Review

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Immunotherapy in Penile Cancer: A Systematic Review

Filipe Fadigas[1,†], Diana Martins[1,2,3,4,†], Fernando Mendes[1,2,3,4,5,*]

[1]Polytechnic University of Coimbra, 3045-093 Coimbra, Portugal
[2]H&TRC-Health & Technology Research Center, Coimbra Health School, Polytechnic University of Coimbra, 3045-043 Coimbra, Portugal
[3]Biophysics Institute of Faculty of Medicine, Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment Genetics and Oncobiology (CIMAGO), University of Coimbra, 3000-548 Coimbra, Portugal
[4]Center for Innovative Biomedicine and Biotechnology, University of Coimbra, 3004-504 Coimbra, Portugal
[5]European Association of Biomedical Scientists, B-1000 Brussels, Belgium
*Correspondence: fjmendes@estesc.ipc.pt (Fernando Mendes)
[†]These authors contributed equally.
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Penile cancer (PeCa) ranks as the 30th most prevalent cancer globally, predominantly affecting populations in developing countries. Phimosis and Human Papillomavirus (HPV) infection are recognized as the primary risk factors. Early-stage diagnosis typically warrants limited excision or non-invasive therapies. However, recent research into the carcinogenesis, tumour microenvironment, and the role of the host immune system in its development suggests that immunotherapy could be a promising treatment for PeCa. The rarity of the disease, combined with the success of standard treatments and the fact that many patients in clinical trials lack alternative options, contributes to the challenges in patient recruitment for these studies. Additionally, the psychological burden stemming from the stigma associated with such an aggressive genital disease and the preference for quicker treatment options, such as surgery with reconstructive procedures, exacerbates these recruitment difficulties. This systematic review aimed to explore various immunotherapy approaches in treating PeCa to elucidate the potential role of immunotherapy in this context. The literature was sourced from freely accessible, full-text randomized controlled trials, non-randomized controlled trials, and original articles published in English between 2017 and 2023. Eligible clinical trials were required to be in phase 2 and have published results. Although only one study met the inclusion criteria—a significant limitation—the objective response rate recorded was 6% across nineteen patients with different tumour histologies, of which only six had PeCa. Currently, other studies are either recruiting or ongoing, necessitating further observation, as results from a single study cannot be generalized to the broader population.

Keywords: penile neoplasms; immunotherapy; immune checkpoint inhibitors; cancer vaccines; tumour microenvironment

Introduction

Penile cancer (PeCa) ranks as the 30th most prevalent cancer and the 31st leading cause of cancer-related deaths worldwide, primarily impacting populations in developing regions such as South America, South Central Asia, and Eastern and Southern Africa [1,2].

Approximately 95% of PeCa cases are squamous cell carcinomas (SCC) [3–5], originating from the inner mucosal lining of the glans, coronal sulcus, or foreskin [6,7]. SCC development follows two distinct etiopathogenic pathways correlated with specific risk factors: Human Papillomavirus (HPV) infection, particularly high-risk HPV, which is implicated in up to 50% of PeCa cases [2,4], and phimosis. When coupled with poor genital hygiene, phimosis can lead to smegma accumulation, fostering chronic inflammation and ultimately resulting in dysplastic epithelial changes [8,9]. The World Health Organization categorizes PeCa as either HPV-related or HPV-unrelated [6,9,10], based on the critical role HPV plays in carcinogenesis through its E6 and E7 oncoproteins, which disrupt cell-cycle regulation, drive autonomous cell proliferation, and contribute to genomic instability; E7 inactivates the retinoblastoma (Rb) protein, while E6 promotes P53 degradation [9,11–17], as illustrated in Fig. 1a.

Surgery remains the gold standard for PeCa treatment, though cytotoxic chemotherapy is employed in cases of unresectable tumours or disseminated metastatic disease, either in a neoadjuvant or adjuvant context [11,12,18–22].

Genomics

Key genetic alterations frequently observed in PeCa include mutations in *Tumour Protein P53* (*TP53*), *Notch Receptor 1* (*NOTCH1*), Phosphatidylinositol-4,5-Biphosphate 3-Kinase Catalytic Subunit Alpha (*PIK3CA*), Hras proto-Oncogene, GTPase (*HRAS*), Cyclin-Dependent Kinase Inhibitor 2A (*CDKN2A*), Caspase 8 (*CASP8*), FAT Atypical Cadherin 1 (*FAT1*), F-Box and WD Repeat

Domain Containing 7 (*FBXW7*), and E1A Binding Protein P300 (*EP300*) [5,10,13,23,24], as depicted in Fig. 1b.

Distinct molecular profiles differentiate HPV-related and HPV-unrelated PeCa: HPV-related tumours typically exhibit *CDKN2A* accumulation without P53 expression, whereas HPV-unrelated variants show high nuclear P53 expression and lack *CDKN2A*. Genomic alterations in the mammalian target of rapamycin (mTOR) pathway, particularly in phosphatase and tensin homolog (PTEN) and Neurofibromatosis type 1 (NF1), have been implicated in tumourigenesis, disease progression, and angiogenesis. These alterations, especially the loss-of-function mutations in PTEN, contribute to deregulated cell growth and heightened mTOR signalling, particularly in the context of concurrent HPV-positive status and *TP53* mutations [5,13,23,25].

Tumours with a higher tumour mutational burden (TMB) are generally more responsive to immune-based therapies. While PeCa's TMB is lower compared to skin melanoma, non-small cell lung cancer (NSCLC), and urothelial bladder cancer, it is comparable to that of head and neck (HNC), cervical, or oesophageal SCC [13,23,26]. A study indicated that immune checkpoint inhibitors (ICI) could be effective in patients with a TMB exceeding 10 mutations per megabase, which accounts for 18% of penile squamous cell carcinomas (PSCC) [27]. Additionally, the amplification of the *Mouse double minute 2 homolog* (*MDM2*) oncogene, associated with hyperprogression—a phenomenon of accelerated tumour growth following ICI treatment—was found to be rare in metastatic PSCC, suggesting a lower risk of hyperprogression in this disease [23,27]. Furthermore, mutations in STK11, known to confer resistance to programmed cell death protein 1 (PD-1) blockade in lung adenocarcinoma, were identified in only about 6% of a cohort of 78 metastatic PSCC samples, indicating a low prevalence of this resistance mechanism in PSCC [23].

Tumour Microenvironment

The tumour microenvironment (TME) of PeCa comprises various innate and adaptive immune cell types that influence the anti-tumour response, either suppressing or promoting tumour progression, as illustrated in Fig. 1c. Tumour-associated macrophages (TAMs) play a significant role in enhancing angiogenesis, increasing tumour cell mobility, modulating immunotolerance, and facilitating metastatic growth. Evidence also suggests that TAMs may reduce the efficacy of anti-PD-1 therapy in lung SCC by hindering CD8+ T cell migration to tumour sites. TAMs are categorized into two groups: M1, which possess anti-tumour properties and express CD68, and M2, which express CD163 and are associated with tumour progression [28]. Research has shown that CD163 expression correlates with high tumour grade and poor survival in non-small cell lung cancer (NSCLC). In contrast, other studies suggest that while a high density of CD163+ TAMs in PeCa is not directly linked to poor survival, it is associated with lymph node metastasis (LNM). Additionally, CD163+ macrophage density is higher in HPV-positive tumours compared to HPV-negative ones [28]. Immunologically, the TME can be classified into three types based on the presence of stromal and intraepithelial cytotoxic T lymphocytes: "immune desert", "immune excluded", and "immune inflamed". PSCC appears to be immune excluded, characterized by a higher number of CD8+ T cells and Forkhead Box P3+ (FOXP3+) regulatory T (Treg) cells in the stroma than within the tumour itself, as depicted in Fig. 1c. Several studies have examined CD3+/CD4+ T cells, CD3+/CD8+ T cells, and CD3+/CD4+/FOXP3+ Treg cells to determine their infiltration patterns and impact on tumour outcomes. In many SCC and urologic malignancies, higher levels of cytotoxic CD8+ T cells are associated with a better prognosis [29]. However, in cases where these cells are predominantly located in the stroma—indicating an immune excluded type—a higher density of CD8+ cytotoxic, antigen-experienced T cells is paradoxically associated with poorer median overall survival (OS). These low levels of stromal CD8+ T cells have also been linked to LNM. Furthermore, elevated levels of tumour-infiltrating FOXP3+ Treg cells are associated with lower odds of disease-free survival. Comparing both PeCa variants, HPV-positive tumours tend to exhibit significantly higher levels of CD8+ T cell infiltration, leading to a more robust anti-tumour response and supporting the observation that patients with this variant generally have a better prognosis [13,23,24,27,30,31].

The PD-1/programmed cell death ligand 1 (PD-L1) axis is crucial in enabling immune evasion across various tumours. T-cell activation triggers an increase in PD-1 expression, which subsequently binds to its ligand, PD-L1, present on the surface of antigen-presenting cells. This interaction leads to T-cell exhaustion. Tumour cells exploit this mechanism by upregulating PD-L1 expression on their surfaces, thereby achieving immune suppression within the TME (Fig. 1c). A report indicates that approximately 40–69% of PSCC cases exhibit PD-L1 expression, predominantly in tumour-infiltrating lymphocytes (TIL) and, more prominently, on tumour cell membranes. Diffuse PD-L1 expression has been associated with higher LNM and poorer survival outcomes compared to cases with border-positive or negative expression [27]. High levels of PD-L1 are frequently observed in PeCa, particularly in the HPV-negative variant, suggesting a more immune-suppressive TME, which may contribute to the observed lower survival rates in these patients [13,32,33].

Immunotherapy

Immune Checkpoint Inhibitors

Tumour cells possess the capability to escape immune surveillance by evading recognition and destruction

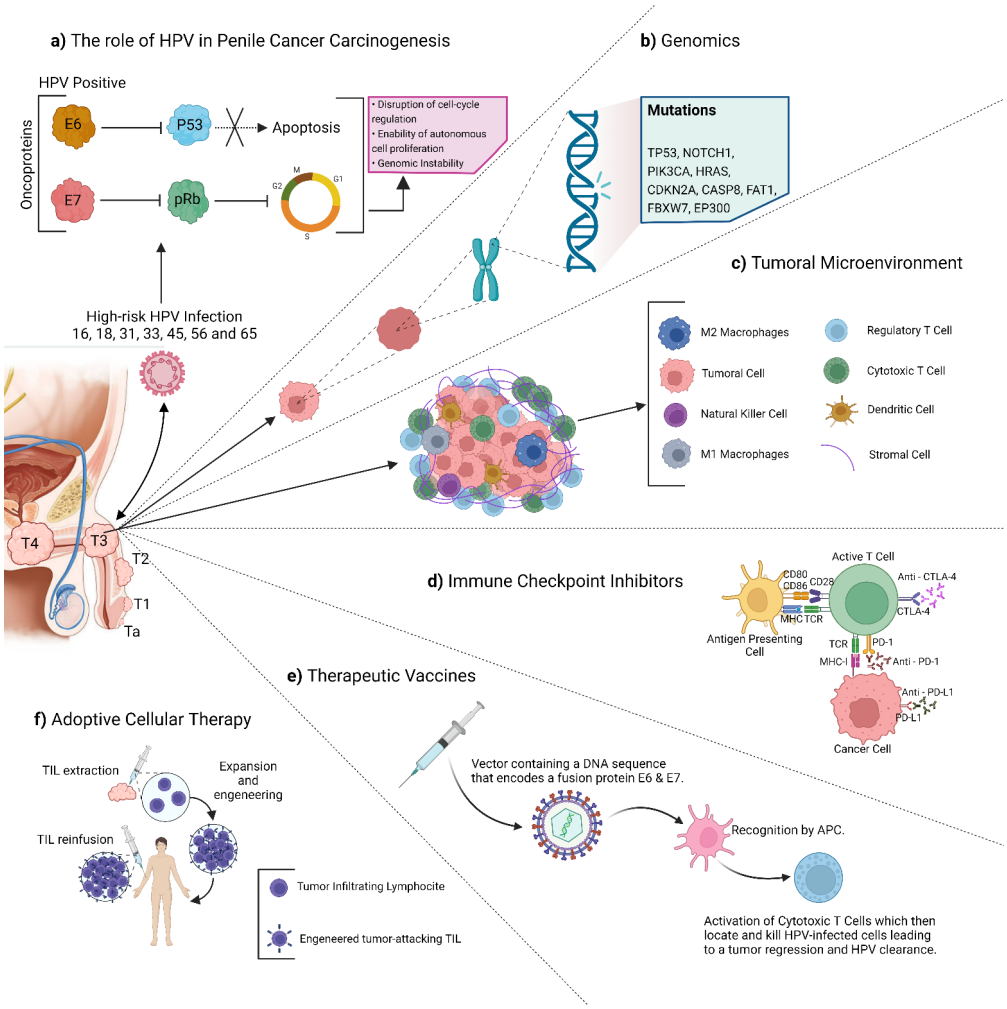

Fig. 1. Tumour staging and grading are critical for selecting appropriate treatment, with diagnosis typically involving the assessment of tumour differentiation and locoregional spread. (a) The World Health Organization (WHO) classifies penile cancer (PeCa) as either Human Papillomavirus (HPV)-related or HPV-unrelated, based on the distinct role HPV plays in PeCa carcinogenesis. HPV-related carcinogenesis is driven by viral E6 and E7 oncoproteins, which disrupt cell-cycle regulation, promote autonomous cell proliferation, and induce genomic instability. The E7 oncoprotein binds to and inactivates the retinoblastoma (Rb) protein, while the E6 oncoprotein facilitates P53 degradation. (b) Genes with a higher overall frequency of alterations that contribute to the development of PeCa. (c) The TME in PeCa includes various immune cells and components that influence the anti-tumour response, either suppressing or promoting tumour progression. PeCa's TME is classified as immune excluded, characterized by a significantly higher number of CD8+ T cells and Forkhead Box P3+ (FOXP3+) regulatory T cells in the stroma compared to the tumour site. TAMs are key players in this environment, divided into M1 macrophages, which express CD68 and have anti-tumour functions, and M2 macrophages, which express CD163 and are associated with high tumour grade and poor survival in NSCLC. M2 macrophages enhance angiogenesis, increase tumour cell mobility, modulate immunotolerance, and support metastatic growth. CD163+ macrophages are more densely present in HPV-positive tumours compared to HPV-negative ones. Additionally, stromal cells provide structural support and nourishment to tumour cells. Natural Killer (NK) cells and dendritic cells perform key anti-tumour functions, with NK cells directly killing tumour cells and dendritic cells presenting antigens to cytotoxic and helper T cells. Both tumour cells and tumour-infiltrating lymphocytes (TIL) often express programmed cell death ligand 1 (PD-L1) to facilitate immune suppression within the TME. Notably, PD-L1 expression tends to be higher in the HPV-negative variant of PeCa. (d) ICI target key pathways that facilitate the development of PeCa. Anti-programmed cell death protein 1 (anti-PD-1), anti-PD-L1, and anti-cytotoxic T-lymphocyte-associated antigen 4 (anti-CTLA-4) therapies work by blocking the tumour's ability to evade the immune system and inhibit T-cell proliferation. (e) Therapeutic vaccines aim to induce an *in vivo* virus-specific T-cell response against the E6 and E7 oncoproteins, thereby boosting cytotoxic T-cell activity and providing both therapeutic and prophylactic effects. (f) Adoptive cellular therapy involves expanding TIL extracted from tumour samples and genetically engineering them. These modified cells are then re-infused into the patient after lymphodepletion to enhance the immune response against the tumour. This figure was created using the BioRender website.

through the co-inhibition of specific pathways [34]. Among the many known immune checkpoints, PD-1, PD-L1, and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) are the most extensively studied. Current research is focused on evaluating the use of immune checkpoint blockade (Fig. 1d) to treat PeCa, building on the success of these therapies in other cancers, such as HNC and lung cancers [34–37].

PD-1 is a transmembrane protein expressed in various cells, including B cells, dendritic cells, macrophages, monocytes, Natural Killer (NK) cells, and activated T CD4+ and T CD8+ cells. It is also highly expressed in tumour-specific T cells [34,37,38]. PD-1 plays a dual role: While it downregulates harmful or ineffective immune responses to maintain immune tolerance, it can also protect tumour cells from the immune system, aiding in their evasion [37,38]. Conversely, PD-L1 is a transmembrane glycoprotein found on some activated T and B cells, dendritic cells (DC), and epithelial cells in inflammatory settings. Tumour cells can also express PD-L1 to escape antitumoural activity [37–39] (Fig. 1d). These immune checkpoints regulate immune tolerance within the TME; When PD-1 binds to PD-L1 on tumour cells, T cells lose their ability to effectively recognize and kill the tumour cells. Additionally, TAMs lose their phagocytic capability, further enhancing tumour immunity [34,37,38].

CTLA-4 is predominantly expressed in intracellular vesicles within FOXP3+ regulatory T (Treg) cells and activated conventional T cells, including CD4+ and CD8+ cells. Its ligands, B7-1 and B7-2, are expressed on antigen-presenting cells (APCs) and are also ligands for CD28 [34]. These ligands are primarily expressed on DC, which, when immature, capture exogenous antigens from blood and tissues. During an immune response triggered by infection or necrosis, dendritic cells mature and migrate to the lymph nodes, where they present antigens to activate both helper and cytotoxic T cells, upregulating MHC and co-signalling molecules such as CD40 and B7 [34]. CD28, a homologous protein to CTLA-4, co-stimulates T cells but differs in being expressed on the plasma membrane [34]. B7-1 and B7-2 can bind to either CD28 or CTLA-4; Binding to CD28 on CD4+ T cells induces their differentiation into T helper cells and promotes CD8+ T cell proliferation through the production of interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), and interleukin 2 (IL-2) [40]. However, when B7 binds to CTLA-4, it downregulates the production of IFN-γ, TNF-α, and IL-2, suppressing T-cell proliferation. In competition, CTLA-4 has a higher affinity for B7-1 and B7-2 than CD28 [34,40] (Fig. 1d). Although CTLA-4 is mainly intracellular, upon engagement and costimulatory signalling through CD28 in T cell receptors, it relocates to the cell surface, outcompeting CD28 and leading to the downregulation of T cell proliferation and activation [34,40]. Tumours can upregulate CTLA-4 to suppress CD4+ T cells, making CTLA-4 blockade a viable immunotherapy option for PeCa treatment. Ipilimumab, a human monoclonal antibody designed to target and block CTLA-4, is an ICI of interest that may prove effective in treating PeCa [32].

Therapeutic Vaccines

Approximately 50% of PeCa cases are HPV-positive, with the E6 and E7 oncoproteins acting as key drivers of tumourigenesis [41,42], as depicted in Fig. 1a. Therapeutic vaccines targeting HPV aim to elicit *in vivo* virus-specific T-cell responses against these oncoproteins, enhancing the systemic activity of cytotoxic T-cells to combat established infections and lesions caused by HPV [10,42] (Fig. 1e). These vaccines offer a promising strategy for treating HPV-related infections and associated diseases, potentially even providing prophylactic benefits against the development of advanced tumours by selectively targeting cells undergoing transformation due to HPV [10,33,41]. Various approaches are under investigation to improve CD4+ and CD8+ T-cell responses, including genetic, protein-based, peptide-based, and DC-based vaccines [42]. Among these, DNA-based vaccines have shown encouraging results in clinical studies. This vaccination strategy involves injecting a plasmid containing a DNA sequence that encodes the target antigen into the tissues. The plasmid transfects both APCs and non-APCs, leading to the *in situ* production of the antigen and subsequent activation of the adaptive immune response, including the activation of cytotoxic and helper T cells [42,43]. Various viral vectors, such as adenoviruses, adeno-associated viruses, alphaviruses, and vaccinia viruses, are being utilized to express E6, E7, and E2 oncoproteins, triggering CD4+ and CD8+ T-cell responses. These vectors can also be engineered to induce the production of interferon-α and present E6 and E7 antigens via DC, leading to the activation of naive T cells in the lymph nodes [42]. In terms of bacterial vectors, Listeria monocytogenes is considered an effective vaccine vector due to its ability to enter APCs, stimulate their maturation, and induce strong innate and adaptive immune responses [17,42].

Adoptive Cellular Therapy

Adoptive cellular therapy (ACT) involves the *ex vivo* expansion of TIL obtained from resected human tumour samples, coupled with the engineering of T cells to express tumour-specific antigens [44,45] (Fig. 1f). These modified T cells are then intravenously re-infused into the patient following lymphodepletion [44]. However, challenges arise when there is insufficient biopsy tissue or an absence of TIL, making the use of native T cells unfeasible. To address these limitations, genetic modification techniques are employed to enhance peripheral blood T cells *ex vivo*, enabling them to express receptors specific to tumour antigens. This modification can involve introducing genes encoding chimeric antigen receptors (CARs)—antibody-based receptors that impart the specificity of a monoclonal antibody onto a T-cell—or conventional T-cell receptors.

Table 1. Medical Subject Headings terms used for literature search in this systematic review.

Medical Subject Headings term	And	Or	Not
Penile Neoplasms	Immunotherapy		Drug therapy, Radiotherapy
Penile Neoplasms	Immune Checkpoint Inhibitors		
Penile Neoplasms	Immunotherapy, Adoptive		
Penile Neoplasms	Cancer Vaccines		
Penile Neoplasms	Tumour Microenvironment		

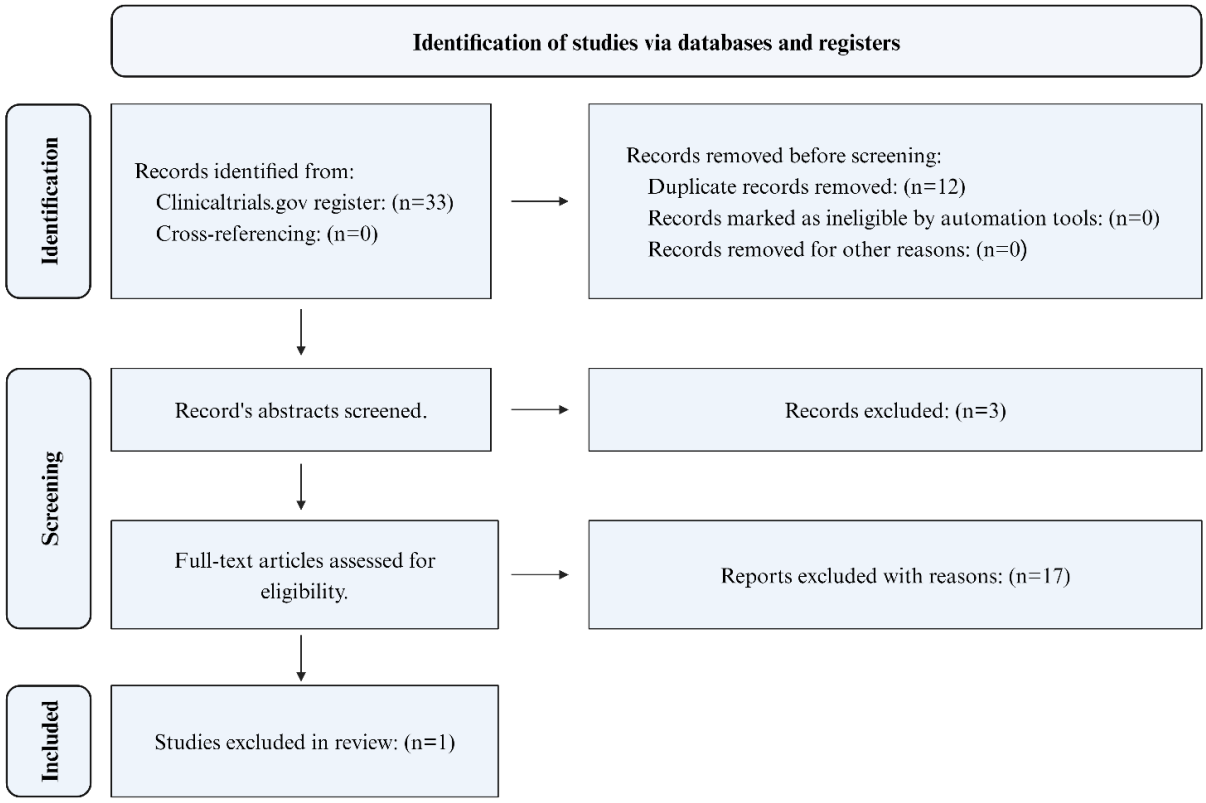

Fig. 2. Preferred Reporting Items for Systematic Reviews. This figure was created using the BioRender website.

CARs have demonstrated superiority over traditional T-cell receptors due to their ability to recognize antigens without any restrictions [45,46].

ACT has gained significant traction because of its capacity to expand specific immune cells and the development of an infrastructure that facilitates the efficient, safe, and quality-assured delivery of cellular products [46]. CD8+ infiltration in PeCa TME is considered a prognostic factor, suggesting that ACT using TIL could be a viable treatment option for patients with PeCa. However, there are notable limitations associated with this therapy. Firstly, ACT is tailored to individual patients, making it a costly treatment option that is challenging to scale if patient numbers are high. Secondly, obtaining tumour samples with isolated TIL is difficult [19,40,47].

Literature Research Strategy

This investigation involved an extensive data search conducted through the "PubMed" and "Clinicaltrials.gov" search engines, which access the "MEDLINE®" database, focusing on the role of immunotherapy in PeCa.

The Medical Subject Headings (MeSH) terms "Penile Neoplasms", "Immunotherapy", "Immune Checkpoint Inhibitors", "Immunotherapy, Adoptive", "Cancer Vaccines", and "Tumour Microenvironment" were selected to filter the most relevant and accurate information for the article (Table 1).

The literature was sourced from freely accessible full-text randomized controlled trials, non-randomized controlled trials, and original research articles written in English and published between 2017 and 2023. The included clinical trials were required to be in phase 2 and have published results, as outlined in our "Preferred Reporting Items

Table 2. Overview of ongoing immunotherapy studies in penile cancer treatment.

Study	Status	Target	Drug	Intervention
NCT03866382 [61]	Recruiting	PD-1, CTLA-4 and VEGFR	Cabozantinib S-malate, Ipilimumab, and Nivolumab	Doses and cycles not established.
NCT04718584 [54]	Recruiting	PD-L1	Human Anti-PD-L1 Monoclonal Antibody Injection (LDP)	10 mg/kg of LDP once every two weeks for a total of 6 weeks. Surgical treatment will be performed within 8 weeks.
NCT03774901 [55]	Recruiting	PD-L1	Avelumab	10 mg/kg every 2 weeks until progression or unacceptable toxicity.
NCT04708470 [58]	Recruiting	TGF-β and PD-L1	Bintrafusp-α, NHS-IL12 and Entinostat	300 mg of Bintrafusp-α independent of body weight. 4, 8, 12, or 16 micrograms/kg of NHS-IL12 every 2 or 4 weeks. Self-administered Entinostat.
NCT03391479 [56]	Recruiting	PD-L1	Avelumab	10 mg/kg, once every 2 weeks
NCT04357873 [60]	Active, not recruiting	PD-1	Pembrolizumab and Vorinostat	200 mg of Pembrolizumab every 3 weeks, up to 35 administrations, and 400 mg of Vorinostat once daily until progression.
NCT03439085 [59]	Active, not recruiting	E6 and E7 oncoproteins of HPV-16/18 genotypes and PD-L1	DNA plasmid-encoding interleukin-12/HPV DNA plasmids therapeutic vaccine MEDI0457 and Durvalumab	DNA plasmid-encoding interleukin-12/HPV DNA plasmids therapeutic vaccine INO-3112 at 1, 3, 7, and 12 weeks, and Durvalumab at 4, 8, and 12 weeks.
NCT03686332 [57]	Active, not recruiting	PD-L1	Atezolizumab	1200 mg of Atezolizumab every 3 weeks.

for Systematic Reviews and Meta-Analyses" (PRISMA) diagram. The selected papers were evaluated using the ROB-VIS risk of bias tool (RoB 2, Risk-Of-Bias VISualization) and were included in the review only if they were determined to have a low risk of bias [48].

The selected data were compiled into a systematic review, written in English, in accordance with the PRISMA checklist guidelines [49].

Immunotherapy in Penile Cancer

Applying the inclusion and exclusion criteria, the authors selected one study that evaluated the efficacy of ICI and presented its results, as illustrated in Fig. 2. A subsequent article, published following the NCT03333616 trial, described this study as a single-arm, phase 2 multicohort investigation assessing the objective response rate (ORR) of a combination therapy involving Ipilimumab, an anti-CTLA-4 monoclonal antibody, and Nivolumab, an anti-PD-1 monoclonal antibody, in treating advanced rare genitourinary cancers, adrenal tumours, platinum-refractory germ cell tumours, PeCa, and prostate cancer with variant histology [50,51].

The study enrolled 57 patients, who were divided into three cohorts, with the final cohort including patients with PeCa.

Within cohort 3, 19 patients, including 6 with PeCa, with a median age of 60, and no prior exposure to PD-1/PDL-1 or CTLA-4 therapies, received 480 mg of Nivolumab intravenously every four weeks, following an initial regimen of 3 mg/kg of Nivolumab and 1 mg/kg of Ipilimumab administered in four doses every three weeks.

The cohort's overall median follow-up was 6.5 months. Imaging evaluations for tumour reduction were conducted on three of the six patients with PeCa: One patient achieved a tumour reduction of 160%, two experienced reductions of approximately 20%, and the remaining patient's tumour increased by approximately 35%.

In this cohort, the ORR was 6%, with a progression-free survival of 2.7 months and an OS of 50%. Patients with PeCa did not respond well to the therapy, with three of the six patients experiencing disease progression and two maintaining stable disease.

More than 10% of patients across all cohorts reported adverse events, the most common being elevated liver enzymes, fatigue, rashes, diarrhoea, thyroid issues, pruritus,

elevated lipase, pulmonary symptoms, hyponatremia, and arthralgias [51].

Discussion

The limited treatment options and poor prognosis associated with PeCa underscore the urgent need for new therapeutic strategies. Immune-based therapies have gained approval for a range of malignancies, and the Food and Drug Admnistration (FDA) has sanctioned the use of ICI for treating various metastatic tumours, including NSCLC, HNC, oesophageal cancer, and cutaneous SCC [13].

Given the potential of immunotherapy as a treatment option for PeCa, the authors sought to clarify its role but found only one relevant study. This scarcity of research raises the question of why so few studies exist. The low incidence of PeCa, which is consistent in countries with both high and low human development indices (HDI) [1,3], significantly contributes to the small number of patients available for clinical trials. Additionally, when PeCa is diagnosed early, surgical intervention offers a 10-year survival rate of 96% for patients with localized tumours and no lymphatic involvement [11]. This high success rate with surgery for early-stage disease diminishes the likelihood of exploring alternative treatments, such as immunotherapy, particularly since patients in clinical trials often have no other viable treatment options due to unresectable or metastatic disease. Moreover, the psychological burden associated with the stigma of having an aggressive genital disease leads many patients with PeCa to prefer swift treatment options like surgery, which can be combined with reconstructive procedures, over other potentially less certain therapies [52]. While it is understandable that patients might favour a proven and rapid treatment like surgery, there remains a strong case for encouraging clinical trials that explore immunotherapy for non-metastatic local PeCa [53].

Despite the challenges in enrolling patients in clinical trials, several studies are currently in the recruiting or active phase, as listed in Table 2 (Ref. [54–61]). For instance, studies such as NCT04718584, NCT03774901, NCT03391479, and NCT03686332 are exploring the use of monoclonal anti-PD-L1 antibodies [54–57]. Additionally, NCT04708470 aims to block PD-L1 activity while also neutralizing TGF-β using Bintrafusp-α, NHS-IL12, and Entinostat [58]. The NCT03439085 study investigates the use of MEDI0457, a DNA plasmid-encoding interleukin-12/HPV DNA plasmids therapeutic vaccine, in combination with Durvalumab, a monoclonal anti-PD-L1 antibody [59]. NCT04357873 focuses on PD-1 inhibition using Pembrolizumab, a monoclonal anti-PD-1 antibody, alongside Vorinostat, a histone deacetylase inhibitor [60]. Finally, NCT03866382 incorporates Nivolumab and Ipilimumab with Cabozantinib S-malate, a tyrosine kinase inhibitor [61].

The planning of these studies represents a significant advancement, particularly because most focus on ICI, which are regarded as a cost-effective option due to the similar expression patterns of PD-L1 in PeCa tumour cells when compared to other cancers where ICI is already in use. ICI also offers scalability, as they do not require complex, patient-specific procedures like ACT, allowing them to potentially benefit a larger number of patients. However, these studies, like the one presented in the results, still face notable limitations. Although the clinical trials aim to evaluate various drug options for PeCa treatment, supported by a solid understanding of their mechanisms of action and the TME, they are often conducted in cohorts with diverse tumour histologies. For instance, the results discussed were derived from a cohort where only six patients had PeCa, which limits the study's ability to draw specific conclusions about this particular cancer. This limitation also extends to the assessment of adverse effects, which were reported in over 10% of patients across all three cohorts but cannot be definitively linked to a specific disease. Another issue identified in the study was the administration of Nivolumab and Ipilimumab without prior quantification of PD-1 and CTLA-4 expression in the tumour cells, which could have provided more targeted insights.

The low incidence of PeCa, coupled with the limited number of studies, restricts our ability to accurately identify and develop innovative immune-based treatments. Nonetheless, the strong association of PeCa with HPV, especially in HPV-positive tumours, suggests that therapeutic HPV vaccines could be a promising treatment modality. Additionally, the high expression of PD-L1 in PeCa tissues underscores the potential for applying ICI in treating this disease [62]. The ongoing clinical trials will be crucial in determining the most effective therapeutic strategies for PeCa.

Conclusions

The combination of Nivolumab and Ipilimumab appears unpromising for patients with PeCa, as none of the five patients responded to the treatment. However, this conclusion should not be generalized to the broader population, given that it is based on a single study. Patients with PeCa were part of a small cohort with diverse tumour histologies, and their TME was not adequately studied, making it difficult to associate the results with a specific disease.

Despite these limitations, several factors suggest that immunotherapy could still be a valuable treatment option for PeCa. Notable among these are the expression of PD-L1 in TIL and PeCa tumour cells, the TMB, and the rarity of STK11 mutations and *MDM2* oncogene amplification.

Future research is expected to identify therapeutic targets that could significantly impact the prognosis of advanced PeCa.

Availability of Data and Materials

The datasets used and/or analysed during the current study were available from the corresponding author on reasonable request.

Author Contributions

FF, DM and FM—designed the study and participated in drafting the manuscript; FF and DM—collected and analyzed the data. All authors conducted the study and contributed to critical revision of the manuscript for important intellectual content. All authors gave final approval of the version to be published. All authors participated fully in the work, took public responsibility for appropriate portions of the content, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or completeness of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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