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HEAD & NECK

Benefit of cetuximab addition to a platinum–fluorouracil-based chemotherapy according to *KRAS-LCS6* variant in an unselected population of recurrent and/or metastatic head and neck cancers

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Abstract

Objectives To evaluate the benefit of cetuximab (Cx) addition to platinum-based and 5-fluorouracil chemotherapy (PFU) in unselected recurrent and/or metastatic head and neck cancer patients (R/MHNC) according to *KRAS-LCS6* variant status.

Methods All patients who received at least two PFU ± Cx cycles from 2004 to 2014 were retrospectively included into to two distinct study periods according to Cx implementation: patients treated by PFU alone before 2009 and those treated by PFU + Cx from 2009. Primary objective was to evaluate the progression-free survival (PFS) between the two groups. Secondary objectives were to analyze the overall survival (OS) between the two groups and the prognostic impact of *KRAS-LCS6* variant. Factors associated with survival were determined by a Cox multivariate analysis including age, WHO performance status (PS), type of treatment, *KRAS-LCS6* variant, Charlson's score and p16 status.

Results Overall, 134 patients were included: 59 (44%) in PFU group and 75 (56%) in PFU + Cx group. Baseline characteristics were well balanced including 30% of patients with 2–3 PS. Median PFS was significantly improved in PFU + Cx group compared to PFU group (6.1 vs 4.4 months, respectively, HR 0.68, $p = 0.02$) and with a trend for better OS. A *KRAS-LCS6* variant was found in 27 (25%) of samples without prognostic impact neither in whole population nor according to treatment. In multivariate analysis, addition of Cx to PFU was the only factor significantly associated with a better PFS ($p = 0.01$, HR 0.6).

Conclusion Our results suggest that PFU + Cx combination may be effective in unselected population of R/MHNC regardless the *KRAS-LCS6* variant status.

Keywords Recurrent and/or metastatic head and neck cancers · Cetuximab · Unselected population · KRAS-LCS6 · Rs61764370 · Prognosis

Introduction

Despite intensive treatment including a combination of surgery, radiotherapy and chemotherapy (CT), patients harboring head and neck squamous cell carcinoma (HNSCC) have poor prognosis. Indeed, loco-regional recurrences are observed in approximately 30–40% of patients treated primarily in curative intent and these recurrences are frequently challenging to treat [1, 2]. Moreover, metastatic evolution may also occur in about 10% of cases at diagnosis and in up to 30% during follow-up [2, 3].

To date, treatment options in inoperable recurrent or metastatic HNSCC (R/MHNC) are based on palliative CT and best supportive care, with a 5-year overall survival (OS)

Part of the results has been presented at the 2017 ESMO Annual Meeting (Abstract 1081P Bastit et al).

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rate of nearly 0% [4]. Platinum plus 5′fluorouracil combination (PFU) is still considered as the backbone of CT in that setting [5]. Due to the involvement of the EGFR pathway in HNSCC carcinogenesis [6], a randomized phase III study in advanced HNSCC patients showed that cetuximab (Cx)—a monoclonal antibody against EGFR—combined to PFU leads to a significant improvement of 3-month OS compared to CT alone [4]. Although PFUCx has become the new standard of care for R/MHNC, a subgroup analysis of this pivotal phase III trial suggested that only patients with Karnofsky ≥ 80 may benefit from PFUCx combination, as for patients treated by radiation + Cx for locally advanced HNC [4, 7]. Thus, considering that patients are highly selected before inclusion in clinical trials, the benefit of addition of Cx to PFU in routine practice needs to be clarified.

Besides the overall efficacy of Cx, new insights in the biology of HNSCC may help to identify predictive biomarkers of response to Cx [8]. The *KRAS-LCS6* variant, a single nucleoid polymorphism (SNP) located on the micro-ARN (miRNA) let-7-binding site, has been recently described as a potential predictive factor of Cx sensitivity in HNSCC [9]. Let-7 is a miRNA that acts as a *KRAS* inhibitor. In case of *KRAS-LCS6* variant, let-7 cannot bind the 3′UTR of *KRAS*, leading to an up-regulation of *KRAS* expression and an oncogenic addiction to EGFR pathway [10]. To date, the predictive value of the presence of *KRAS-LCS6* variant for Cx efficacy has not been clearly established and conflicting results are available in R/MHNC [9, 11–13].

In this context, the primary objective of the present retrospective study was to evaluate the impact on the prognosis of PFUCx combination vs PFU alone in unselected R/MHNC patients. Furthermore, we also assessed the impact of treatment schedules according to *KRAS-LCS6* variant status.

Patients and methods

Patients

This monocentric retrospective study included patients treated as first line by at least two cycles of PFU ± Cx from 2004 to 2014 for R/MHNC. Patient characteristics were systematically collected into their computerized medical file before the start of treatment including age, sex, comorbidities, alcohol and tobacco consumption, the WHO performance status (PS), tumor location, TNM stage and AJCC classification, and previous oncologic treatment. A physical examination, laboratory test and the monitoring of treatment-related toxicities were also systematically performed and recorded at each visit. A chest and abdominal CT scan was performed at baseline and every 3 months during treatment. All patients signed a form allowing conservation and analysis of tumor samples, and the study was approved by the Institutional Review Board of the Henri Becquerel Centre (registering number 1605B).

Treatment

Patients were planned to be treated by six cycles of treatment. The PFU protocol was delivered every 3 weeks and consisted of the association of cisplatin (100 mg/m^2) or carboplatin (area under curve or AUC 5 at mg/mL/min) at day 1 with 5′fluorouracil (5FU) in continuous infusion (1000 mg/m^2/day from day 1 to day 4). Cisplatin or carboplatin was used at the discretion of the physician. As reported, the PFUCx protocol was based on the PFU schedule associated with Cx infusion at initial dose of 400 mg/m^2 followed by weekly infusion at 250 mg/m^2. Dose modifications were performed according to physician's choice. Patients without progression after six cycles of PFUT ± Cx received maintenance by Cx monotherapy at 500 mg/m^2 every 2 weeks until disease progression, unacceptable toxicity or death.

P16 and *KRAS-LCS6* variant determination

Tumor expression of p16 was determined with the CINtec® Histology kit (Roche diagnostics, Switzerland). Tumor p16 staining was considered positive if a diffuse nuclear and cytoplasmic staining was observed on at least 75% of tumors cells.

For *KRAS-LCS6* analysis, DNA was extracted on 10-µm section of paraffin-embedded from tumor and adjacent non-tumoral tissues. After 12 h of paraffin dissolution at 70 °C with the Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Promega Corp, Wisconsin, USA), extraction and purification were performed automatically by a DNA/RNA Maxwell 16® (Ref AS2000) (Promega Corp, Wisconsin, USA). Sample quality was assessed by spectrophotometric analysis on Thermo Scientific™ NanoDrop™ One Spectrophotometers (Thermo Fisher Scientific, Massachusetts, USA). Genotyping of *KRAS-LCS6* 3′UTR (rs61764370) was performed by a first step of amplification by PCR followed by a pyrosequencing analysis restricted on 146 bp of the region of interest. PCR was realized with the GoldStar® Mix kit (Eurogentec®, Belgium) on Eppendorf Mastercycler® (Eppendorf, Germany). Forward primer was 5′-[Btn]-GTC TCGAACTCCTGACCTCA-3′ and reverse 3′-TGGTGA CTGGCATCTGGTAG-5′. Specificity of PCR products was verified on agarose gel before pyrosequencing. Sequencing was performed on a Pyromark Q24® (QIAGEN®, Germany) with PyroMark Q24 Gold Q24 Reagents ® (QIAGEN®, Germany) and the sequencing primer: 3′-ACAGGTTTATGA GGCCAAGG-5′.

Statistical analysis

The main objective of the study was to assess the progression-free survival (PFS) between PFUCx and PFU alone. Secondary objectives were to analyze the overall survival (OS) between PFUCx and PFU alone; and the impact of *KRAS-LCS6* variant in the whole population and according to treatment arm. For the purpose of the study, patients were divided into two study periods according to Cx use in routine practice. The PFU group corresponded to patients treated from 2004 to 2009 and PFUCx group corresponded to those treated from 2009 to 2014. Considering that the pivotal trial [4] reported a 23% difference in 6-month PFS in PFUCx group compared to PFU alone, at least 122 patients needed to be included in our study to observe similar difference, assuming a 5% alpha risk and a 20% beta risk. The PFS was defined as time between the date of first cycle of CT and the date of progression or death, and OS was defined as time between the date of first cycle of CT and the date of death from any cause. Survival curves were realized by Kaplan–Meier method and compared by log-rank test. Univariate analyses tested the influence on the prognosis of Cx addition, PS, age, Charlson's score, p16 status, *KRAS-LCS6* status and, among patients receiving cetuximab, grade 2–3 cutaneous toxicity. Multivariate analyses were performed by Cox/ proportional hazards regression method. Qualitative data were compared using the chi-square test and quantitative data by Student's *t* test. Mean values were presented as "mean [95% confidence interval (CI95)]" and median values as "median (lowest value – highest value)". *P* value < 0.05 was considered significant. Statistical analyses were carried out using the MedCalc Software© v12.1.4.0.

Results

Patients

A total of 134 patients was included: respectively, 59 (44%) treated by PFU between 2004 and 2009 and 75 (56%) treated by PFUCx between 2009 and 2014. Patient characteristics are summarized in Table 1, showing that the two groups were well balanced except for the inclusion of older patients with a higher Charlson's score in PFUCx group [respectively, 57.9 (56.3–59.5) vs 54.8 (52.7–56.8) $p = 0.02$; and 7 (6.6–7.4) vs 6.3 (5.7–6.9), $p = 0.05$]. First-line palliative chemotherapy was mostly indicated for metastatic evolution (80%), rather than for non-curable loco-regional evolution (20%). Overall, 30% of the patients had a performance status of 2 or more. There was also a non-significant trend for more oropharyngeal cancer in PFUCx group ($p = 0.07$). Of note, most of the patients had an alcohol–tobacco addiction, while a p16 tumoral expression was observed in 14 of the 110 evaluable cases (13%) with no difference between the two groups of treatment (six patients (9.3%) in the PFUCx arm and eight (17.4%) in the PFU arm, $p = 0.3$). Overall biological samples were available for 110/134 of the patients (82%) and were equally distributed between the two treatment groups [64 (84%) in PFUCx group and 46 (78%) in PFU group ($p = 0.4$)].

Effect of the addition of cetuximab to a PFU chemotherapy

In univariate analysis, median PFS in the whole population was 5 months (1.5–32.2), with a significant better PFS in the PFUCx group compared to the PFU group (6.1 vs 4.4 months respectively, HR 0.68 CI95 (0.5–0.97), $p = 0.02$, Fig. 1a and Table 2). Median OS in the whole population was 9.5 months (1.6–44). OS seems better for patients treated by PFUCx compared to PFU alone although this difference did not reach significativity [median OS of 11.1 months vs 9.1, respectively, HR 0.8 CI95 (0.6–1.1), $p = 0.2$, Fig. 1b].

A performance status of 0–1 compared to 2–3, an age ≤ 55, a Charlson's score ≤ 6 or a tumoral p16 expression was not related to a better outcome in PFS (Table 2). However, patients with PS 0 or 1 had a trend of better OS compared to patients with PS 2 or 3 [median OS of 11.2 months (1.6–44.1) vs 8.8 (1.8–37.3) respectively; HR 1.43 CI95 (0.9–2.2) $p = 0.06$].

Among the patients treated by PFUCx, a grade 2–3 cutaneous reaction was associated with a significantly better PFS [HR 0.6 CI95 (0.4–0.9) $p = 0.008$; Fig. 1 c and Table 2) and a non-significant trend over a better OS [HR 0.7 CI95 (0.4–1.1) $p = 0.1$; Fig. 1d].

KRAS-LCS6 prevalence and relation to outcome under PFU or PFUCx

Among the 110 cases available, 27 (25%) had a *KRAS-LCS6* variation. There was a 100% concordance between the non-tumoral and matched tumoral samples for the *KRAS-LCS6* status, i.e. all the *KRAS-LCS6* variations were related to constitutive variants and not to somatic mutations. Patient characteristics are summarized in supplementary table. No significant difference was observed for *KRAS-LCS6* status according to patient characteristics or tumoral p16 expression. Overall, 17/27 patients (63%) with *KRAS-LCS6* variant were treated by PFUCx while the others were treated by PFU. In the whole population, no difference neither in PFS nor in OS was observed between patients with *KRAS-LCS6* variant and the others (respectively, 6.1 months vs 4.8 $p = 0.7$; Table 2 for PFS and 9.3 months vs 8.1; $p = 0.8$, for OS). Furthermore, *KRAS*

Table 1 Demographic and tumoral characteristics of patients treated by PFUCx or PFU

	PFUCx + 56% ($n = 75$)	PFU 44% ($n = 59$)	Significance, p
Male	85%	83%	n.s.
Age	57.9 (56.3–59.5)	54.8 (52.7–56.8)	0.02
Tobacco use	97%	97%	n.s.
Alcoholic addiction	87%	95%	n.s.
Initial cancer location			
Oral cavity	26%	31%	n.s.
Oropharynx	40%	24%	0.07
Hypopharynx	23%	35%	n.s.
Larynx	4%	5.08%	n.s.
Primitive adenopathy	8%	6.78%	n.s.
Stage AJCC I	0	0	
II	4%	7%	n.s.
III	8%	8%	n.s.
IVa	51%	49%	n.s.
IVb	13%	15%	n.s.
IVc	24%	20%	n.s.
Previous treatment received			
Neoaduvant CT	8%	3%	n.s.
Surgery	1%	5%	n.s.
Surgery + po RT	17%	24%	n.s.
Surgery + po RTCT	36%	24%	n.s.
RT	4%	5%	n.s.
RTCT	15%	20%	n.s.
None	27%	22%	n.s.
Indication of palliative care			
Non-curable LR evolution	16%	25%	n.s.
Metastatic evolution	56%	49%	n.s.
Metastatic and LR evolution	28%	25%	n.s.
Performance status			
OMS: 0	21%	20%	n.s.
OMS: 1	51%	47%	n.s.
OMS: 2	25%	27%	n.s.
OMS: 3	3%	5%	n.s.
Charlson score (mean)	7 (6.6–7.4)	6.3 (5.7–6.9)	0.05
Albumin g/L (mean)	41.2 (40.4–42)	40 (38.8–41.2)	n.s.

n.s. Non-significant, *CT* chemotherapy, *RT* radiotherapy, *RTCT* radio and chemotherapy, *Po* post-operative, *LR* loco-regional

status was not related to a peculiar outcome neither for patients exposed to PFUCx (Fig. 2c, d) nor for patients exposed to PFU (not shown).

Multivariate analysis

In a multivariate analysis including PS ≥ 2, addition of Cx, *KRAS* status, Charlon's score, p16 status and age ≥ 55 as variables, addition of Cx to PFU was the only factor related to a better PFS ($p = 0.01$, Table 2).

Discussion

The present retrospective study was based on a historical comparison including 134 unselected metastatic or recurrent HNSCC patients. Our results showed that PFUCx combination was associated with a significant improvement in PFS compared to PFU alone regardless of *KRAS-LC6* variant status. Moreover, considering the degree of related comorbidities and that 30% of patients had a PS 2–3, these findings support that adding Cx may also be

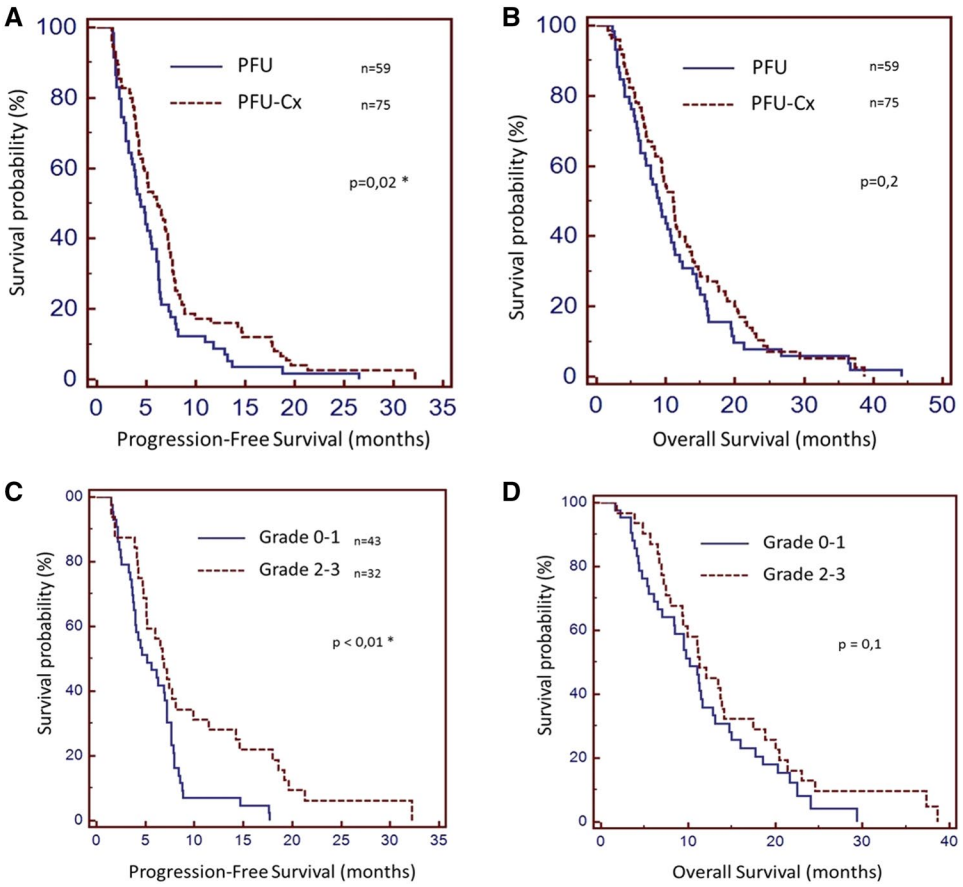

Fig. 1 Survival analysis **a** Progression-free survival (PFS) according to treatment received; **b** overall survival (OS) according to treatment received; **c** PFS of patient treated with cetuximab according to cutaneous reaction; **d** OS of patient treated with cetuximab according to cutaneous reaction. *PFU*: platinum–5′fluorouracil, *PFU-Cx* PFU plus cetuximab. *$p < 0.05$ are considered significant

Table 2 Prognostic factors of progression-free survival

Variables	Univariate analysis			Multivariate analysis[a]		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Addition of Cx	0.68	0.5–0.97	0.02	0.6	0.4–0.9	0.01
PS ≤ 1	1	0.7–1.4	0.9	1.1	0.7–1.6	0.8
Age ≤ 55	1.2	0.8–1.7	0.3	1.2	0.8–1.8	0.3
Charlson's score ≤ 6	1.10	0.8–1.6	0.6	1.2	0.8–1.9	0.4
P16+[a]	0.9	0.5–1.6	0.7	0.7	0.4–1.3	0.3
KRAS-LCS6[a]	0.9	0.6–1.4	0.7	0.9	0.5–1.4	0.5
Grade 2–3 cutaneous reaction to Cx[b]	0.6	0.4–0.9	0.008	NI		

Cx cetuximab, *PS* performance status, *NI* not included in the model

[a]Analysis on the 110 cases with histological data available

[b]Analysis on 75 patients treated by cetuximab

considered as a relevant option in this frail population. To our knowledge, although some retrospective studies have already reported the effect of PFUCx regimen in unselected patients (Table 3), none of them used a PFU control arms as in our work and their sample size was also relatively smaller than in our study ($n = 21$ and 22) [14, 15]. Interestingly, results of the prospective observational study of real-life exposure to the PFUCx regimen (ENCORE study) were presented at the 2018 ESMO congress. Among the 221 patients treated by a PFUCx-based regimen, comparable results to ours were observed (Le Tourneau et al. Abstract 1057).

Of course, the retrospective design of our study may induce some bias. Some very frail patients were unable to be a candidate to chemotherapy limiting the "unselected" character of our study; furthermore, patients who refused chemotherapy could be a significant part of the R/M SCCHN population [8]. This study cannot replace a prospective

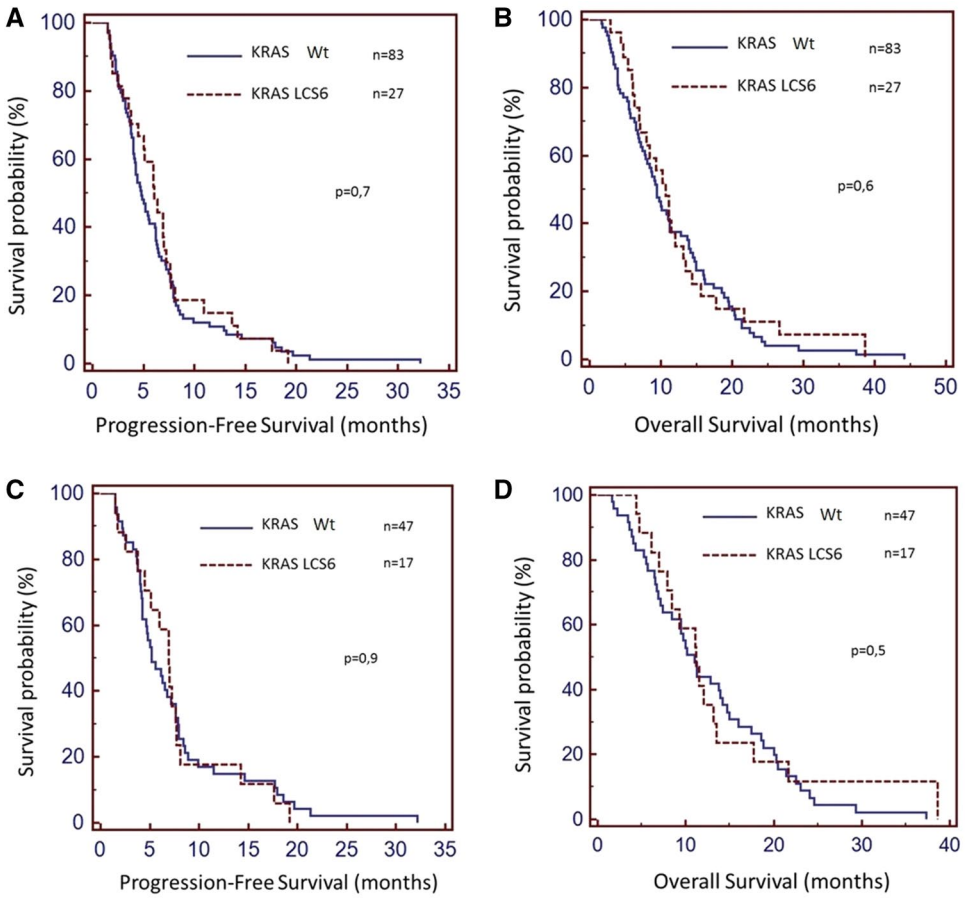

Fig. 2 Survival analysis according to *KRAS* status: **a** progression-free survival (PFS) according to *KRAS* status in the whole population; **b** overall survival (OS) according to *KRAS* status in the whole population; **c** PFS according to *KRAS* status among patients receiving PFUCx; **d** OS according to *KRAS* status among patients receiving PFUCx

randomized study, but such comparative study is hardly feasible since Vermorken's results, especially due to ethical aspect. Thus, only an indirect comparison of historical cohorts could assess the impact of cetuximab addition. Of note, beside addition of cetuximab to PFU, no other major change in R/MHNSCC management occurred between 2004 and 2014, including in supportive care treatment. Furthermore, no patient in the PFUCx was included in a clinical trial nor received immunotherapy.

Likewise the slight differences in baseline characteristics between PFU and PFUCx groups cannot explain the PFS difference since patients who received PFUCx were older ($p = 0.02$) and had more comorbidities ($p = 0.05$) compared to patients receiving PFU. Moreover, our results are similar to those already published by Vermorken et al. [4] who reported a PFS improvement from 3.3 to 5.6 months after the addition of Cx, HR = 0.54 CI95(0.43–0.67), $p < 0.001$. Regarding OS, we observed a non-significant difference favoring PFUCx, in line with Vermorken study. Of note, considering our sample size and the 0.8 HR observed in Vermorken trial, our analysis was not powered to detect a statistically significant difference in OS. Furthermore, the presence of a PS 2–3 population can lower the OS results, but it was precisely the aim of the study to confirm the effectiveness of this protocol in a non-selected population and, therefore, in worse general condition.

Baseline characteristics of this cohort are similar to those described in that setting [1]. The trend toward an increased proportion of oropharyngeal cancer in PFUCx group vs PFU group (40% and 24%, respectively, $p = 0.07$) can be explained by the worldwide increase of oropharyngeal cancer incidence in the meantime [1, 3]. Indeed, median year of inclusion of the PFU group was 2007 compared to 2012 for PFUCx group. Of note, p16 tumoral expression was comparable in both groups limiting potential bias in survival analysis.

Currently, the severity of skin reaction induced by Cx is the only established marker of good response to this monoclonal antibody. Herbst et al. were the first to describe this association in the phase II trial which tested the PFUCx schedule [19]. In the present study, even if its retrospective design may have generated biases for skin toxicity monitoring, our results confirmed the predictive value of skin reaction under Cx ($p = 0.008$). Of note, this observation remains useless due to the lack of pre-therapeutic test, and the identification of a predictive biomarker of Cx efficiency is still an unmet need.

Table 3 Published studies on the efficiency of EXTREME protocol (platinum–5FU–cetuximab), in the first line of palliative chemotherapy, for head and neck carcinomas, after publication of Vermorken's study

Author/date	Context	Method	*n*	Survival in months (CI 95%)	Authors' conclusion
Chang et al. (2010) [16]	To investigate the efficacy of cetuximab in Betel nut's chewer in 1st and 2nd line of palliative chemotherapy	Uncontrolled retrospective study	$n=13$[a]	OS: 28[a] PFS: 4.8[a]	Cetuximab is effective. No difference on Betel nut's chewer
Yoshino et al. (2013) [17]	To study the effectiveness of the EXTREME protocol in the Japanese population in view of marketing approval	Open-label, single-arm, multicentre, phase II trial on selected cohort	n = 33	PFS: 4.1 (4,0–5,5) OS: 14.1 (10,2–15,4)	Efficiency of EXTREM protocol in Japanese population
De Mello et al. (2014) [15]	To study the effectiveness of the EXTREME protocol in daily practice	Uncontrolled retrospective study on unselected population (PS 2–3 = 15%)	$n=21$	OS: 11 (8.68–13.31) PFS: 8 (6.5–9.9)	Efficiency of EXTREM protocol even in an unselected population; tolerable toxicity
Lynggaard et al. (2015) [14]	To study the effectiveness of the EXTREME protocol in daily practice	Uncontrolled retrospective study on unselected population (but PS 2–3 = 0%)	$n=22$	PFS: 5.8 (4.3–7.3) OS: 7.3 (5.0–9.7)	Too small benefit to cetuximab addition. Too much toxicity. High cost
Van der Linden et al. (2016) [8]	To describe all 1st-line chemotherapies used in Netherlands and their cost	Retrospective and descriptive study	$n=40$[a]	PFS: 4.8 (3.2–6.4)[a] OS: 6.7 (4.4–8.9)[a]	Poor survival regardless of chemotherapy for a too high cost
Soulières et al. (2016) [18]	To compare 2 cetuximab manufacturing techniques to authorize cetuximab in the US	Prospective, randomized, double-blind study on selected population. No control arms without cetuximab	$n=145$	OS: 9.2 (7.1–11.8) vs 9.5 (6.9–11.4) ns PFS: 4.7 (3.5–5.8) vs 5.65 (4–6.5) ns	No difference on survival between the two manufacturing techniques

None of these studies had control arms without cetuximab

PFS Progression-free survival, *OS* overall survival, *ns* not significant

[a]Only the results for the EXTREME protocol in first line are reported

The *KRAS-LCS6* variant was first described in 2008 as a risk factor for non-small cell lung cancer by Chin et al. [10]. This constitutive variation is observed in 15–30% of the population, which is comparable to the 25% found in our cohort. Following conflicting results regarding a possible association between *KRAS-LCS6* variant and cancer incidence, a large study analyzed 513 HNSCC cancer cases and 537 controls [12], and two recent meta-analyses including various primary tumors (breast, colorectal, lung, ovary, head and neck) did not find any relationship between *KRAS-LCS6* variant and cancer incidence [20, 21].

In contrast, the prognostic role of *KRAS-LCS6* variant is still debated. While Smits et al. [22] reported a better prognosis in early colorectal cancer patients harboring the variant ($n=409$), Graziano et al. [23] found a worse prognostic of the variant in advanced colorectal cancer ($n=134$). Among gynecologic cancers, a large study including 15,357 ovarian cancers and 37,640 breast cancers did not find any association between *KRAS-LCS6* and OS [24]. Concerning HNSCC, de Ruyck et al. [11] found a better survival in case of *KRAS-LCS6* variant in oropharyngeal cancers ($n=122$), while Christensen et al. [12] reported a worse prognosis in oral cavity cancer patients harboring this variant and no effect on survival in pharyngeal cancer ($n=344$).

More recently, a small retrospective study on 26 R/M HNSCC patients treated by PFUCx reported a better PFS in *KRAS-LCS6* variant patients treated by Cx ($p=0.03$) compared to non-variant group ($p=0.57$) [9]. In localized disease, an ad hoc analysis of the RTOG 0522 study investigated response to Cx according to *KRAS* status in HNSCC patients treated by radiotherapy + cisplatin ± Cx ($n=413$). In this study, patients treated by Cx and harboring a *KRAS-LCS6* variant had a better PFS at 1 year ($p=0.04$) but not thereafter ($p=0.28$), and a better OS at 2 years ($p=0.03$) but not thereafter ($p=0.23$), while there was no benefit of Cx in non-variant group [13]. These results are hardly comparable to ours, since the populations were different (curable HNSCC treated by multimodal therapies vs metastatic HNSCC treated by chemotherapy only). Furthermore, Weidhass et al. [13] results are positive only at early time but not thereafter which may indicate that the influence of the *KRAS-LCS6* variant is probably low and may disappear in R/M disease. In our study and whatever the location, *KRAS-LCS6* variant was not associated with a peculiar outcome neither in OS ($p=0.6$) nor PFS ($p=0.7$). Although the number of patients with KRAS LCS6 remains relatively low in our study, it is higher than that of Chung et al. [9] which presented only seven patients mutated and treated with cetuximab. Moreover, their results on these few cases have never been confirmed or reversed in the same population. Taken together, all these findings hardly support a potential clinical usefulness of the *KRAS-LCS6* variant in HNSCC.

Moreover, at a biological level, if the effects of this genetic polymorphism are still discussed, most in vivo studies found a link between *KRAS-LCS6* variant and hyperexpression of *KRAS* [9, 10, 25]. Lack of binding of miR Let7 within the RNA-induced silencing complex (RISC) and the *KRAS* mRNA would induce a loss of negative control over KRAS synthesis. This lack of Let7 fixation is associated with a decrease of Let7 level in variant cell lines [26]. Therefore, more than KRAS amount itself, loss of Let7 and of its other regulatory activities on mRNA (*cMyc, CDK4-6, BAX2* ...) could explain the abnormalities observed in *KRAS-LCS6* variant cells. Thus, therapies targeting the KRAS pathway may not be efficient in tumors harboring these alterations. Whatever, as shown in our work, hypotheses made on biological data need to be confirmed at HNSCC patient level.

Conclusion

This retrospective study confirmed the effectiveness of the PFUCx protocol on PFS in an unselected population of R/M HNSCC patients and regardless of *KRAS-LCS6* variant. Predictive factors of response to Cx are still awaited according to the overall small benefit and the cost of this treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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