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Targeted Therapeutic Approaches in Vulvar Squamous Cell Cancer (VSCC): Case Series and Review of the Literature

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Therapeutic options in recurrent or metastasized vulvar squamous cell cancer (VSCC) not amenable to radiotherapy or radical surgery are limited. Evidence for the use of targeted therapies is sparse. All patients with VSCC treated at the Gynecological Cancer Center Hamburg-Eppendorf 2013–2019 were retrospectively evaluated for targeted therapeutic approaches. Furthermore, a MEDLINE, EMBASE, Web of Science, Scopus, and OVID database search was performed using the terms: "vulvar cancer" AND "targeted therapy," "erlotinib," "EGFR," "bevacizumab," "VEGF," "pembrolizumab," or "immunotherapy." Twelve of 291 patients (4.1%) with VSCC received at least one targeted therapy at our institution. Previously, one or more platinum-based chemotherapy was applied to all patients [median 3.5 previous lines (range 2–5)]. In the erlotinib subgroup, two of five patients (40%) achieved stable disease (SD), while two patients (2/5, 40%) experienced partial response (PR). Treatment was given as monotherapy in second/third line for a median of 3.4 months (range 2–6 months). Bevacizumab (n = 9) was given as maintenance therapy after platinum-based first-line chemotherapy (9/9); best response was complete response (CR) (n = 2/9 22.2%). Median duration of treatment was 7 months (range 4–13 months) with two patients still under ongoing treatment. Best response in the pembrolizumab (n = 3) subset was SD (n = 1/3 33%). Treatment was given as monotherapy in second/third line for a median of 3.3 months (range 3–4 months). Nine of 12 patients (75%) experienced treatment-related adverse events (TRAEs), most commonly grade 1/2. Rapidly evolving antibody treatments have proven clinical benefit especially in HPV-driven tumor entities; however, clinical investigations in VSCC are still limited. These reported cases provide evidence for the clinical utility and feasibility while ensuring an acceptable safety profile.

Key words: Vulvar cancer (VC); Targeted therapy; EGFR targeting; VEGF signaling pathway; Immuno-oncology

INTRODUCTION

Despite still being a rare disease of mostly elderly patients (median age of 70 years at diagnosis), the incidence of vulvar cancer (VC) is constantly on the rise to currently 3–5/100,000/year in Europe, whereas the age of onset is decreasing[1–3]. Almost 90% of VC are vulvar squamous cell cancer (VSCC); however, they remain clinically and pathologically heterogeneous. So far, two etiologies have been proposed, human papillomavirus (HPV)-associated and HPV-independent disease[4]. Approximately 40% of VSCC are related to high-risk HPV infections characterized by p16 overexpression, mostly arising in younger women. The majority of VSCC evolves based on HPV-independent pathways, often harboring *TP53* mutations, preferably affecting postmenopausal patients[5]. Furthermore, a third subtype (p16-/p53-) has just recently been suggested based on the AGO-CaRE-1 translational data[6]. Prognosis is mainly determined by the tumor stage at initial diagnosis[7–11]. Both overall survival (OS) and progression-free survival (PFS) are strongly dependent on nodal involvement (3-year PFS rate of 35.2% and OS rate of 56.2% in node-positive patients compared

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to 75.2% and 90.2% in node-negative patients)[9,12,13]. In case of recurrent or metastasized disease not amenable to radiotherapy or radical surgery, therapeutic options are extremely limited. Especially after first-line treatment with platin-based combination regimens, response rates to the often used monochemotherapies are poor and range 0–15%[14,15]. Determination of the best therapeutic regimen with the least toxicity is difficult as there are only very few studies with heterogeneous populations. Current recommendations therefore rely on scarce and often controversial evidence instead of randomized data.

Consequently, no improvement in survival could be achieved in the last two decades for locally advanced, recurrent, or metastatic disease—as reflected in a 1-year survival rate of only 15–30%[16]. A targeted approach to treatment has become of high clinical and scientific interest in order to improve therapeutic options. However, only little is known regarding underlying genetic and molecular alterations in VC[17,18]. Current therapeutic targets of interest are therefore mainly adopted from other entities like head and neck cancer and cervical cancer and focus on the epidermal growth factor receptor (EGFR) signaling cascade, VEGF-/angiogenesis-related markers, as well as immune checkpoints[4]. However, with the exception of erlotinib, data on the efficacy of these therapies in VC is very limited. We therefore analyzed a small cohort of patients with advanced VC treated with targeted agents at our own institution and conducted a review of literature, summarizing the emerging data.

MATERIALS AND METHODS

Patients

Between 2013 and 2019, n = 291 patients with VSCC were treated at our gynecologic oncology center. A retrospective evaluation regarding the application of targeted therapy was performed. Targeted therapy was recommended to a total of 16 patients (5.5%) with recurrent or metastasized VSCC not amenable to radical surgery or definitive radiotherapy, and 12 patients (4.1%) finally received one or more of the of the following drugs: erlotinib, bevacizumab, or pembrolizumab (Table 1). The remaining four patients chose different therapeutic options due to deterioration, or their health insurance did not cover the cost of treatment. Before the treatment was applied, all patients had received one or more prior lines of platinum-based chemotherapy. Duration of response, treatment tolerance, time to progression, and time to death after the beginning of targeted treatment was evaluated. Therefore, medical charts and pathological reports were reviewed. Previously, informed consent had been obtained from all included patients according to our investigational review board and ethics committee guidelines (Ethics Committee of the Medical Board Hamburg reference number 190504). Drug-related side effects were evaluated according to the National Cancer Institute Common Terminology for adverse events, CTCAE version 4.0. The following methods have been applied to classify the expression of the different molecular targets. In order to predict the responsibility to PD-L1 antagonist pembrolizumab, the combined positivity score (CPS) was evaluated—a score that represents the number of PD-L1 staining cells divided by the total number of viable tumor cells, multiplied by 100. According to the FDA approval criteria for pembrolizumab, a combined positivity score (CPS) ≥1 is mandatory. EGFR mutational status was analyzed by PCR, and HPV status was classified by analyzation of proliferation markers such as p16. The prognostic role of the HPV status and the use of immunohistochemical p16 overexpression as surrogate marker of HPV-induced transformation in VSCC are discussed controversially[19,20]; however, a recent study revealed a significant correlation between detection of HPV DNA and p16 overexpression ($p < 0.001$) in patients with VSCC. Furthermore, a significant correlation between p16 status and tumor stage ($p = 0.003$) could be observed as well as the association between p16 overexpression and higher tumor stage (>T2)[21]. Accordingly, in other squamous cell carcinomas, especially in oropharyngeal and anal cancers, overexpression of p16 by immunohistochemical staining (IHC) has been shown to be associated significantly with HPV positivity by PCR or in situ hybridization[22,23]. Furthermore, p16 overexpression has been found to be of independent prognostic value for the response to radiation treatment[24–26]. In accordance with oropharyngeal squamous cell carcinomas, scoring criteria for p16 in our study were no expression (negative), weak expression (<30% positive cells), moderate expression (31–50% positive cells), and strong expression (>50% positive cells). Samples scored as moderate or higher were considered as positive for p16[27].

CASE SERIES AND REVIEW OF THE LITERATURE

Medline (Pubmed), EMBASE, Web of Science, Scopus, and OVID were searched for articles on targeted therapy in VC independent of publication date. We selected only studies reporting on VSCC. Search terms were “vulvar cancer” AND “targeted therapy,” “bevacizumab,” “VEGF,” “erlotinib,” “EGFR,” “pembrolizumab,” “checkpoint inhibitor,” or “immunotherapy.” In addition, we paired the search term “vulvar cancer” with different molecular markers involved in cell cycle, apoptosis, and angiogenesis.

Table 1. Summary of All Patients Who Received One or More Targeted Therapeutic Agents

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age at FD	74	42	58	46	57	37
First-line therapy/prior treatment before targeted treatment	1. Surgery, adj. RT; 2. Adj. RCTX; 3. CTX	1. Surgery, adj. RCTX; 2. Surgery, RCTX	1. Surgery; 2. RT; 3. CTX	1. Surgery, adj. RT; 2. Surgery; 3. CTX	1. Surgery, adj. RT; 2. Surgery, RCTX; 3. CTX	1. Surgery, RCTX; 2. Surgery; 3. Surgery; 4. CTX
Targeted therapeutic agents (in applied order)	1. Bevacizumab 2. Erlotinib	1. Erlotinib 2. Bevacizumab	Erlotinib	Erlotinib	Erlotinib	1. Bevacizumab 2. Pembrolizumab
HPV status/EGFR Mut/PDL-1 status	HPV unknown/EGFR unknown	HPV unknown/EGFR wild type (PCR)	HPV unknown EGFR wild type (PCR)	HPV negative EGFR wild type (PCR)	HPV negative (p16−)/EGFR wild type (PCR)	HPV negative; PD-L1: CPS 1-5
Best response	Bevacizumab: PR Erlotinib: SD	Erlotinib: PR; Bevacizumab: PD	SD	PD	PR	Bevacizumab and pembrolizumab: PD
Side effects	Bevacizumab: grade 3 CTCAE: high blood pressure Erlotinib: grade 1 CTCAE: exanthema	Bevacizumab: no Erlotinib: grade 2 CTCAE: diarrhea, elevated liver enzymes, skin alterations (facial comedo)	Grade 2 CTCAE: Skin problems, elevated liver enzymes	Grade 3 CTCAE: diarrhea with C. difficile infection, elevated liver enzymes Grade 2 CTCAE: skin problems (cutaneous rhagades)	None	Bevacizumab: grade 2 CTCAE: high blood pressure Pembrolizumab: grade 2 CTCAE: fatigue, lymphedema
Dose reduction	Bevacizumab: yes, due to high blood pressure resistant to therapy; Erlotinib: no	Erlotinib and Bevacizumab: no	No	Yes, from 150 to 100 mg due to elevated liver enzymes	No	Bevacizumab and pembrolizumab: no
Time to progression (months)	Bevacizumab: 4 Erlotinib: 2	Erlotinib: 3; Bevacizumab: 4	6	2	4	Bevacizumab and pembrolizumab: 4
Time to death from FD (months)	Unknown	36	19	47	Unknown	Unknown
Cause of death	Unknown	Tumor progression	Tumor progression	Kidney failure, tumor progression	Unknown	Unknown

Table 1 (Continued)

	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Age at FD	61	26	64	58	49	52
First-line therapy/prior treatment before targeted treatment	1. Surgery; 2. CTX	1. Surgery, RCTX; 2. Surgery; 3. CTX	1. Surgery; 2. Surgery; 3. CTX	1. Surgery; 2. RCTX; 3. Surgery + CTX; 4. Surgery; 5. RCTX 6. CTX	1. Surgery, adj. local ablative RT; 2. RT; 3. stereotactic irradiation; 4. CTX	1. Surgery; 2. RCTX; 3. CTX
Targeted therapeutic agents (in applied order)	1. Bevacizumab 2. Pembrolizumab	1. Bevacizumab 2. Pembrolizumab	Bevacizumab	Bevacizumab	Bevacizumab	Bevacizumab
HPV status/EGFR Mut/PDL-1 status	HPV unknown/PD-L1 CPS 60	HPV negative/PD-L1 CPS unknown	HPV negative	HPV unknown	HPV unknown	HPV unknown
Best response	Bevacizumab: CR Pembrolizumab: SD	Bevacizumab: PD Pembrolizumab: PD	SD	CR	SD	SD
Side effects	Bevacizumab: peri-cardial effusion Pembrolizumab: none	Bevacizumab: grade 3 CTCAE: deep vein thrombosis Pembrolizumab: grade 2 CTCAE: lymphedema, hypothyroidism				
	Grade 2 CTCAE: diarrhea, lym-phedema, high blood pressure	Grade 3 CTCAE: arterial bleed-ing right groin 2 months after end of bevacizumab	None	None		
Dose reduction	Bevacizumab and pembrolizumab: no	Bevacizumab: yes, end of bevacizumab due to deep vein thrombosis Pembrolizumab: no	No	No	No	No
Time to progression (months)	Bevacizumab: 13 Pembrolizumab: 3	Bevacizumab: 6 Pembrolizumab: 3	Ongoing treatment	12	Ongoing treatment	6
Time to death from FD (months)	Unknown	40	NA	48	NA	Unknown
Cause of death	Unknown	Tumor progression	NA	Tumor progression	NA	Unknown

adj., adjuvant; CPS, combined positive score; CTCAE, Common Terminology Criteria for Adverse Events; EGFR, epidermal growth factor receptor; HPV, human papilloma virus; loc, local, dist, distant; rec, recurrence; PD, progressive disease; RD, recurrent disease; SD, stable disease; PR, partial response; FD, first diagnosis; CTX, chemotherapy; RT, radiotherapy; CRTX, chemoradiation.

EGFR Targeting: Erlotinib

The EGFR is expressed on the surface of both normal and cancer cells and represents a key member of the family of receptor tyrosine kinases (TK), involved in cellular proliferation, migration, and differentiation. Being an EGFR inhibitor, erlotinib reversibly and selectively blocks EGFR-TK activity, leading to inhibition of intracellular phosphorylation and prevention of further downstream signaling. As a result, cell death is induced, while dissemination of tumor cells is reduced. The most commonly reported drug-related adverse reactions (>20%) are rash, fatigue, dyspnea, cough, nausea, and diarrhea. Erlotinib is applied orally, and recommendations regarding the dosage vary between 100 and 150 mg/day[28].

Increased expression of EGFR has been detected in 40–67% of all VC[29], and EGFR copy number increase was observed in 39.9%[30]. Moreover, amplification of EGFR is suggested to be associated with advanced tumor stages ($p < 0.001$), lymph node metastases ($p = 0.02$), and HPV negativity ($p = 0.04$) in VSCC[30]. In a prospective phase II trial with erlotinib, Horowitz et al. enrolled a total of 41 patients with VC either suitable for surgery or chemoradiation[31]. No information regarding the tumors' mutational status or FISH results for amplification of EGFR were made available in the publication. Patients received erlotinib 150 mg orally per day; 28 days of treatment were considered to be one cycle. A remarkable clinical benefit rate of 67.5% was observed including 27.5% partial response (PR) and 40% stable disease (SD). Besides these promising results, a relatively short response duration with a median of 3 months was revealed, while toxicity with $n = 10$ grades 3 and 4 adverse events including renal failure ($n = 2$) was serious. However, the authors concluded that given the poor prognosis and the lack of treatment options in recurrent or metastasized VSCC, blocking the EGFR-signaling pathway by erlotinib may serve as one of the most promising therapeutic approaches available in this indication[31].

In addition, encouraging results regarding the effect of erlotinib have been described in a few selected cases. Olawaiye et al. enrolled two elderly patients with locally advanced VC who were treated with oral erlotinib 150 mg/day; both patients experienced substantial clinical benefit with one CR and one PR[32]. More recently, another case of a 76-year-old patient with recurrent VSCC FIGO stage IIIA was published first showing a long-term response of 9 months under treatment with erlotinib[33].

In our case series, five patients were treated with erlotinib (Table 2). All patients had received platin-based chemotherapy before and received erlotinib as second/third line treatment. Median age at treatment was 56 years (range 42–74). In 4/5 of patients (80%), EGFR mutational status analyzed by PCR were negative. Best responses were partial response (PR) in 2/5 of patients (40%) and stable disease (SD) in 2/5 of patients (40%). Median time to progression was 3.4 months (range 2–6). During the treatment with erlotinib, the patients experienced diarrhea, grade 2 (n = 1) and grade 3 including one *Clostridium difficile* infection. Further clinically relevant side effects were skin problems: facial comedo (n = 1), cutaneous rhagades (n = 1), or exanthema (n = 2), and elevated liver enzymes (n = 3)—in this context, dose reduction from 150 to 100 mg was necessary in one patient.

VEGF-Signaling Pathway: Bevacizumab

The rationale using antiangiogenetic treatment in cancer is supposed to depend on the presence of hypoxia in cancer tissue; the reduction of oxygen induces the transcription of the vascular endothelial growth factor receptor (VEGF-R). Subsequently, binding of VEGF to its receptors induces angiogenesis in the form of endothelial cell proliferation and new blood vessels. The inhibition of microvascular growth is therefore supposed to reduce the growth of all tissues, including metastatic tissue. Hence, the VEGF signaling pathway serves as an important mediator of tumor angiogenesis, an event directly correlating with the extent of disease and inversely correlating with survival.

The most commonly reported adverse reactions (>10%) caused by bevacizumab are epistaxis, headache, hypertension, proteinuria, and dry skin. Recent warnings furthermore include gastrointestinal perforations and fistula, wound healing complications, as well as arterial and venous thromboembolic events. Bevacizumab is administered as an intravenous infusion preferably every 3 weeks, and the recommended dosage usually ranges 10–15 mg/kg[34].

Angiogenesis inhibitors like bevacizumab have been approved by the FDA for treatment in various cancer types (e.g., colorectal cancer, non-small cell lung cancer, and glioblastoma). Regarding gynecologic malignancies, bevacizumab has shown promising results especially in cervical and also in ovarian cancer. The approval of bevacizumab for women with recurrent and metastatic cervical cancer was granted in 2014 based on the second interim analysis of the phase III Gynecologic Oncology Group (GOG) 240 trial[35]. Herein, the addition of bevacizumab to combination chemotherapy consisting of cisplatin and paclitaxel extended median OS by 3.7 months (17.0 vs. 13.3 months; HR: 0.71; 98% CI 0.54–0.95, $p = 0.004$) and resulted in higher response rates (48% vs. 36%, $p = 0{,}008$). More recently, in June 2017, the final OS analysis of GOG-240 was published by Tewari et al. and showed continued benefit of the addition of bevacizumab to chemotherapy in patients with metastatic, persistent, or recurrent cervical carcinoma (median OS of 16.8 months vs 13.3 months, HR = 0.77, $p = 0.007$)[36].

Table 2. Erlotinib

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at FD	74	42	58	46	57
First diagnosis (MM/YY)	11/17	07/12	01/13	05/09	10/15
History of disease/time to recurrence (months)	1. surgery, adj. RT/6; 2. loc rec > surgery R0/2; 3. loc rec -> surgery, R0, adj RCTX; 3. loc rec under ongoing RT -> CTX carboplatin/paclitaxel/ bevacizumab/4; 4. dist rec -> erlotinib	1. surgery, adj. RCTX/8; 2. local and dist rec(parallel) -> surgery, RCTX/4; 3. loc PD -> erlotinib/3; 4. Loc dist. PD -> CTX/4 (carboplatin/ paclitaxel/bevacizumab)	1. surgery -> R0/9; 2. loc rec -> surgery, RT/6; 3. distant rec -> CTX carboplatin/paclitaxel/5; 4. dist rec -> erlotinib/6; 5. dist PD -> best supp care	1. surgery, adj RT/12; 2. loc rec -> surgery R0/10; 3. dist rec -> CTX cisplatin/ topotecan/6; 4. dist PD - > CTX paclitaxel/4; 5. dist PD -> erlotinib/2; 6. dist rec -> best supp care	1. surgery, adj. RT/10; 2. local and dist rec -> surgery, RCTX (cisplatin)/4; 3. dist PD -> CTX paclitaxel/3; 4. loc PD -> erlotinib/4; 5. loc PD -> elec-trochemotherapy + erlotinib
Disease at indication (tumor load)	Local PD (left groin)	Local PD (right groin)	Distant metastasis (bone)	Distant metastasis (liver, bone)	Local PD (left groin)
HPV status/EGFR Mut (HPV+/p16)	HPV unknown/EGFR unknown	HPV unknown/EGFR wild type (PCR)	HPV unknown/EGFR wild type (PCR)	HPV negative/EGFR wild type (PCR)	HPV negative (p16−)/ EGFR wild type (PCR)
Best response	SD	PR	SD	PD	PR
Side effects	Grade 1 CTCAE: exanthema	Grade 2 CTCAE: diarrhea, elevated liver enzymes, skin alterations (facial comedo)	Grade 2 CTCAE: Skin problems, elevated liver enzymes	Grade 3 CTCAE: diarrhea with *C. difficile* infection, elevated liver enzymes grade 2 CTCAE: skin problems (cutaneous rhagades)	None
Dose reduction	No	No	No	Yes, from 150 to 100 mg due to elevated liver enzymes	No
Time to progression	2 months (8 weeks)	3 months (12 weeks)	6 months (24 weeks)	2 months (8 weeks)	4 months (16 weeks)
Time to death from FD	Unknown	36 months	19 months	47 month	Unknown
Cause of death	Unknown	Tumor progression	Tumor progression	Kidney failure, tumor progression	Unknown

adj., adjuvant; CTCAE, Common Terminology Criteria for Adverse Events; EGFR, epidermal growth factor receptor; HPV, human papilloma virus; loc, local; dist, distant; rec, recurrence; MM/YY FD, month/year of first diagnosis; PD, progressive disease; RD, recurrent disease; SD, stable disease; PR, partial response; FD, first diagnosis; CTX, chemotherapy; RT, radiotherapy; CRTX, chemoradiation; R0, tumor free margins; R1, microscopic tumor residual.

To date, antiangiogenic treatment has not been investigated in VC. However, there are a few analyses examining the role of the VEGF pathway in this rare disease. According to the data from previous studies, VEGF is supposed to be prognostically relevant in VC as serum concentration of VEGF protein is associated with tumor stage[37] and patients with increased VEGF expression were reported to have significantly worse OS rates[38].

Based on the data mentioned above, we recommended bevacizumab to nine of our patients (Table 3). All of these patients received bevacizumab concomitantly to, and as maintenance approach after, platinum-based combination therapy. Therefore, isolated response to bevacizumab cannot be reported. Median age at treatment was 51.4 years (range 26–74). Median time to progression was 28 weeks (range 16–52 weeks), while two patients are still under ongoing treatment and are doing well. Best response was CR in 2/9 of cases (22.2%) followed by PR in 1/9 of cases (11.1%), and SD in 3/9 of cases (33.3%). In 2/9 of cases (22.2%), treatment with bevacizumab had to be stopped due to thromboembolic event and elevated blood pressure resistant to therapy.

Checkpoint Inhibition: Pembrolizumab

Pembrolizumab is a monoclonal, programmed cell death 1 (PD-1) binding antibody on the surface of activated T cells specifically blocking the interaction between PD-1 and programmed death ligand 1 (PD-L1), predominantly found on tumor cells of several cancer types. Thereby, T-cell proliferation is enhanced and PD-1 pathway-mediated inhibition of the adaptive immune response is released. Overexpression of PD-L1 as well as high microsatellite instability (MSI-H) seem to be predictive factors regarding the response to targeted PD-1/PD-L1 inhibitors in a variety of tumor types[39–41]; however, in other studies, no positive association between PD-L1 expression and response to immune checkpoint inhibitors or OS could be observed[42]. In VC, PD-L1 expression has been found in 12/103 of patients with VC (11.65%), and association with HPV negativity as well as with poor prognosis was observed (recurrence-free survival HR: 3.029 CI 1.228–8.471, $p = 0.0018$)[43]. However, more recently published data showed a statistically significant correlation between PD-L1 expression and low tumor stage, but association with the HPV status or OS in patients with VC could not be confirmed[44,45].

The most frequent pembrolizumab-related side effects (reported in ≥20% of patients) are fatigue, musculoskeletal pain, and pruritus in case it is applied as a single agent; when given in combination with chemotherapy, nausea, constipation, diarrhea, rash, cough, and peripheral neuropathy are reported to have arisen the most. In addition, one should also be aware of immune-mediated complications such as pneumonitis, colitis, hepatitis, as well as endocrinopathies when treating patients with PD-1/PD-L1 antagonists. Pembrolizumab is administered as an intravenous infusion over 30 min[46], and the recommended dosage varies between 200 and 300 mg q3w (every 3 weeks).

Given the similarities especially in HPV-associated tumorigenesis, the recent approval of pembrolizumab in cervical as well as head and neck cancer (HNSCC) might be seen as a predictor for efficacy in VC. Efficacy results in patients with recurrent or metastatic cervical cancer in the KEYNOTE-158 study revealed an ORR of 14.3% in PD-L1-positive disease (95% CI: 7.4%, 24.1%) with complete and partial response rates of 2.6% and 11.7%, respectively[47]. According to the FDA approval criteria for pembrolizumab, a combined positivity score (CPS) ≥1 is mandatory—a score that represents the number of PD-L1 staining cells divided by the total number of viable tumor cells, multiplied by 100. In June 2019, pembrolizumab was furthermore approved for the treatment of patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy based on the results of the phase III KEYNOTE-048 trial[48]. Herein, pembrolizumab/chemotherapy achieved superior OS in patients with PD-L1 CPS ≥1 disease and the total population with comparable safety; furthermore, pembrolizumab (alone) reached superior OS rates in the CPS ≥1 population, as well as noninferior OS in the total population with favorable safety.

As of today, only a few studies have been published evaluating the therapeutic impact of pembrolizumab in patients with advanced VC. Recently, Shields et al. reported a case of a 61-year-old patient with recurrent VC who was successfully treated with pembrolizumab for the first time[49]. In order to identify patients with potentially higher likelihood of response to anti-PD-L1 therapies, the KEYNOTE-028 trial most recently evaluated 471 patients with over 20 solid cancer types regarding PD-L1 expression, T-cell-flamed gene expression (GEP), and tumor mutational burden (TMB)[50]. Eighteen patients with advanced VC and PD-L1$^+$ tumors were treated with pembrolizumab 10 mg/kg every 2 weeks for 2 years or until confirmed disease progression or unacceptable toxicity occurred. The primary end point was ORR, while the second end points included PFS, OS, and safety. For the cohort of heavily pretreated VC patients, the ORR was 6% with a median PFS of 3.1 months and relatively short median duration of OS with 3.8 months. PD-L1 expression by CPS was available for eight VC patients, and statistical testing revealed significant correlation between PD-L1 CPS and both ORR ($p = 0.018$) and PFS ($p = 0.005$).

In our case series, three patients with median age of 41 years (range 26–61) received pembrolizumab (Table 4). All were heavily pretreated; 2/3 patients (66.6%) had

Table 3. Bevacizumab

	Patient 6	Patient 7	Patient 8	Patient 9	Patient 1
Age at FD	37	61	26	64	74
MM/YY FD	11/17	03/16	04/15	11/17	11/17
History of disease/ time to recurrence (months)	1. surgery, RCTX/5; 2. loc rec -> surgery/2; 3. loc rec -> surgery/3; 4. loc rec -> CTX (carboplatin/ paclitaxel/ bevacizumab)/4; 5. loc and dist rec-> ctx with mitomycin/ capecitabine/3; 6. loc PD: pembrolizumab/4; 7. loc and dist PD: best supp care	1. surgery, RT/11; 2. dist rec -> CTX (cisplatin/ topotecan)/9; 3. loc PD -> CTX (paclitaxel/ bevacizumab)/13; 4. dist rec -> pembrolizumab/3; 5. dist PD -> best supp care	1. surgery, RTX/10; 2. loc rec -> surgery R0/3; 3. dist rec -> CTX (carboplatin/paclitaxel/ bevacizumab)/6; DVT: end of bevacizumab, start pembrolizumab/3; 4. dist. rec -> CTX vinorelbine	1. surgery/11; 2. loc rec -> surgery (R1) -> RT/10; 3. loc and dist rec -> CTX (carboplatin/ paclitaxel/ bevacizumab)	1. surgery, adj. RT/6; 2. loc rec -> surgery R0/2; 3. loc rec -> surgery, R0, adj RCTX; 4. loc rec under ongoing RT -> CTX carboplatin/paclitaxel/ bevacizumab/4; 5. dist rec -> erlotinib
Disease at indication (tumor load)	Local PD (Vulva)	Local PD (Vulva)	Distant metastasis (bone)	Local and distant PD (right groin, liver)	Local PD (vulva)
HPV status	Unknown	Unknown	Unknown	Negative (p16–)	Unknown
Best response	PD	CR	PD	SD	PR
Side effects	Grade 2 CTCAE: high blood pressure	Grade 2 CTCAE: pericardial effusion	Grade 3 CTCAE: DVT	Grade 2 CTCAE: diarrhea, lymphedema, high blood pressure	Grade 3 CTCAE: high blood pressure
Dose reduction	No	No	Yes, end of bevacizumab (DVT)	No	Yes, end of bevacizumab (high blood pressure resistant to therapy)
Time to progression	4 months	13 months	6 months	Ongoing treatment	4 months
Time to death from FD	Unknown	Unknown	40 months	Unknown	Unknown
Cause of death	Unknown	Unknown	Tumor progression	Unknown	Unknown

Table 3 (Continued)

	Patient 2	Patient 10	Patient 11	Patient 12
Age at FD	42	58	49	52
MM/YY FD	07/12	03/16	01/16	01/18
History of disease / time to recurrence (months)	1. surgery, adj. RCTX/8; 2. local and dist rec/4 (parallel) -> surgery, RCTX; 3. loc PD -> erlotinib/3; 4. Loc dist. PD -> CTX (carboplatin/ paclitaxel/bevacizumab)/4	1. surgery/10; 2. loc rec -> RCTX/6; 3. loc rec -> surgery (R0) + CTX (cisplatin, paclitaxel)/10; 4. loc rec -> surgery (R1) -> RCTX/7; 5. loc PD -> CTX (carboplatin/ paclitaxel/bevacizumab)/12; 6. loc PD -> CTX (paclitaxel mono)	1. surgery, adj. local ablative RT/8; 2. dist rec -> RT/7; 3. dist rec -> stereotactic irradiation/9; 4. dist PD -> CTX cisplatin/ paclitaxel/bevacizumab	1. surgery -> RCTX/7; 2. loc rec -> CTX (carboplatin/paclitaxel/bevacizumab)/6 carboplatin/paclitaxel intolerance, bevacizumab maintenance; 3. loc rec -> olaparib (known BRCA mutation)
Disease at indication (tumor load)	Local and distant PD (right groin, skin)	Local PD (right groin)	Distant metastasis (lung, liver)	Local PD (left groin)
HPV status	Unknown	Unknown	Unknown	Unknown
Best response	PD	CR	SD	SD
Side effects	None	Grade 3 CTCAE: arterial bleeding right groin 2 months after end of bevacizumab	None	None
Dose reduction	No	No	No	No
Time to progression	4 months	12 months	Ongoing treatment	6 months
Time to death from FD	36 months	48	NA	Unknown
Cause of death	Tumor progression	Tumor progression	NA	Unknown

adj., adjuvant; CTCAE, Common Terminology Criteria for Adverse Events; HPV, human papilloma virus; loc, local; dist, distant; rec, recurrence; MM/YY FD, month/year of first diagnosis; PD, progressive disease; RD, recurrent disease; SD, stable disease; CR, complete response; PR, partial response; FD, first diagnosis; CTX, chemotherapy; RT, radiotherapy; CRTX, chemoradiation; R0, tumor-free margins; R1, microscopic tumor residual; DVT, deep vein thrombosis.

Table 4. Pembrolizumab

	Patient 6	Patient 7	Patient 8
Age at FD	37	61	26
MM/YY FD	11/17	03/16	04/15
History of disease/time to recurrence (months)	1. surgery, RCTX/5; 2. loc rec -> surgery/2; 3. loc rec -> surgery/3; 4. loc rec -> palliative CTX (carboplatin/paclitaxel/bevacizumab)/4; 5. loc and dist rec-> ctx with mitomycin/capecitabine/3; 6. loc PD: pembrolizumab/4; 7. loc and dist PD: best supp care	1. surgery, RT/11; 2. dist rec -> CTX (cisplatin/topotecan)/9; 3. loc PD -> CTX (paclitaxel/bevacizumab)/13; 4. loc rec -> pembrolizumab/3; 5. dist PD -> best supp care/erlotinib;	1. surgery, RTX/10; 2. loc rec -> surgery (R0)/3; 3. dist rec -> CTX (carboplatin/paclitaxel/bevacizumab)/6, DVT: end of bevacizumab, start pembrolizumab/3; 4. dist rec -> ctx vinorelbine; 5. erlotinib
Disease at indication (tumor load)	Local PD (left groin, vulva)	Local PD (left groin)	Distant metastasis (bone)
HPV status/PD-L1 status	HPV negative/PD-L1: CPS 1-5	HPV unknown/PD-L1 CPS 60	HPV negative/PD-L1 CPS unknown
Best response	PD	SD	PD
Side effects	Grade 2 CTCAE: fatigue, lymphedema	None	Grade 2 CTCAE: lymphedema, hypothyroidism
Dose reduction	No	No	No
Time to progression	4 months	3 months	3 months
Time to death from FD	Unknown	Unknown	40 month
Cause of death	Unknown	Unknown	Tumor progression

adj., adjuvant; CPS, combined positive score; CTCAE, Common Terminology Criteria for Adverse Events; HPV, human papilloma virus; loc, local; dist, distant; rec, recurrence; MM/YY FD, month/year of first diagnosis; PD1-L1, programmed cell death ligand; PD, progressive disease; RD, recurrent disease; SD, stable disease; CR, complete response; PR, partial response; FD, first diagnosis; CTX, chemotherapy; RT, radiotherapy; CRTX, chemoradiation; R0, tumor-free margins; R1, microscopic tumor residual; DVT, deep vein thrombosis; CPS, combined positive score.

HPV negative and PD-L1 positive (CPS 1 and CPS 60, respectively) tumors. Median time to progression was 3.3 months (range 3–4), and the best response rate was SD in one patient (33.3%), while the remaining two patients experienced progressive disease (66.6%). However, tolerance was fairly good as only one patient suffered from moderate hypothyroidism induced by pembrolizumab.

DISCUSSION

Although considerable improvement in the surgical management of VC was obtained within the last two decades, these achievements could not have been mirrored in the treatment for patients with advanced or metastasized VC. As mentioned in the National Comprehensive Cancer Network (NCCN) guidelines, treatment in recurrent settings strongly depends on the localization of the recurrence as well as on previous treatment[51]. Subsequent surgery and (chemo)radiation can be considered in case of local recurrence. However, mutilating results due to radical surgeries and higher cutaneous toxicity as well as elevated complication rates for surgery following (chemo)radiation eventually lead to increased morbidity and reduced quality of life in these often already elderly patients[52]. In patients not amenable to surgery or radiotherapy, systemic approach to treatment should be taken into consideration. However, as of today, no standard chemotherapy regimens exist for recurrent or metastatic VC. The NCCN guidelines therefore preferably suggest treatments applied in other HPV-driven cancers, mainly cervical cancer, including cisplatin, paclitaxel, mitomycin-C, 5-fluorouracil, and vinorelbine. Paclitaxel weekly has shown only slight activity in a phase II trial of 31 VC patients represented in an RR of 14%, PFS of 2.6 months, and median OS of 6.8 months, indicating a lower effectiveness in single-agent treatment in comparison to a platinum-based combination therapy[53]. Furthermore, chemotherapy in a recurrent setting appeared to be less effective than in a neoadjuvant setting as patients are mostly pretreated, and recurrence in previously treated fields is common[13,52,54,55]. Moreover, chemotherapy has proven to be less effective in VC compared with other HPV-induced tumor entities[53]. Nevertheless, the treatment of choice in primary recurrence is more or less standardized in the form of platinum-based (combination) chemotherapy, whereas in second-line settings, standardized treatment recommendations are lacking. In this context, as second-line treatment option, targeted agents have become of increasing clinical and scientific interest.

Especially, EGFR has been studied extensively and seems to be one of the most promising targets for HPV-independent VC when EGFR gene amplifications is observed[31]. Whereas Johnson et al. demonstrated better survival in patients with low EGFR levels compared with patients with high EGFR levels (DFS of 25% in patients with EGFR levels >90% vs. DFS of 54% in patients with EGFR levels <90%)[29], a study of EGFR expression in 197 patients showed an association between high EGFR protein expression and increased depth of invasion as well as the presence of lymph node metastases (OR 2.12, 95% CI 1.09–4.10)[56]. Besides confirming these data by pointing out the relationship between EGFR overexpression, high tumor stage, and the number of metastatic lymph nodes ($p<0.001$, $p=-0.02$, respectively), an analysis of 183 patients furthermore revealed a statistical correlation between EGFR protein expression and EGFR gene copy numbers as well as significant association between EGFR overexpression and HPV negativity ($p<0.05$, $p=0.04$, respectively)[30]. Growdon et al. additionally determined that high levels of EGFR amplification are linked to poor OS in VC ($p<-0.025$)[57], and results from a study published by Dong et al. underline these findings by showing a negative correlation between EGFR expression and p16 and a positive association between p53 and EGFR[58]. Given the increased expression of EGFR in VC (40–67%) and its potential association to faster progression of the disease, anti-EGFR-targeted therapies are of high therapeutic interest in a subset of advanced VC. However, all this information provides only limited use when it comes to anticipate the response to EGFR-targeted treatment as especially protein expression does not serve as a reliable marker in this setting[31]. As known from other entities (e.g., lung cancer and HNSCC), immunohistochemistry of EGFR is difficult and not suitable to predict response to treatment, which is usually performed by mutational analysis. Therefore, in VC, the detection of EGFR mutation status has increasingly become of clinical interest as a molecular predictor of response to treatment with significant impact on prognosis. In order to determine whether EGFR TKIs have different efficacies in patients with and without EGFR mutations, Liu et al. enrolled 30 patients with advanced VC, performed EGFR genetic testing, and evaluated the clinical efficacy in both patients with and without EGFR mutation[59]. Treatment consisted of oral gefitinib (250 mg once daily), another anti-EGFR-targeted agent; the mutation rate was 30% (9/30), and EGFR wild-type (wt) patients accounted for 70% (21/30). The results demonstrated statistically significant higher efficacy of gefitinib in patients with EGFR mutations compared with patients with wt-EGFR (ORR 44.5% vs 14.3%, $p=0.096$; median PFS 108 vs. 49 days $p=0.42$), suggesting that targeted therapy based on EGFR mutation status might improve the prognosis of patients with advanced VC[59]. In addition, antibodies against the EGF receptor like cetuximab have been reported to be associated with increased clinical benefit in patients with advanced VC when combined with cisplatin chemotherapy and radiotherapy (PR 5 months)[60]. These findings underline the potential utility of EGFR inhibitors

as single agent treatment or in combination with chemotherapy as a promising therapeutic approach. Therefore, further investigations may also focus on the evaluation of combining anti-EGFR targets with chemoradiation, other targeted therapies (antiangiogenic or PI3K inhibitors), or cytotoxic agents in order to improve the outcome in a subset of patients with advanced VC.

PD-L1 expression has been detected in up to 73% of tumors in VC, and moderate or strong expression was revealed in 27%[61]. As these data confirm PD-L1 overexpression in a substantial subset of patients with VC in all stages and independent of HPV, immune checkpoint inhibition (ICI) serves as another suitable therapeutic target. Currently, pembrolizumab has been one of the best investigated agents in this context[50]. As mentioned earlier, the KEYNOTE-028 recently evaluated PD-L1 expression, TMB, and T-Cell GEP in 471 patients treated with pembrolizumab across 20 advanced solid cancer entities presenting with PD-L1$^+$ tumors. A closer look toward gynecologic malignancies revealed rather disappointing results in VC with an ORR of 6% and OS of 3.8 month. Other HPV-driven tumor entities like cervical and anal cancer achieved twice as high results regarding the ORR with 17% and 16%, respectively. Nivolumab is another PD-1 agent currently being under investigation in a phase I/II study in 24 patients with recurrent or metastatic cervical, vaginal, and VC[62]. While preliminary data demonstrated encouraging disease control rates of 70.8% in all three tumor entities, responses were exclusively observed in patients with cervical cancer (ORR 26.3%) regardless of PD-L1 or HPV status or number of prior therapies. As for the available data, nivolumab provided similar results as pembrolizumab in regard to safety and toxicity with 12.5% treatment-related adverse events grade 3 or 4. In the light of these results, the future of immune oncology in VC will not be monotherapy with anti PD-1/L1 antibodies but combined and preferable early treatment in advanced disease. In this context just recently, an interim analysis of the phase I/II Checkmate-358 study has been presented at the ESMO 2019; herein, the combination of nivolumab and ipilimumab (CTLA-4 antagonist) showed durable clinical activity in patients with recurrent or metastatic (R/M) cervical cancer, regardless of tumor PD-L1 expression[63]. Noteworthy, ORR was higher in patients without prior systemic therapies (PST) compared with patients without PST (45.8% vs. 31.6%). As a result, the combination of checkpoint inhibitors could also provide an effective treatment alternative in patients with other HPV-driven cancers at an early point of recurrent/metastastic disease.

In conclusion, the management of advanced VC continues to be challenging, and data from clinical trials regarding therapeutic options are scarce due to the low incidence of the disease. Furthermore, small number of studies, heterogenous patient cohorts, and diverse treatment regiments impede comparing the available data. While the current "state-of-the-art" treatment in primary recurrent settings without radiotherapeutic or surgical options is platin-containing combination chemotherapy, in second-line treatment targeted agents can be used to improve clinical outcome.

To date, erlotinib is the best investigated targeted agent in VC. As high rates of EGFR expression and increased EGFR copy numbers have been found in VC, consequent EGFR mutation testing should be performed to predict treatment response in advanced VC. Bevacizumab was the first targeted agent to improve OS in a gynecologic cancer as shown in the GOG-240 trial; herein, adding bevacizumab to chemotherapy prolonged OS by 3.4 months in patients with cervical cancer. Application of bevacizumab in VC analogous to cervical cancer is feasible, as shown by our case series. However, data confirming the activity in VC will probably never be available. The role of immunooncology for VC will have to be determined in the coming years. Pembrolizumab monotherapy showed only very modest antitumor activity with an ORR of 6% and median PFS duration of 3.1 month in patients with advanced VC and PD-L1$^+$ tumors. Centralized clinical observations, translational research, and new study designs such as basket trials will be needed to individualize therapy by identifying effective molecular and biological markers for subtype characterization, prognosis, and predication of treatment response as well as to reduce the rates of recurrence and concurrently improve the survival.

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