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ORIGINAL PAPER

The roles of PTEN, cMET, and p16 in resistance to cetuximab in head and neck squamous cell carcinoma

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Abstract

There is no established biomarker for cetuximab efficacy in recurrent head and neck squamous cell carcinoma (HNSCC). The aim of the present study was to evaluate the prognostic and predictive impact of PTEN, cMET, and p16 expression in recurrent HNSCC. In this retrospective study, 112 patients with recurrent HNSCC received chemotherapy (CT) alone ($n=37$) or chemotherapy with cetuximab ($n=75$). PTEN, cMET, and p16 protein expression were evaluated by immunohistochemistry. The median overall survival (mOS) for the patients treated with cetuximab + CT versus CT alone was 11.4 months and 7.0 months, ($p=0.949$). The median progression-free survival (mPFS) was 6.2 months versus 3.0 months ($p=0.154$). Patients with PTEN loss exhibited a mOS of 5.8 months versus 10.5 months ($p=0.002$) and a mPFS of 3.2 months versus 4.7 months ($p=0.019$). A multivariate analysis identified an independent association between PTEN loss and OS (HR 2.27; 95% confidence 95% CI 1.27–4.08; $p=0.006$) and with PFS (HR 1.85; 95% CI 1.09–2.99; $p=0.022$). A negative prognostic impact of PTEN loss was observed in the patients treated with cetuximab + CT, and not in the CT only group. Expression of cMET and p16 showed no impact on OS or PFS. The present findings confirm that PTEN is a prognostic factor for metastatic HNSCC and they support further studies of PTEN expression to evaluate its predictive value to cetuximab response.

Keywords Cetuximab resistance · Head and neck squamous cell carcinoma · PTEN · MET · P16 · Predictive factors · Prognostic factors

Introduction

Squamous cell carcinoma (SCC) from the oral cavity, oropharynx, hypopharynx, and larynx will affect 64,690 individuals in the United States and 22,370 individuals in Brazil [1, 2]. Most patients are diagnosed with locally advanced disease and approximately 50% will experience recurrence within the first 3 years of follow-up [3].

Platinum-based chemotherapy (CT) in combination with cetuximab has been considered the standard first-line palliative treatment for head and neck squamous cell carcinoma (HNSCC) for the last 10 years [4]. However, despite the recent extensive molecular characterization of HNSCC [5–7] with identification of the role of human papillomavirus (HPV) in its carcinogenesis and prognosis [8, 9], as well as the emergence of immunotherapy as an effective treatment for platinum refractory HNSCC [10], predictive biomarkers for anti-EGFR therapy remain lacking and the field has advanced little within the past years [11].

Resistance to anti-epidermal growth factor receptor (EGFR) targeted therapy may be due to intrinsic activation of EGFR or a downstream component of the EGFR pathway, such as *PIK3CA* mutations or inactivation of phosphatase and tensin homolog (PTEN). Activation of parallel tyrosine

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kinase receptor pathways, such as hepatocyte growth factor receptor (cMET), may also be responsible for resistance [11]. Amplification of *EGFR* is not related to response to anti-EGFR therapy [12], and *EGFR* mutations with intrinsic activity are very rare events in the mutational landscape of HNSCC [6].

PTEN is a negative regulator of the EGFR-PI3K-Akt-mTOR pathway and low PTEN expression is present in approximately 10–30% of HNSCCs [6, 7, 13–15]. It is associated with worse prognosis in localized disease [16, 17]. We previously demonstrated a relationship between low PTEN expression and worse progression-free survival (PFS) and overall survival (OS) in a cohort of HNSCC patients treated with chemotherapy (CT) plus cetuximab [18].

cMET is a receptor for hepatocyte growth factor and is able to activate downstream effectors of the EGFR pathway [19]. Overexpression of cMET has been associated with more advanced lymph node dissemination [20], worse prognosis in locally advanced disease [21], and in patients treated with CT + cetuximab for incurable recurrences [22]. In contrast, HPV-related HNSCC has a better prognosis both in localized and metastatic disease [8, 23]. Nonetheless, the frequency of HPV-related tumors detected in incurable recurrent disease is low [24], and in phase III anti-EGFR therapy trials for recurrent disease, HPV status could not predict cetuximab benefit [24].

To the best of our knowledge, few biomarker analyses have been published from anti-EGFR phase III trials. Biomarker studies of patients treated with and without cetuximab to better differentiate predictors of response from prognostic biomarkers are also scarce. In Brazil, patients with HNSCC who lack private insurance are treated according to public health standards and they do not have access to cetuximab-containing regimens. The aim of this study was to evaluate predictive biomarkers for cetuximab response in a cohort of HNSCC patients with incurable recurrent disease who were treated with chemotherapy with and without cetuximab.

Materials and methods

Patients

A cohort of 112 patients who received a diagnosis of primary HNSCC in their oral cavity, oropharynx, larynx, or hypopharynx and were treated at the A.C.Camargo Cancer Center (São Paulo, Brazil) between 2007 and 2015 was retrospectively evaluated. All patients presented with recurrent or metastatic disease and were treated with CT with or without cetuximab. Patients without available tumor samples for immunohistochemistry (IHC) analysis were excluded.

Treatment

First-line CT for metastatic disease at our institution involves a combination of 5-fluorouracil (5FU) at a dose of 1000 mg/m^2/day for 4 days plus cisplatin at a dose of 100 mg/m^2; or carboplatin at an area under the curve of 5 mg/ml/min on day 1 and cetuximab at a loading dose of 400 mg/m^2 followed by 250 mg/m^2 weekly. Patients receive a maximum of six cycles of CT, those who achieve at least stable disease status are kept on weekly cetuximab until disease progression or unacceptable toxicity is observed. Patients covered by public health insurance in Brazil do not have access to cetuximab and are usually treated with first-line CT which includes carboplatin at an area under the curve of 5 mg/ml/min plus paclitaxel (175 mg/m^2) on day 1 in 21-day cycles; or a combination of 5FU (1000 mg/m^2/day) that is administered for 4 days plus cisplatin (100 mg/m^2) or carboplatin at an area under the curve of 5 mg/ml/min on day 1 in a 21-day cycle. Other CT regimens that were used as second-line therapy or later lines of therapy included weekly methotrexate (40 mg/m^2) and paclitaxel (60 mg/m^2).

Clinical data

Demographic, clinical, and pathologic characteristics, as well as treatment and outcomes, were collected from the patients' medical records. A history of smoking was recorded if any tobacco use was described in the medical records. Locoregional recurrence was defined as recurrence at the primary site or in the lymph nodes of the neck.

Tissue samples

All available formalin-fixed and paraffin-embedded tumor tissue samples were used to construct a tissue microarray (TMA) as previously described [18].

Immunohistochemistry

TMA sections were stained with primary antibodies recognizing PTEN (AMB2052 Clone amb 2052 [Cascade, -]; dilution 1:500) and cMET (C-12 Clone sc-10 [Santa Cruz Biotechnology, Dallas, TX, USA]; dilution 1:100). A Polymer Detection System was used to detect the staining reactions (Novolink Max Polymer, Novocastra). IHC to detect p16 was performed by using an automated immunohistochemical system (BenchMark XT, Ventana Medical Systems, Tucson, AZ, USA) and the iVIEW DAB detection kit (Ventana Medical Systems)

with a primary antibody recognizing p16 (Clone E6H4, prediluted; CINtec Histology Kit, MTM Laboratories, Heidelberg, Germany).

Immunohistochemical stainings were analyzed under a light microscope and slides were interpreted by the same experienced head and neck pathologist who was blinded to the clinical data. For PTEN and cMET, a histologic score (H-score) was applied. Briefly, a continuous score from 0 to 300 was calculated by taking into account both staining intensity and the percentage of stained cells. Cytoplasmic stainings of both PTEN and cMET were evaluated. The percentage of cells with different staining intensities were determined by visual assessment and a score was calculated according to the formula: $1 \times (\%$ of $1+$cells$) + 2 \times (\%$ of $2+$cells$) + 3 \times (\%$ of $3+$cells). An H-score < 10 was considered to indicate low PTEN expression. For cMET expression, an H-score higher than the median H-score for the cohort was considered to indicate high cMET expression. For statistical analysis, p16 samples were classified in a binary manner as either negative or positive (with strong and uniform cytoplasmic and nuclear staining observed in more than 70% of stained cells).

Statistical analysis

Frequencies were used to describe categorical variables. Median and interquartile range (IQR) values were used to describe continuous variables. Pearson's χ^2 test was used to compare baseline characteristics between the patients treated with CT + cetuximab versus CT alone, to test the association between biomarker expression and the primary sites, to test the association between one biomarker and another, and to test the association of overall response rate (ORR) with clinical and pathological characteristics.

Response to treatment was collected as registered by the treating physician on medical charts and divided into response if complete response or partial response were assigned, and no response if stable disease of progression disease were assigned.

For patients treated with cetuximab for recurrent disease, PFS was calculated from the date of the first cetuximab infusion until disease progression or death by any cause. Similarly, OS was calculated from the date of the first cetuximab infusion until death by any cause. For patients who did not receive cetuximab, their first or later treatments were analyzed in order to maintain the same proportion of patients that were evaluated at first-line treatment or at further treatments in parallel with the cetuximab group. PFS was calculated from the date of the first CT infusion until disease progression or death by any cause. The Kaplan–Meier method was used to plot survival curves and the log-rank test was used to evaluate the impact of each clinical and biologic variable on PFS and OS.

All clinical and pathological variables were tested in univariate analyses of OS and PFS by using a Cox proportional hazards model. Variables with a p-value less than 0.20 in the univariate analysis were selected for the multivariate analysis. Results were considered statistically significant when a p-value less than 0.05 was obtained. Statistical analyses were performed with SPSS (v.23, SPSS, Chicago, IL, USA).

This study was approved by the Ethics Committee of the A.C.Camargo Cancer Center (#1911/14).

Results

Data for 112 patients with recurrent HNSCC were retrospectively reviewed. Seventy-five patients were treated with CT + cetuximab and 37 patients were treated with CT alone. The median age of the cohort was 59.0 year and the most frequent primary tumor site was the oral cavity (38.4% of cases), followed by the oropharynx (25.9%), and larynx (20.5%). Sixty percent of the patients presented with distant metastasis as an incurable recurrence.

The median age of the cetuximab group was higher than the CT alone group (60 year vs. 53 year, respectively; $p < 0.001$). In the CT alone group, the oral cavity was more often the primary site of disease and more patients in this group received paclitaxel with a platinum compound. In the cetuximab group, the most often chemotherapy associated to platinum compound was 5FU (Table 1).

Low PTEN expression was detected in 18.8% of the tissues examined, while p16 expression was detected in 12.5% of the tissues examined. There were slightly more patients in the cetuximab group who presented low PTEN expression, and slightly more patients in the CT only group showed p16 positivity. More patients in the CT only group had high cMET expression (Table 1).

In 8 out of 8 patients with the hypopharynx as the primary tumor site, cMET expression was above the median level of cMET expression ($p = 0.003$). In addition, 8 out of 14 patients with p16-positive tumors had the oropharynx as their primary tumor site ($p = 0.010$). No other associations between the three biomarkers and patient characteristics were observed, nor were there any associations observed between the three biomarkers themselves.

Overall survival

After a median follow-up of 27.0 months, 88 patients had died. The median OS was 10.2 months (95% CI 8.1–12.3 months) (Fig. 1). Patients treated with cetuximab had an OS of 11.4 months compared to 7.0 months for patients in the CT only group ($p = 0.949$) (Fig. 1).

Table 1 Baseline characteristics of our cohort ($n = 112$)

Characteristic	Frequency (%)		p-value
	CT + cetuximab	CT	
Number of patients	75 (67.0)	37 (33.0)	
Median age, year (IQR)	60.0 (53.0–70.0)	53.0 (46.0–63.0)	0.010
Gender			
Male	60 (80.0)	29 (78.4)	0.513
Female	15 (20.0)	8 (21.6)	
ECOG performance status			
0	54 (73.0)	21 (60.0)	0.172
≥ 1	20 (27.0)	14 (40.0)	
Smoking history			
Current smoker	53 (74.6)	30 (81.1)	0.452
No history of smoking	18 (25.4)	7 (18.9)	
Primary tumor site			
Oral cavity	26 (34.7)	17 (47.2)	0.437
Oropharynx	19 (25.3)	10 (27.8)	
Larynx	16 (21.3)	7 (19.4)	
Hypopharynx	10 (13.3)	2 (5.6)	
Unknown	4 (5.3)	0 (0.0)	
Site of disease recurrence			
Locoregional	27 (36.0)	17 (45.9)	0.311
Distant	48 (64.0)	20 (54.1)	
First-line cetuximab treatment			
No	64 (85.3)	32 (86.5)	0.870
Yes	11 (14.7)	5 (13.5)	
Chemotherapy			
Cisplatin and 5-FU	30 (40.5)	3 (8.1)	< 0.001
Carboplatin and 5-FU	32 (43.2)	0 (0.0)	
Platinum and taxanes	8 (10.8)	29 (78.4)	
Other	4 (5.4)	5 (13.5)	
p16 expression			
Yes	8 (11.0)	6 (16.7)	0.402
No	65 (89.0)	30 (83.3)	
Low PTEN expression[a]			
Yes	16 (21.6)	5 (13.9)	0.333
No	58 (78.4)	16 (21.6)	
High cMET expression[b]			0.001
Yes	30 (66.7)	11 (30.6)	
No	15 (33.3)	25 (69.4)	

CT chemotherapy, *IQR* interquartile range; *ECOG* Eastern Cooperative Oncology Group

[a]Low PTEN expression is defined as an H-score ≤ 10

[b]High cMET expression is defined as an H-score > the median value for the cohort (H-score = 45)

ECOG performance status ≥ 1 and low PTEN expression had a negative impact on the OS curves (Online Resource 1 and Fig. 2). The median OS for patients with an ECOG performance status of 0 versus ≥ 1 was 11.8 months and 7.7 months ($p = 0.053$). The median OS for patients with high versus low PTEN expression was 10.5 months and 5.8 months ($p = 0.002$). In contrast, none of the other IHC markers had an impact on OS (Online Resource 2). In the subgroup analysis of p16 status in the 29 patients with the oropharynx as a primary tumor site, no effect on OS was observed ($p = 0.941$).

In a Cox univariate analysis, smoking history, performance status ≥ 1, site of recurrence, and low PTEN expression were associated with a worse OS ($p \leq 0.200$ for each)

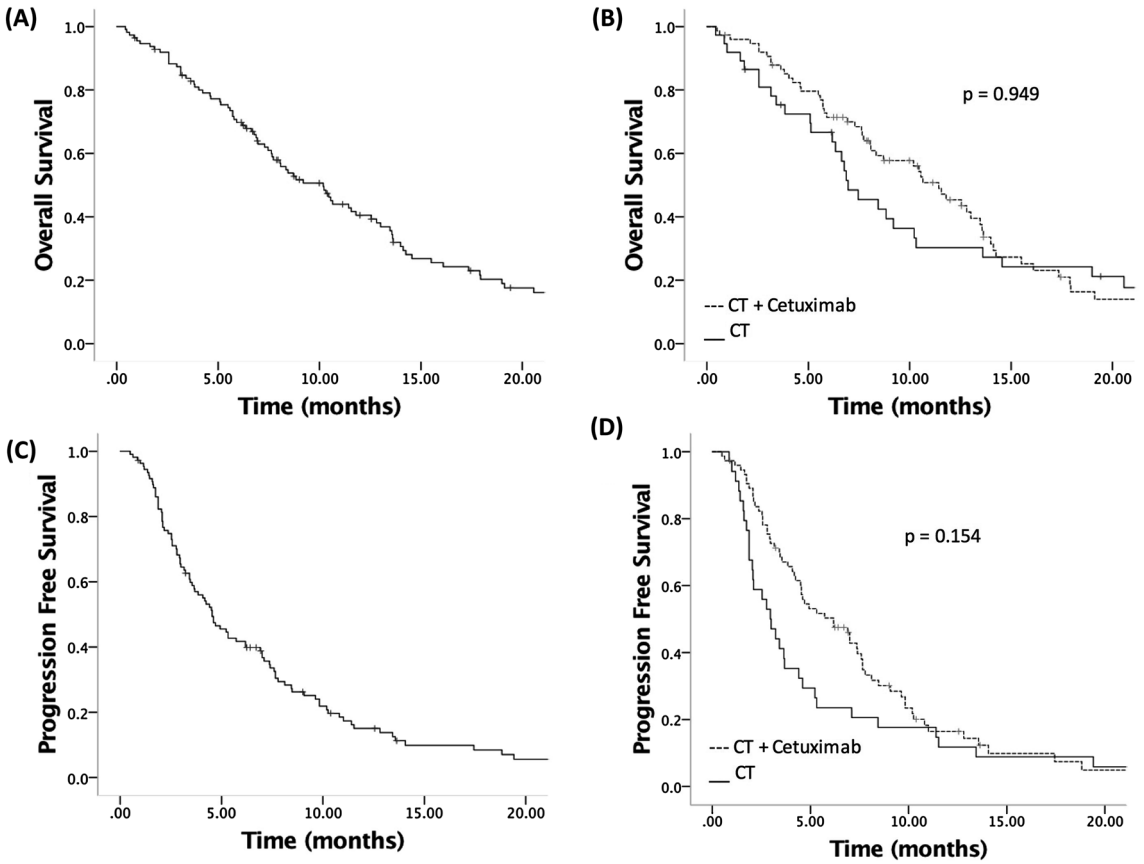

Fig. 1 Kaplan–Meier curves for OS and PFS for the entire cohort (**a, c**) and according to cetuximab treatment (**b, d**) are presented, respectively, in each case

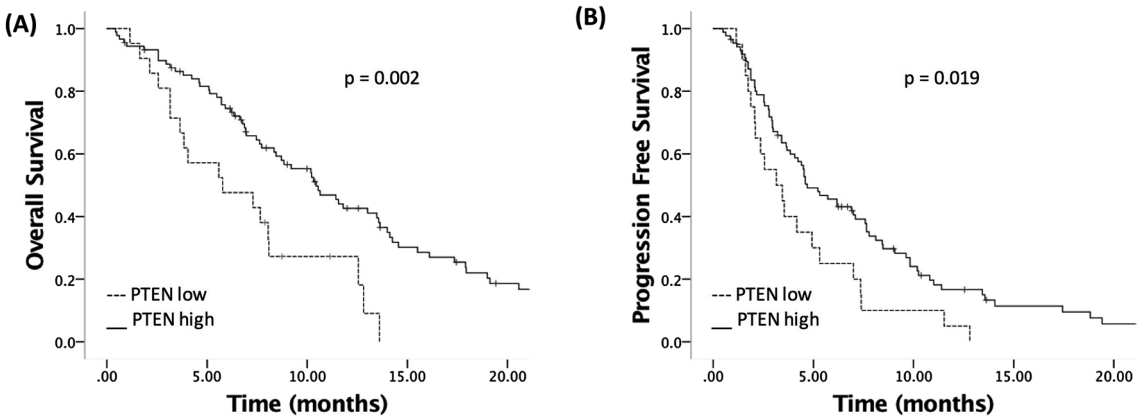

Fig. 2 Kaplan–Meier curves for OS (**a**) and PFS (**b**) according to low versus high PTEN expression

(Online Resources 1, 3). When these factors were included in a multivariate analysis, ECOG performance status ≥ 1 and low PTEN expression remained significantly associated with worse OS (Table 2).

Progression-free survival

Disease progression was observed in 97% of the patients in our cohort and the median PFS was 4.5 months (95% CI 3.5–5.6 months) (Fig. 1). Patients treated with cetuximab

Table 2 Multivariate Cox regression analysis for OS

Characteristic	HR (95% CI)	*p*-value
History of smoking		
No	1	0.705
Yes	1.12 (0.62–2.03)	
Site of disease recurrence		
Locoregional	1	0.088
Distant	1.52 (0.94–2.44)	
ECOG performance status		
0	1	0.035
≥ 1	1.69 (1.04–2.75)	
Low PTEN expression[a]		
> 10	1	0.006
≤ 10	2.27 (1.27–4.08)	

The final Cox regression model included 104 patients and 83 events

CT chemotherapy, *IQR* interquartile range; *ECOG* Eastern Cooperative Oncology Group

[a]Low PTEN expression is defined as an H-score ≤ 10

Table 3 Multivariate Cox regression analysis of PFS

Characteristic	HR (95% CI)	*p*-value
Patient age		
≤ 65 year	1	0.075
> 65 year	0.68 (0.44–1.04)	
Low PTEN expression[a]		
> 10	1	0.022
≤ 10	1.85 (1.09–2.99)	

The final Cox regression model included 98 patients and 87 events

HR hazard ratio, *CI* confidence interval

[a]Low PTEN expression is defined as an H-score ≤ 10

had an PFS of 6.2 months versus 3.0 months for those not treated with cetuximab ($p = 0.154$) (Fig. 1).

Patients older than 65 years old and whose tumors had low PTEN expression exhibited a significantly worse PFS (Online Resource 1 and Fig. 2). Median PFS was 3.7 months for patients younger than 65 years old versus 6.9 months for patients older than 65 years old ($p = 0.033$). Median PFS was 4.7 months for PTEN high versus 3.2 months for patients with PTEN low ($p = 0.019$). None of the other IHC markers had an impact on PFS.

In a univariate analysis, performance status ≥ 1 and low PTEN expression were associated with a worse PFS (Online Resource 4). Performance status, PTEN status, patient age, and cetuximab treatment were included in the final multivariate model, only performance status ≥1 and low PTEN expression remained related to PFS (Table 3).

Overall response rate

The ORR for this cohort was 40.7%. The factors that were found to be associated with a better ORR were an ECOG performance status of 0 compared to ECOG ≥ 1 (ORR 46.9% vs. 20.7%, respectively; $p = 0.016$) and use of cisplatin and 5FU compared to other treatment options (ORRs: cisplatin + 5FU = 67.9%, carboplatin + 5FU = 29.0%, platinum + taxane = 27.6%, other drugs 37.5%; $p = 0.011$).

PTEN as a predictive factor of cetuximab benefit

Among the patients treated with cetuximab, the median OS for patients with low PTEN expression was 7.3 months versus 11.8 months for patients with high PTEN expression ($p = 0.003$). Among the patients treated with CT alone, the median OS for patients with low PTEN expression was 3.2 months versus 7.5 months for patients with high PTEN expression ($p = 0.052$) (Fig. 3). The *p*-value for interaction between PTEN expression and cetuximab use for OS was 0.392. In the group of patients not treated with cetuximab, there were only five patients with low PTEN expression hampering this analysis.

Among the patients treated with cetuximab, the median PFS for low PTEN expression was 3.5 months versus 7.0 months for high PTEN expression ($p = 0.004$). Among the patients treated with CT alone, the median PFS for low PTEN expression was 1.9 months versus 3.0 months high PTEN expression ($p = 0.727$) (Fig. 3). The *p*-value for interaction between PTEN expression and cetuximab use for PFS was 0.521. In the group of patients not treated with cetuximab, there were only three patients with low PTEN expression hampering this analysis.

Discussion

Our study identified PTEN as a prognostic factor for both OS and PFS among patients with recurrent HNSCC. In subgroup analyses, the prognostic impact of PTEN was statistically significant only for the cetuximab-treated group.

PTEN mutations or deletions were found in 12% of HPV negative and 6% of HPV positive tumors according to The Cancer Genome Atlas Network [6]. In other studies, the frequency of PTEN loss of function has been reported to range from 7 to 30% in HNSCC tumors [7, 14, 15, 25]. In accordance with these data, low PTEN expression was detected in 18.8% of the patients in the present cohort.

A negative prognostic impact of PTEN loss was previously reported for localized HNSCC patients who underwent surgery and radiotherapy [17, 26], while other authors have suggested that PTEN overexpression could be related to worse prognosis [16]. In a previous study conducted by

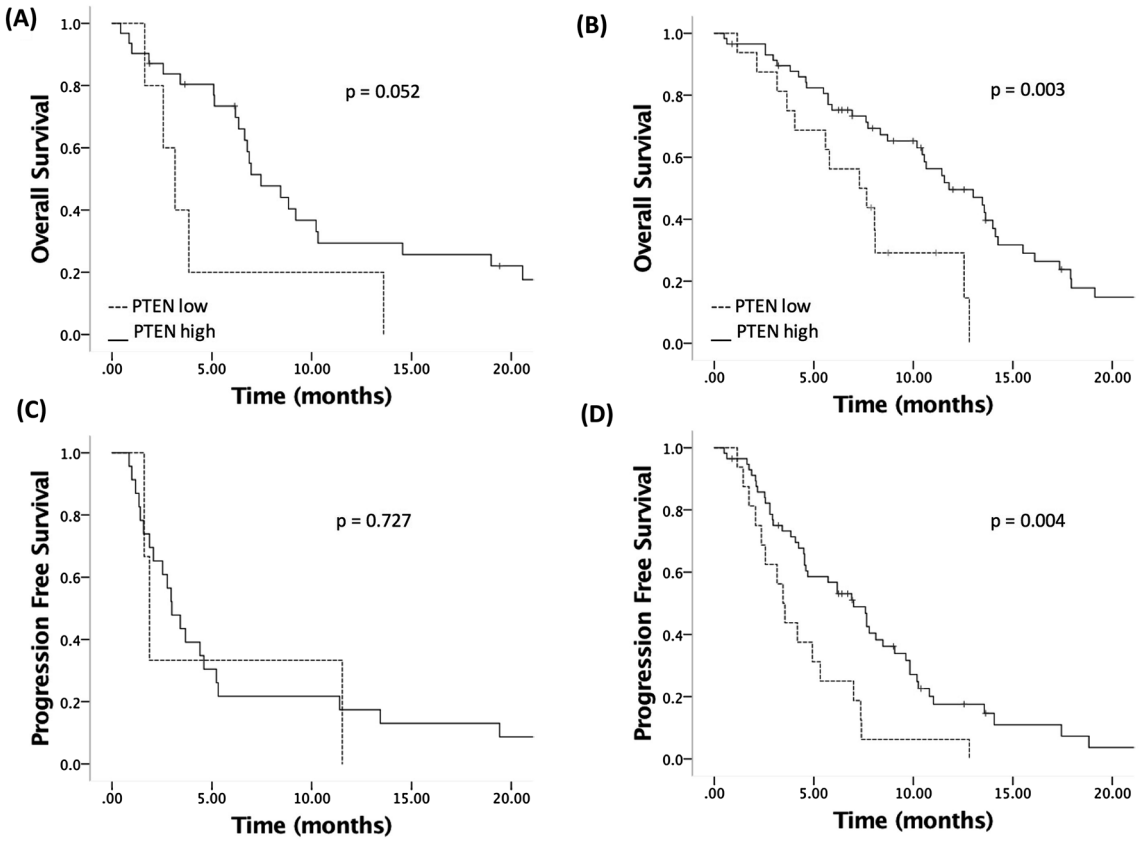

Fig. 3 Kaplan–Meier curves for OS and PFS according to PTEN expression in patients treated with CT alone (**a, c**) versus CT + cetuximab (**b, d**), respectively, in each case

our group, we observed a negative prognostic impact of low PTEN expression in a cohort of 61 HNSCC patients with recurrent disease who were treated with palliative CT plus cetuximab [18]. In the present study, low PTEN expression was associated with a clear negative impact on OS and PFS after adjusting for other prognostic factors in a multivariate analysis. Thus, the present findings confirm that PTEN is a major prognostic factor in metastatic/recurrent HNSCC. PI3K-Akt-mTOR pathway targeting has been explored within the last decade with disappointing results in clinical trials compared to preclinical findings [27]. The present findings may facilitate a better selection of patients for PI3K-Akt-mTOR pathway targeting.

In other primary tumors, such as breast and colon, loss of PTEN function has been associated with a worse response to both anti-HER2 and anti-EGFR targeted therapies [28, 29]. In HNSCC tumors, at least one phase II clinical trial suggests predictive role of PTEN expression for afatinib response [30].

To the best of our knowledge, the role of PTEN as a predictive biomarker of response to cetuximab treatment in metastatic HNSCC has not been evaluated. In a subgroup analysis of the present study, the prognostic impact of PTEN was found to be statistically significant only for the patients who received cetuximab. However, due to the small number of patients with low PTEN expression in the group of patients who were not treated with cetuximab, we cannot definitively conclude that these data indicate a predictive role for PTEN. Indeed, the p-value for the interaction between PTEN expression and cetuximab use was not significant for either OS or PFS.

In our study, p16 was positive in 12.6% and showed no prognostic impact. HPV-related HNSCC is known to have better prognosis in localized disease [8]. In patients with recurrent disease, the frequency of HPV positivity is generally lower, between 10 and 18% [23]. In two phase III trials involving anti-EGFR treatment for metastatic HNSCC, the p16-positive patients exhibited a better overall prognosis, although p16 status was not found to be predictive of anti-EGFR therapy response [24]. A prognostic impact for p16 expression was not observed in the present study, even when patients with oropharynx tumors were analyzed alone. However, the small number of patients with p16-positive tumors may explain the results.

Overexpression of cMET is present in more than 80% of HNSCC [19]. At least one retrospective study has showed

a negative prognostic impact of cMET among a cohort of HNSCC patients who were treated with CT + cetuximab [22]. The latter study included 57 patients and all of them were treated with cetuximab. However, they used a different primary antibody (SP44 clone, Ventana Medical Systems, USA) from the one used in the present study, and the cutoff value in the latter study was determined based on a receiving operating curve. Alternatively, we used the median value of the H-score as a cutoff value for defining overexpression. In a sensitive analysis that was performed which included testing of different cutoff values and performing a subgroup analysis of the CT + cetuximab and CT only groups (data not shown), no association between cMET expression and OS or PFS was found. Ongoing interest regarding the potential for cMET to serve as a prognostic and predictive marker is related to the existence of targeted drugs which are already in use for other primary cancers, such as crizotinib [31] and cabozantinib [32]. However, the results of the present study do not support a role for cMET in the prognosis of HNSCC, nor as a predictive factor for anti-EGFR therapy.

We did observe that cetuximab promoted a non-statistically significant improvement in OS and PFS in the present study. The observation that the median OS for patients treated with cetuximab was longer than the median OS for patients not treated with cetuximab (11.4 months versus 7.0 months, respectively) is consistent with the findings of a phase III trial where the median OS was longer for the cetuximab-treated group (10.1 months vs. 7.4 months, respectively). Moreover, while the present study included 112 patients, the latter phase III trial included 442 patients. Thus, the present study may have been underpowered in its analysis of the impact of cetuximab.

Our study has limitations due to its retrospective nature. Selection bias for treatment could be an issue since the decision of treatment modality was not randomized. Patients were treated with chemotherapy alone or with cetuximab based on the health insurance policy and not due to patient's intrinsic characteristics. This minimizes selection of healthier patients to the three drug therapy group. Indeed, the two groups were significantly different only for older age in the cetuximab group and the chemotherapy regimen used. Older age in the cetuximab group would favor the non-cetuximab group, and there is evidence of no different efficacy for platinum plus 5FU compared to carboplatin and paclitaxel as first-line palliative treatment for HNSCC [33]. Conversely, it is important to highlight a strength of the present study which is that we included patients that were treated and not treated with cetuximab as a palliative treatment for HNSCC within the same time period. This study design allowed us to not only analyze the prognostic impact of the biomarkers of interest, but also to evaluate their predictive value. To date, data regarding cohorts that include both of these types of patient groups are scarce.

In conclusion, this larger study confirms the findings of our previous study [18] regarding the prognostic impact of low PTEN expression in recurrent cases of HNSCC treated with palliative CT. In addition, the present findings highlight the importance of the PI3K-Akt-mTOR pathway in mediating alterations in HNSCC, and this may contribute to the selection of patients who are more sensitive to drugs that target this pathway. While a clear predictive impact of PTEN expression for cetuximab efficacy was not demonstrated, our findings do indicate a possible predictive role for PTEN expression. This should be further examined in a larger cohort of patients which includes patients that are treated and not treated with cetuximab. cMET showed no prognostic or predictive impact on OS or PFS and p16 expression was found to be less frequent in recurrent/metastatic HNSCC.

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Compliance with ethical standards

Conflict of interest None to declare.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

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