17.1 months (34 months) |
| Pizzocaro et al., 2009 [127]| Prospective; n=6                     | • TPF; n=6                                  | • Grade 3 toxicities were most common observed toxicities | Median OS of 7.1 months (14.5 months) |
| Zou et al., 2014 [129]     | Retrospective; n=24                  | • Bleomycin + methotrexine + CIS; n=24      | • ORR of 62.5% observed | 45.8% 5-year OS (NA) |
| Djajadiningrat et al., 2015[27] | Prospective; n=26                  | • TPF; n=26                                 | • Safety data not available | Median OS of 10.1 months (30 months) |
| Nicolai et al., 2016 [19]  | Retrospective; n=28                  | • TPF; n=28                                 | • 1 CR and 11 PR observed | 7.1% 2-year DFS (NA) |

Bleo, bleomycin; BMP, bleomycin, methotrexate, and cisplatin; CIS, cisplatin; CPT-11, irinotecan; CR, complete response; DFS, disease-free survival; MTX, methotrexate; NAC, neoadjuvant chemotherapy; NA, not applicable; ORR, objective response rate; OS, overall survival; PBM, bleomycin, cisplatin, and methotrexate; PC, paclitaxel; PR, partial response; and carboplatin; SD, stable disease; TIP, paclitaxel, ifosfamide, and cisplatin; TPF, docetaxel or paclitaxel, cisplatin, and 5-fluorouracil; VBM, bleomycin, vincristine, and methotrexate; 5-FU, 5-fluorouracil; VIN, vincristine.
focus of investigations in other malignancies, predictive biomarkers used prior to NAC or at mid-treatment evaluations could enable us to predict responders versus non-responders to NAC and offer alternative therapies earlier in the disease course. Currently in the setting of penile cancer, there are no such predictive biomarkers used in the clinical setting. In this regard, given that approximately 50% of penile cancers are HPV-positive and reports of a positive correlation between HPV-positivity and survival, HPV status may have potential as a predictive biomarker of treatment response [3,31–33]. In fact, a recent retrospective study showed that positive HPV status predicted improved locoregional control of nodal metastases via adjuvant chemoradiotherapy (CRT) in comparison to HPV-negative patients [34]. To validate these findings, prospective multi-institutional collaborations are necessary to enable and statistically power such investigations.

2.2. Adjuvant chemotherapy

In the setting of locally advanced penile cancer, current NCCN guidelines recommend adjuvant chemotherapy in the form of either TIP or 5-fluorouracil (5-FU) in patients who did not receive first-line NAC and exhibited ≥2 positive nodes or extranodal extension at the time of ILND [13]. In slight contrast, the EAU guidelines recommend adjuvant chemotherapy in the form of 5-FU plus cisplatin and a taxane-based agent for pN2/N3 disease irrespective of whether or not the patient has previously received NAC [14]. Currently, there is no prospective evidence to support these guidelines; collectively, these recommendations rely on retrospective studies that have demonstrated improvements in OS following adjuvant therapy [18],[19,20]. Specifically, a large multicenter analysis of 171 patients by Necchi and colleagues [20] demonstrated a significant improvement in OS following adjuvant chemotherapy in patients with positive pelvic nodes at the time of surgery. However, a similar significant survival advantage was not shown following adjuvant therapy in patients who exhibited extranodal extension at the time of surgery [20]. Of note, only 15.8% of patients in this study received 5-FU plus cisplatin, and none received TIP. In another multicenter analysis of 36 patients by Sharma and colleagues [18], median OS was 21.7 months versus 10.1 months among patients who received adjuvant chemotherapy versus in patients who did not, respectively. Similar to the study by Necchi and colleagues [20], only a combined 25% of patients in this study received either TIP or 5-FU plus cisplatin. In another recent study looking at adjuvant chemotherapy for nodal disease, TPF was the regimen used [19]. Thus, it is difficult to ascertain which chemotherapy regimens are most effective in the adjuvant setting due to the small study size and the heterogeneity in treatment regimens used. At the present time, there are no ongoing prospective trials investigating adjuvant chemotherapy for penile cancer. A summary of studies on adjuvant chemotherapy are provided in Table 2.

With respect to predictive and prognostic factors of response and survival, respectively, there is currently a lack of such data. In a small study of 21 patients who received adjuvant TPF, p53 expression was associated—albeit non-significantly—with poorer disease-free survival and OS on both univariate and multivariate analysis [36]. However, in addition to the small sample size, this study is limited by variations in the extent of lymph node dissection and the administration of TPF. Thus, future prospective, multicenter studies on adjuvant chemotherapy should incorporate analyses that allow for the identification and validation of predictive/prognostic factors in a more neatly defined cohort of patients.

Within the setting of locally advanced penile cancer, there is a lack of studies comparing neoadjuvant versus adjuvant chemotherapy. In principle, NAC offers advantages over adjuvant chemotherapy including a reduction in disease burden enabling more effective surgical consolidation, systemic intervention earlier in the disease course to address micro-metastatic disease, and the provision of prognostic information based on the response to NAC as evaluated at the time of surgery. In penile cancer, Nicolai and colleagues [19] performed a retrospective comparison of OS and progression-free survival between 28 patients that received neoadjuvant TPF and 19 patients that received adjuvant TPF. While their analysis was limited by a small sample size, no significance difference in outcomes was observed between the two groups. Further studies are warranted to validate these findings.

3. Chemotherapy in relapsed and/or metastatic penile cancer

As noted previously, survival outcomes for penile cancer patients with relapsed and/or stage IV metastatic disease are dismal [21]. In addition, response rates to salvage therapy have historically been very poor. For instance, in a study of 19 patients who experienced progression or recurrence following NAC with TIP, only three patients demonstrated an objective response to salvage treatment and the median OS was less than 6 months [21]. Thus, there is an absence of consensus guidelines on how to effectively manage disease recurrence, especially with respect to the optimal utilization of second-line chemotherapy [37]. In this regard, data from prospective studies on chemotherapy for relapsed disease are limited. In one phase II, single-arm multicenter trial, Di Lorenzo and
While multi-agent regimens such as TIP are effective in reducing toxicity, these regimens may result in significant and unmanageable toxicity in the second-line or subsequent salvage setting, and thus, a greater emphasis should be placed on identifying a chemotherapeutic agent that can be used safely and taking into consideration prior received therapies. For example, while 2/5 patients demonstrated response to a multi-agent regimen comprising of bleomycin, methotrexate, and cisplatin, one of the patients experienced a treatment-related death [21].

With respect to the systemic management of penile cancer patients with distant metastatic disease, the NCCN recommends chemotherapy as a first-line therapy in the form of either TIP or 5-FU plus cisplatin, followed by consolidative surgery (when applicable) for responders or subsequent salvage systemic therapy. The recommendation to use chemotherapy in the first-line setting for distant metastatic disease arises from low-level evidence from a very limited number of retrospective and prospective studies that are heterogeneous in regard to the chemotherapy regimens used and patient characteristics. Collectively, these studies have demonstrated only modest response rates and survival advantages while often reporting significant toxicities [25,26,40–43]. Thus, due to

| Study                  | Study type; total number of patients | Chemotherapy agent(s); number of patients | Safety                                                                 | Key finding                                                                 |
|------------------------|-------------------------------------|------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Pizzocaro and Piva, 1988 [130] | Retrospective; n=12                 | ● VBM; n=12                              | ● Study reported mild toxicity (CTCAE grading not available)            | ● 11/12 patients demonstrated no evidence of disease at a median follow-up of 42 months |
| Hakenberg et al., 2006 [131]    | Retrospective; n=8                  | ● PBM; n=8                               | ● Treatment-related death in one patient                               | ● 3/8 patients demonstrated no evidence of disease at a median follow-up of 54 months |
| Noronha et al., 2012 [132]   | Retrospective; n=19                 | ● Paclitaxel + cisplatin; n=15           | ● Treatment-related death in one patient                               | ● Significantly improved median DFS in patients who completed chemotherapy versus those that did not (23.1 months vs. 2.16 months, respectively; p=0.0001) |
| Sharma et al., 2015 [18]   | Retrospective; n=36                 | ● TPF; n=18                              | ● Neutropenia was the most common Grade 3 toxicity                      | ● 2-year DFS of 36.8%                                                       |
| Nicolai et al., 2016 [19]   | Retrospective; n=19                 | ● TPF; n=19                              | ● Neutropenia was the most common Grade 3 or greater toxicity           |                                                                             |
| Necchi et al., 2019 [20]   | Retrospective; n=171                | ● Unspecific regimen; n=83              | ● NA                                                                   | ● Significant improvement in OS among patients with positive pelvic nodes   |

CTCAE, common terminology criteria for adverse events; DFS, disease-free survival; NA, not applicable; OS, overall survival; PBM, bleomycin, cisplatin, and methotrexate; TIP, paclitaxel, ifosfamide, and cisplatin; TPF, docetaxel or paclitaxel, cisplatin, and 5-fluourouracil; TPG, paclitaxel, cisplatin, and gemcitabine; VBM, bleomycin, vincristin, and methotrexate; 5-FU, 5-fluourouracil.
poor outcomes in this group and the lack of higher-level evidence for treatment guidelines, the NCCN recommends participation in clinical trials due to the lack of evidence in the second-line setting [13]. Similarly, the EAU provides little guidance on how to manage these patients [14]. Patients are encouraged to undergo molecular testing to evaluate their eligibility for alternative systemic therapy options such as immune-checkpoint blockade and HPV-directed therapeutic vaccines through clinical trials.

4. Novel systemic therapy options for penile cancer

4.1. Immune-checkpoint inhibitors (ICIs)

In recent decades, our growing understanding of the role of the programmed cell death protein (PD-1), programmed death-ligand 1 (PD-L1), and the cytotoxic T-lymphocyte-associated protein 4 (CTLA4) in immunosuppression and tumor immune evasion has led to the development of an entirely new class of systemic agents called ICIs [44–48]. Currently, many anti-PD-1, anti-PD-L1, and anti-CTLA4 inhibitors are approved for use across a broad range of tumor types, including several squamous cell carcinomas (SCCs) [49]. However, in the setting of penile cancer, the NCCN only recommends the use of ICIs subsequent-line treatment, exclusively for patients with unresectable relapsed or metastatic disease and tumors that are characterized as mismatch repair-deficient, microsatellite instability-high, or tumor mutational burden (TMB)-high (TMB >10 bases per megabase) [13].

This being said, the first step in exploring the broader utility of ICIs in penile cancer is improving our understanding of the tumor immune microenvironment in this disease context [50,51]. In this regard, multiple studies have shown that tumor-infiltrating lymphocytes (TILs) such as CD8+ cytotoxic T-cells and other immune cell types—such as FoxP3+ Tregs and tumor-associated macrophages—are not only present in the tumor immune microenvironment of penile cancer but also have prognostic value with respect to disease-specific survival and the incidence of lymph node metastases [52–55]. In addition, 40%–67% of primary penile cancer tumor samples demonstrate PD-L1 expression [54,56–59]; prognostically, diffuse PD-L1 expression has been associated with poorer disease-specific survival and a greater incidence of lymph node metastases [53,54,57,59]. Taken together, these studies underscore the relevance of the PD-1/PD-L1 axis in penile cancer.

At the present time, however, current data on ICI use for penile cancer are limited to case reports in the setting of relapsed or metastatic disease and pre-clinical studies [60]. Treatment responses have been mixed across these studies [61–64]. For example, while McGregor and colleagues [62] did not demonstrate an objective response to a combined regimen of ipilimumab (anti-CTLA4) and nivolumab (anti-PD-1) in all five patients treated in a basket trial, other case reports have shown complete and partial responses using subsequent-line ICI therapy in patients with chemo-refractory metastatic disease [63,64]. Currently, multiple clinical trials are investigating ICI therapy for penile cancer, mainly in the setting of relapsed and/or metastatic disease (Table 3).

As noted earlier, because outcomes for patients who fail first-line multimodal treatment are poor, there is an unmet need to improve the efficacy of existing neoadjuvant systemic therapies used in the first-line setting and expand the range of treatment options. In this regard, ICI therapy may be an effective neoadjuvant substitute for NAC. In addition to demonstrating a more tolerable safety profile as compared to standard-of-care chemotherapy across a wide range of malignancies [65–68], ICI therapy can be administered in the outpatient setting. Given that the current TIP regimen requires patients to receive treatment in the inpatient setting, the relative safety and feasibility of ICI therapy may reduce the burden on community medical practices that lack the resources to administer TIP and/or lack the expertise to manage complex chemotherapy-related toxicities. Additionally, neoadjuvant ICI therapy has demonstrated superior efficacy as compared to NAC in non-small cell lungcancer. Specifically, 38% of patients exhibited a major pathologic response and an additional 38% demonstrated a complete response to a combined regimen of nivolumab plus ipilimumab in the phase II NEOSTART trial, in a disease where historical rates of major pathologic response in NAC are between 7% and 27% [69]. Thus, there is sufficient evidence across multiple tumor types to support future studies on neoadjuvant ICI therapy in penile cancer [70–73]. Alternatively, rather than replacing NAC with neoadjuvant ICI, combining the two therapies may lead to improved outcomes. In principle, the cytotoxic activity of chemotherapeutic agents may lead to an increased release of tumor-associated antigens. Given that the anti-tumor immune response relies on these tumor-associated antigens, stimulation of the immune system via ICI therapy and increased tumor-associated antigen release may lead to enhanced treatment outcomes [74,75].

While currently a novel theoretical treatment approach in penile cancer, combination NAC plus ICI is an approved approach in triple-negative breast cancer. In addition, early results from the phase 3 CheckMate-816 trial comparing neoadjuvant nivolumab plus NAC versus NAC alone in non-small cell lung-cancer demonstrated a significant increase in pathological complete response rates among patients who received nivolumab plus NAC versus NAC alone (24.0% vs. 2.2%, p<0.0001). Notably, this increase was observed in the "TMB-low", "TMB-high", "PD-L1<1%", and "PD-L1≥1%" groups; these findings support the utility of a combination therapy approach in a broad range of patients based on TMB status and PD-L1 status. In addition, smaller percentages of patients who received nivolumab plus NAC experienced treatment- and surgery-related Grade 3-4 AEs compared to those who received NAC alone [76]. Thus, based on available data from other malignancies, further examination of ICIs is warranted for penile cancer patients earlier in the disease course, either as a replacement for or addition to NAC [77]. However, at the present time, there is an absence of such ongoing prospective studies.

4.2. Adaptive and engineered T-cell therapies

In addition to stimulating cytotoxic T-cell activity through the use of ICIs, a number of strategies have been developed in recent years to enhance cytotoxic T-cell function via
| ClinicalTrials.gov identifier; study phase | Planned accrual; status | Setting | ICI agent(s) | Combination therapy (if applicable) | Primary endpoint | Basket trial (yes or no) |
|------------------------------------------|------------------------|---------|-------------|-------------------------------------|------------------|------------------------|
| NCT03391479; phase II | n=24; recruiting | Unresectable or metastatic disease in chemo-refractory or chemo-ineligible patients | Avelumab (anti-PD-L1) | None | ORR | No |
| NCT03686332; phase II | n=32; recruiting | Advanced, unresectable disease | Atezolizumab (anti-PD-L1) | Radiotherapy | PFS | No |
| NCT03774901; phase II | n=32; recruiting | Maintenance therapy following platinum-based chemotherapy | Avelumab (anti-PD-L1) | None | PFS | No |
| NCT04224740; phase II | n=33; recruiting | First-line therapy for unresectable disease | Pembrolizumab (anti-PD-1) | Cisplatin or carboplatin plus 5-FU | ORR | No |
| NCT044321981; phase II | n=18; not recruiting | Advanced unresectable or metastatic disease | INCMGA00012 (anti-PD-1) | None | ORR | No |
| NCT02496208; phase I | n=152; not recruiting | Locally advanced or metastatic disease | Nivolumab (anti-PD-1) +/- ipilimumab (anti-CTLA-4) | Cabozantinib-s-malate | Phase II dose and AE incidence | Yes |
| NCT02721732; phase II | n=225; not recruiting | Unresectable or metastatic disease | Pembrolizumab (anti-PD-1) | None | Non-progression rate | Yes |
| NCT02834013; phase II | n=818; closed to accrual | Relapsed disease | Nivolumab (anti-PD-1) +/- ipilimumab (anti-CTLA-4) | None | ORR | Yes |
| NCT03333616; phase II | n=100; recruiting | Unresectable advanced or metastatic disease | Nivolumab (anti-PD-1) +/- ipilimumab (anti-CTLA-4) | None | ORR | Yes |
| NCT03427411; phase II | n=57; not recruiting | Locally advanced or metastatic HPV-associated disease | M7824 (also called bintrafusp alfa); M7824 is a bi-functional fusion protein targeting TGF-β trap and anti-PD-L1 | None | ORR | Yes |
| NCT03517488; phase I | n=154; recruiting | Advanced disease | XmAb^®20717 (bi-specific anti-PD-1 and anti-CTLA-4) | None | Safety | Yes |
| NCT03866382; phase II | n=224; recruiting | Metastatic disease | Nivolumab (anti-PD-1) +/- ipilimumab (anti-CTLA-4) | Cabozantinib | ORR | Yes |
| NCT03457873; phase II | n=111; recruiting | Relapsed or metastatic disease | Pembrolizumab (anti-PD-1) | Vorinostat | ORR | Yes |
| NCT04718584; phase II | n=127; recruiting | Advanced disease | LDP (anti-PD-L1 injection) | None | pCR and ORR | Yes |

AE, adverse event; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HPV, human papilloma virus; ICI, immune checkpoint inhibitor; LDP, human anti-PD-L1 monoclonal antibody injection; 5-FU, 5-fluourouracil; ORR, overall response rate; pCR, pathologic complete response; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TGF-β, transforming growth factor-beta.
ex vivo expansion of autologous TILs and ex vivo engineering of T-cells that target tumor-associated antigens. The latter group includes engineered T-cell receptor (TCR) therapy and chimeric antigen receptor (CAR) T-cell therapy.

With respect to autologous TILs, the aim of this approach is to autologously re-infuse the TILs grown ex vivo back into the patient, a process termed adoptive T-cell therapy (ACT). Within the setting of penile cancer, Aydin and colleagues [78] were recently successful in expanding TILs from 11/12 patient-derived lymph nodes that were positive for penile cancer. In addition, in 5/11 cases, expanded TILs demonstrated anti-tumor reactivity against tumor samples derived from the same patient. Taken together, these findings are a proof of concept for the use of ACT in penile cancer. Notably, neither prior NAC nor HPV-status led to a difference in the ability to expand TILs, further suggesting that ACT may benefit both HPV-positive and HPV-negative patients as a subsequent-line treatment [78].

However, one of the major limitations associated with ACT using naturally occurring TILs is that this therapy is restricted to a single patient, making the process expensive and difficult to scale. Another major limitation of ACT is having access to a tumor sample from the patient from which the TILs are isolated. Engineered T-cell therapy addresses both of the limitations, as it enables isolation of normal T-cells from the peripheral blood of patients and genetic modification of these T-cells to contain specific TCRs or CARs that have optimal affinity for specific extra- or intra-cellular tumor-associated antigens. In addition, T-cells isolated from a single patient can be modified and expanded to treat other patients with matching human leukocyte antigen alleles in the case of TCR-therapy, wherein CAR T-cell therapy is not limited by MHC-class-I alleles in the case of TCR-therapy, expanded to treat other patients with matching human T-cells isolated from a single patient can be modified and intra-cellular tumor-associated antigens. In addition, TCRs or CARs that have optimal affinity for specific extra- or genetic modification of these T-cells to contain specific normal T-cells from the peripheral blood of patients and having access to a tumor sample from the patient from ACT using naturally occurring TILs is that this therapy is Notably, neither prior NAC nor HPV-status led to a difference in the ability to expand TILs, further suggesting that ACT may benefit both HPV-positive and HPV-negative patients as a subsequent-line treatment [78].

However, one of the major limitations associated with ACT using naturally occurring TILs is that this therapy is restricted to a single patient, making the process expensive and difficult to scale. Another major limitation of ACT is having access to a tumor sample from the patient from which the TILs are isolated. Engineered T-cell therapy addresses both of the limitations, as it enables isolation of normal T-cells from the peripheral blood of patients and genetic modification of these T-cells to contain specific TCRs or CARs that have optimal affinity for specific extra- or intra-cellular tumor-associated antigens. In addition, T-cells isolated from a single patient can be modified and expanded to treat other patients with matching human leukocyte antigen alleles in the case of TCR-therapy, wherein CAR T-cell therapy is not limited by MHC-class-I matching [79–81]. Notably, in addition to modifying T-cells to contain specific TCRs and CARs, T-cells can be edited in other ways to improve their efficacy, including encoding genes that make the T-cells more resistant to apoptosis, more responsive to co-stimulatory molecules, and more effective at proliferating [82].

However, in general, engineered T-cell therapies are limited by their monoclonal specificity for tumor-associated neoantigens, optimal selection of the fittest T-cell population, and potential off-target effects of treatment which may lead to cytokine storms in patients. In addition, within the context of penile cancer, a major limitation of both ACT therapy using autologous TILs and engineered T-cell therapy is the lower TMB of penile cancer as compared to other malignancies where ACT has been successful such as melanoma [83–86]. A lower TMB limits the range of mutated tumor-associated antigens that can be recognized by the immune system. Additionally, while ACT and engineered T-cell therapies have shown promise in hematological malignancies, these approaches require efficient trafficking to the site of the solid tumor which may impair their efficacy [87–90]. Finally, both approaches require the patient to receive lymphodepletion via chemotherapy or radiotherapy, thus potentially preserving ACT and engineered T-cell therapies for patients with better performance status.

Currently, there are no prospective data available on the utility of these therapies in penile cancer. However, in a recent phase I trial, Nagarsheth and colleagues [91] investigated TCR therapy targeting the HPV-16 E7 oncoprotein in 12 patients, of which 11 had SCCs. Of these 12 patients, 6/12 patients demonstrated an objective response and 5/12 patients showed stable disease following treatment. With respect to safety and tolerability, no dose-limiting toxicities, off-target tissue damage, or treatment-related deaths were observed. In another basket phase I/II study in nine patients with SCCs and three with adenocarcinomas of an HPV-16 E6-targeting TCR, 2/12 patients (anal SCC) exhibited an objective response; in addition, no off-target toxicities or dose-limiting toxicities were observed [92]. While limited, these data are promising results for the potential safety and efficacy in other SCCs like penile cancer. At the present time, NCT02379520 is a large basket trial investigating the utility of E6- and E7-targeting TCRs in a broad range of HPV-positive tumors, including penile cancer [93]. Of note, this trial is also evaluating a combination approach involving TCR therapy plus ICI in the form of nivolumab.

4.3. Tyrosine kinase inhibitors (TKIs) and other targeted therapies

In recent years, there has been considerable effort to define the molecular landscape of penile cancer and advance our knowledge of oncogenesis and tumor development. For example, studies have found frequently altered genes in penile cancer include PI3KCA, NOTCH1, CDK2NA, HRAS, KRAS, CCND1, TP53, and STK11, resulting in changes in signaling across several pathways including the PI3K-AKT-mTOR, RTK/RAS/MAP-kinase, and tyrosine kinase pathways [83,94–108]. However, due to the rarity of penile cancer, our current understanding is derived from a limited number of retrospective studies with heterogeneous patient cohorts. Nonetheless, these studies provided valuable insight into potential molecular targets for TKIs and other targeted therapies.

In this regard, epidermal growth factor receptor (EGFR) expression has been examined in penile cancer. For example, Chaux and colleagues [109] demonstrated low to high EGFR expression in 88% (99/112) of invasive penile cancer cases upon immunohistochemical analysis. In another cohort of thirty patients, Di Lorenzo and colleagues [110] found that all patients expressed EGFR upon immunohistochemical, and a portion of patients expressed cytosolic and/or nuclear phosphorylated-EGFR. In addition, Di Lorenzo and colleagues [110] found cytosolic phosphorylated-EGFR expression to be significantly associated with poor survival and an increased risk of recurrence upon multivariate analysis. In light of these findings and the efficacy of TKI therapy in a range of solid tumors, anti-EGFR targeting agents have been investigated in penile cancer (Table 4).

In the retrospective setting, Carthon and colleagues [111] examined outcomes among patients with relapsed locally advanced/metastatic disease who were treated with a single anti-EGFR agent (cetuximab, erlotinib, or gefitinib) alone or in combination with a platinum chemotherapy/TIP. In this cohort, only patients treated with either cetuximab alone or in combination with cetuximab plus cisplatin/TIP showed a radiographic response.

Prospectively, Necchi and colleagues [112] evaluated the anti-EGFR agent panitumumab in 11 patients with
metastatic disease who had progressed following chemotherapy. Of these patients, three patients demonstrated an objective response and two patients demonstrated stable disease, with Grade 2 skin toxicity being the most frequent AE. These five patients experienced a median OS of 9.5 months compared to 7.6 months among non-responders.

In a separate phase II study, Necchi and colleagues [113] examined dacomitinib—a pan-human EGFR (HER) TKI that is capable of inhibiting EGFR, HER2, and HER4—in a prospective cohort of 28 chemo-naïve patients with N2-M1 disease. An ORR of 32.1% with only three Grade 3 AEs was observed. Notably, among chemo-naïve patients with locally advanced disease—which would be a group comparable to some extent with patients eligible for TIP as NAC—the median OS was 20 months compared to 17.1 months for TIP as reported by Pagliaro and colleagues [15].

Overall, it is difficult to make direct comparisons and draw conclusions from these limited studies due to the small size of these patient cohorts and the heterogeneity between patients with respect to the extent of disease and prior treatments. Broadly, it appears that anti-EGFR and pan-HER TKI agents are tolerable and result in fewer toxicities as compared to standard of care chemotherapy regimens like TIP. Thus, these agents may be worth investigating as alternative therapies for patients with low performance status or as combination therapy in patients who are eligible to receive chemotherapy. In addition, based on these data, a pan-HER TKI that is capable of binding multiple targets may prove more effective than a single-target anti-EGFR TKI. However, these claims must be further investigated and validated in prospective, randomized-controlled trials within more homogenous patient cohorts when possible.

### 4.4. HPV-targeting vaccines

As mentioned previously, approximately 50% of penile cancers are HPV-positive [3]. In addition, studies have shown that HPV infection plays a role in penile cancer development through the activity of the E6 and E7 oncoproteins, which remain constitutively active in HPV-positive cells as drivers of tumorigenesis [114–118].

In light of these findings, therapeutic vaccines have emerged in recent years as a potential systemic treatment strategy for HPV-associated malignancies, including penile cancer [119–121]. HPV-targeting therapeutic vaccines aim to elicit an immune response against the E6 and E7 oncoproteins, and as a result, enhance the systemic activity of cytotoxic T-cells. Given our previous discussion of E6/E7-targeting engineered TCR therapies, the E6 and E7 oncoproteins are important tumor-associated antigens in HPV-positive penile cancer at a time when the molecular etiology of this disease is not well understood. While there are no current data available on the use of therapeutic vaccines in penile cancer, HPV-targeting therapeutic vaccines have demonstrated efficacy in other tumor types and premalignant lesions, including cervical cancer and intraepithelial lesions [122–124].

Currently, therapeutic HPV-vaccines are being investigated via multiple prospective basket trials that include...
| ClinicalTrials.gov identifier; study phase | Planned accrual; status | Setting | HPV-targeting agent(s) | Combination therapy (if applicable) | Primary endpoint |
|------------------------------------------|-------------------------|---------|------------------------|--------------------------------------|------------------|
| NCT02379520; phase I                    | n = 32; not recruiting | Recurrent HPV+ disease or HPV+ disease ineligible for SOC treatment | HPV-16/18 E6/E7-specific T lymphocytes | Cytoxan, fludarabine, and nivolumab (anti-PD-1) | Incidence of DLT |
| NCT02858310; phase I/II                 | n = 180; recruiting    | Recurrent or metastatic HPV+ disease | HPV-16 E7-targeting TCR T-cells (E7 TCR) | Aldesleukin, fludarabine, and cyclophosphamide | Phase II dosage |
| NCT03418480; phase I/II                 | n = 44; not recruiting | Recurrent HPV+ disease or disease-free patients | HPV anti-CD40 RNA vaccine | None | Incidence of DLT |
| NCT03439085; phase II                   | n = 77; recruiting     | Recurrent or metastatic HPV+ disease | INO-3112 (HPV vaccine) | Durvalumab (anti-PD-L1) | ORR |
| NCT04180215; phase II                   | n = 200; recruiting    | Relapsed or treatment-refractory HPV+ disease | HB-201 ± HB-202 (both express antigenic HPV-16 E6/E7 fusion protein) | None | Incidence of DLT and phase II dose |
| NCT04287868; phase I/II                 | n = 56; recruiting     | Recurrent or metastatic HPV+ disease | PDS0101 (HPV vaccine) | M7824 (bintrafusp alfa and bi-functional fusion protein; TGF-β trap and anti-PD-L1) and NHS-IL12 | ORR |
| NCT04432597; phase I/II                 | n = 76; recruiting     | Recurrent or metastatic HPV+ disease | PRGN-2009 (HPV vaccine) | M7824 (also called bintrafusp alfa); M7824 is a bi-functional fusion protein targeting TGF-β trap and anti-PD-L1 | Phase II dose and safety |

DLT, dose-limiting toxicity; HPV, human papilloma virus; ORR, overall response rate; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SOC, standard-of-care; TCR, T-cell receptor; TGF-β, transforming growth factor-beta.
HPV-positive penile cancer (Table 5). Of note, therapeutic vaccines are being evaluated as both monotherapies as well as in combination with ICIs. In this regard, while a direct relationship between these two therapeutic approaches remains to be elucidated, it is possible that the combined immuno-stimulatory activity of therapeutic vaccines and ICIs may lead to an enhanced anti-tumor immune response.

5. Conclusion

Currently, chemotherapy is the mainstay systemic therapy option in the first-line setting for penile cancer patients with locally advanced or metastatic disease. However, while only a subset of patients benefit, subsequent-line treatment options for progressive disease are scarce and long-term outcomes remain very poor. In recent years, a number of studies have sought to define the molecular landscape of penile cancer and elucidate drivers of disease progression, potential drug targets, and molecular indicators of potential eligibility for novel systemic therapy approaches that have been discussed previously; specifically, ICIs, adoptive or engineered T-cell therapies, targeted therapies, and HPV-directed therapeutic vaccines.

While ICIs and targeted therapies have demonstrated some efficacy in patients with advanced and/or treatment-refractory penile cancer, larger prospective studies are needed to further evaluate the utility and sequencing of these systemic therapy options. For example, while unexplored in penile cancer, there is evidence from triple-negative breast cancer and non-small cell lung-cancer to support ICI use in the neoadjuvant setting in combination with NAC. However, while there are several ongoing clinical trials investigating ICI therapy in penile cancer, the majority of studies are examining ICI use later in the disease course or in the setting of unresectable disease, either as single-agents or in combination with other ICIs. Thus, future studies on ICI should explore its use in the first-line setting as well as in combination with other systemic therapies.

At the present time, both ACT-based therapies and HPV-directed therapeutic vaccines have not been evaluated in penile cancer. However, pre-clinical studies in penile cancer have demonstrated the feasibility of expanding patient-derived TILs. Further studies in larger, prospective cohorts are warranted to study the utility of ACT-based therapies in penile cancer, especially given the unique challenges associated with administering TCR and CAR T-cell therapies in solid tumors. With respect to HPV-directed therapeutic vaccines, limited clinical studies in other pre-malignant and malignant conditions have exhibited some efficacy of HPV-directed therapeutic vaccines. Currently, a number of vaccines are being investigated in penile cancer through basket trials for HPV-positive cancers.

To summarize, there is an urgent need to address the dismal outcomes associated with advanced penile cancer and develop more effective lines of systemic treatment. While promising, the novel systemic therapy approaches discussed in this review require prospective validation through randomized-controlled trials. Due to the rarity of penile cancer, basket trials involving other SCCs should be pursued when possible.

Author contributions

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All authors reviewed and approved the final version of the manuscript.

Conflicts of interest

Vidhu B. Joshi and Juskaran Chahda report no conflicting financial interests; Jad Chahoud reports that he provided advisory board consultations for Pfizer, Aveo, and Exelixis.

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