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p16, HPV, and Cetuximab: What Is the Evidence?

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Abstract

Squamous cell carcinoma of the head and neck (SCCHN) is the sixth most common cancer worldwide. It has recently been appreciated that human papillomavirus (HPV) status (or p16 status, which is a frequently used surrogate for HPV status) is prognostic for oropharyngeal SCCHN. Here, we review and contextualize existing p16 and HPV data, focusing on the cetuximab registration trials in previously untreated, locoregionally advanced, nonmetastatic SCCHN (LA SCCHN) and in recurrent and/or metastatic SCCHN (R/M SCCHN): the IMCL-9815 and EXTREME clinical trials, respectively. Taken together, the available data suggest that, while p16 and HPV are prognostic biomarkers in patients with LA SCCHN and R/M SCCHN, it could not be shown that they are predictive for the outcomes of the described cetuximab-containing trial regimens. Consequently, although HPV status provides prognostic information, it is not shown to predict therapy response, and so is not helpful for assigning first-line therapy in patients with SCCHN. In addition, we discuss assays currently used to assess p16 and HPV status, as well as the differentiation between these two biomarkers. Ultimately, we believe HPV E6/E7 polymerase chain reaction–based mRNA testing may represent the most informative technique for assessing HPV status in patients with SCCHN. While p16 is a valid surrogate for HPV status in oropharyngeal carcinoma (OPC), there is a higher risk of discordance between p16 and HPV status in non-OPC SCCHN. Collectively, these discussions hold key implications for the clinical management of SCCHN. ***The Oncologist*** 2017;22:811–822

Implications for Practice: Human papillomavirus (HPV) status (or its commonly utilized surrogate p16) is a known prognostic biomarker in oropharyngeal squamous-cell carcinoma of the head and neck (SCCHN). We evaluated implications of the available evidence, including cetuximab registration trials in previously untreated locoregionally advanced (LA) SCCHN and recurrent and/or metastatic (R/M) SCCHN. We conclude that, although p16 and HPV are prognostic biomarkers for both LA and R/M SCCHN, they have not been shown to be predictive of response to the described cetuximab-containing regimens for either indication. Thus, current evidence suggests that benefits of cetuximab are observed in both p16-/HPV-positive and -negative SCCHN.

Introduction

Squamous-cell carcinoma of the head and neck (SCCHN) is one of the most frequently diagnosed cancers, with an annual global incidence of more than 500,000 new cases and a death toll of approximately 300,000 patients per year [1, 2]. At the time of diagnosis, the majority of patients with SCCHN present with stage III or IVA-B disease. Nevertheless, because relatively few patients present with incurable distant metastatic disease, most patients with locally advanced SCCHN can still be treated with curative intent.

Generally, the clinical management of patients with locally advanced stage III and stage IV SCCHN is dependent on the extent of disease and the primary site [3, 4]. Patients with previously untreated, locoregionally advanced (LA), nonmetastatic SCCHN who are treated nonsurgically should typically receive radiotherapy (RT) in combination with high-dose cisplatin. An alternative option, RT plus cetuximab, is used in those patients for whom RT plus high-dose cisplatin is not appropriate because of absolute or relative contraindications or in whom it

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is deemed unacceptable after a physician–patient discussion [5–7]. No formal comparison exists to date between cisplatin and cetuximab in combination with RT. Other treatment options for patients with LA SCCHN include, but are not limited to, surgery with or without postoperative RT and with or without cisplatin. In some selected cases, induction chemotherapy with docetaxel, cisplatin, and 5-fluorouracil (5-FU) followed by RT with or without platinum or cetuximab could be considered [6]. Current guidelines recommend that patients with an acceptable performance status who have recurrent and/or metastatic (R/M) SCCHN are treated with a platinum (either cisplatin or carboplatin) plus 5-FU plus cetuximab [8].

Although the SCCHN field has historically been plagued by a dearth of informative biomarkers, it has recently been appreciated that human papillomavirus (HPV) status has prognostic value in patients with oropharyngeal SCCHN, with patients with HPV-positive tumors characterized by improved outcomes relative to patients with HPV-negative disease [9–11]. Indeed, HPV-associated oropharyngeal cancer represents a distinct disease entity. p16 status has emerged as a commonly utilized surrogate biomarker for HPV status because of the cost effectiveness of testing for its presence or absence in tumor cells [12]. Although this technique is commonly deployed in oropharyngeal carcinoma (OPC), concordance between the two biomarkers is far less than 100% in non-OPC SCCHN. It is therefore important to ensure appropriate specificity and clarity of terminology when describing p16 and HPV analyses. In the current literature, these terms are often used interchangeably, and this could lead to potentially inconsistent conclusions between studies in non-OPC SCCHN and between analyses of OPC and non-OPC patient populations.

Irrespective of these terminological considerations, the importance of the observed prognostic value of p16 and HPV status is further underscored by the increasing incidence of HPV-positive SCCHN, particularly in patients with OPC. Additionally, it is now believed that HPV is a causative agent for the majority of cases of OPC in many developed countries [13–17]. Indeed, 45%–90% of newly diagnosed OPC is HPV-positive, which represents nearly twice the prevalence recorded during the late 1990s [13, 15, 18–20]. In the United States, 63.8% of patients with OPC enrolled in the Radiation Therapy Oncology Group (RTOG) 0129 study had tumors that were HPV-positive [9]. In a German prevalence rate analysis and a European validation study, 34.4% and 54.6% of patients with OPC had tumors that were p16-positive, respectively [10, 17]. Based on recent studies in Scandinavia, incidence rates of HPV-associated OPC have been rising by 3.5%–5% per annum, with the number of cases expected to double within a decade in this region [21, 22]. However, it is apparent that epidemiologic trends in p16 and HPV prevalence are subject to variation in geography and local economic status [13–15, 17]. Patients with p16-positive non-OPC SCCHN had superior outcomes relative to those of patients with p16-negative non-OPC SCCHN in an analysis of data from the RTOG 0129, 0234, and 0522 studies [23], suggesting that the prognostic influence of p16 status does not appear to be exclusively confined to patients with OPC; however, the generalizability of these observations in non-OPC SCCHN remains somewhat controversial and requires further studies to confirm. Finally, it has been appreciated that the incidence of HPV-positive SCCHN is substantially higher in LA versus R/M SCCHN, a difference that may—at least in part—reflect the superior prognosis of patients with HPV-positive tumors (i.e., patients with HPV-negative tumors are more likely to experience recurrences) [24–26].

In consonance with this line of thinking, there is robust empirical evidence that the biology of HPV-positive SCCHN differs fundamentally from that of HPV-negative SCCHN. For example, patients with HPV-positive SCCHN are characterized by less or no tobacco exposure, more lifetime sex partners, fewer comorbidities, and a unique molecular signature compared with patients with HPV-negative disease [14]. Furthermore, HPV-positive tumors are more commonly characterized by loss of TNF receptor–associated factor 3 and hyperactive phosphoinositide-3 kinase pathway, while HPV-negative tumors present with amplifications of *CDKN2A*, *CCND1*, *EGFR*, and *MYC* and loss of *TP53* [1]. Nevertheless, it should be noted that both HPV-positive and HPV-negative SCCHN tumors contain CD8-positive tumor-infiltrating lymphocytes [27]; moreover, smoking status (which has not always been collected in SCCHN clinical trials) is an important risk modifier even in HPV-positive disease, although there is no consensus yet regarding an optimal pack-years threshold [11, 28].

Despite the impressive progress regarding comprehension of the etiology, epidemiology, biology, and prognostic impact of HPV, the extent to which HPV status may be predictive of response to common regimens used in the treatment of LA and R/M SCCHN remains incompletely understood. As alluded to earlier, the anti–epidermal growth factor receptor (EGFR) monoclonal antibody cetuximab is used to treat both patients with LA SCCHN and those with R/M SCCHN. More specifically, in patients with LA SCCHN in the phase III IMCL-9815 trial, the addition of cetuximab to RT improved locoregional control (LRC), overall survival (OS), and progression-free survival (PFS) without increasing the frequency of grade 3 mucositis or dysphagia [29–31]. Furthermore, as established by the phase III EXTREME trial, adding cetuximab to first-line platinum plus 5-FU improved OS, PFS, disease control, and response rate in patients with R/M SCCHN and provided additional symptom relief and better physical functioning without showing a deleterious effect on quality of life [32–34]. Notably, in addition to direct receptor blockade, cetuximab can elicit antibody-dependent cellular cytotoxicity (ADCC), and prior evidence suggests that cetuximab can synergize with RT and various chemotherapeutic agents in SCCHN model systems [35–40]. Differences in these attributes—as well as their different affinities for EGFR—serve to distinguish cetuximab from several other monoclonal antibodies and tyrosine kinase inhibitors targeting EGFR [41, 42].

In this article, we review and discuss available methodologies for evaluating HPV status, as well as current evidence involving the prognostic and potential predictive value of p16 and HPV status in patients with LA or R/M SCCHN treated with cetuximab combination regimens, with an emphasis placed on recent subgroup analyses of the phase III IMCL-9815 and EXTREME trials. Because very limited data on HPV analyses for cetuximab monotherapy in heavily pretreated refractory R/M SCCHN patients suggest that cetuximab may be less effective in HPV-related disease than in HPV-unrelated SCCHN [43–45], we focus on randomized HPV data available to assess the effect of the addition of cetuximab to standard SCCHN therapy. It must be noted that p16 and HPV analyses of IMCL-9815 and EXTREME were performed retrospectively and are therefore subject to limitations commonly associated with such analyses. Due to the broad range and variability between available studies, we decided that this topic would be better addressed by a nonsystematic, rather than systematic, review process.

Table 1. Trial designs for IMCL-9815 and EXTREME

Trial, *n* Extent of disease	IMCL-9815, *n* = 424 LA SCCHN	EXTREME, *n* = 442 R/M SCCHN
Trial design	Phase III, randomized	Phase III, randomized
Arm 1	RT	Platinum + 5-FU
Arm 2	Cetuximab + RT	Cetuximab + platinum + 5-FU
Tumor sites included	• Hypopharynx • Larynx • Oropharynx	• Hypopharynx • Larynx • Oral cavity • Oropharynx
Primary endpoint	LRC	OS
Selected secondary endpoints	• OS • PFS • Safety	• PFS • Response rate • Safety
p16 evaluation	• Assessed in all evaluable patients from the ITT population (*n* = 311) and all evaluable patients in the OPC subgroup (*n* = 182) • Assay: IHC • Threshold for positivity: strong and diffuse nuclear and cytoplasmic staining in ≥70% of the tumor cells	• Assessed in all evaluable patients in the ITT population (*n* = 416) and all evaluable patients in the OPC subgroup (*n* = 136) • Assay: IHC • Threshold for positivity: >70% of tumor cells displaying moderate or strong and diffuse nuclear staining (irrespective of cytoplasmic staining intensity)
p16 distribution (ITT population)	• p16-positive: 83 (27%) • p16-negative: 228 (73%)	• p16-positive: 41 (10%) • p16-negative: 340 (82%) • Inconclusive: 35 (8%)
p16 distribution (OPC subgroup)	• p16-positive: 75 (41%) • p16-negative: 107 (59%)	• p16-positive: 24 (18%) • p16-negative: 112 (82%)
HPV evaluation	• Assessed in all evaluable p16-positive samples from the ITT population (*n* = 69) and the OPC subgroup (*n* = 63) • Assay: ISH • Threshold for positivity: specific staining of tumor cell nuclei	• Assessed in all evaluable patients in the ITT population (*n* = 416) and all evaluable patients in the OPC subgroup (*n* = 110) • Assay: Cervista FRET-based test • Threshold for positivity: HPV signal-to-noise ratio ≥2 and a pre-specified internal control signal-to-noise ratio
HPV distribution (ITT population)	• HPV-positive: 54 (78%) • HPV-negative: 15 (22%)	• HPV-positive: 24 (6%) • HPV-negative: 297 (71%) • Inconclusive: 70 (17%) • Assay failed: 25 (6%)
HPV distribution (OPC subgroup)	• HPV-positive: 49 (78%) • HPV-negative: 14 (22%)	• HPV-positive: 18 (16%) • HPV-negative: 92 (84%)
Concordance between p16-positivity and HPV-positivity	• ITT population: 78% (54/69 patients) • OPC subgroup: 78% (49/63 patients) • Non-OPC subgroup: 83% (5/6 patients)	• ITT population: 56% (19/34 patients) • OPC subgroup: 80% (16/20 patients) • Non-OPC subgroup: 21% (3/14 patients)

Abbreviations: 5-FU, 5-fluorouracil; FRET, fluorescence resonance energy transfer; HPV, human papillomavirus; IHC, immunohistochemistry; ISH, in situ hybridization; ITT, intention to treat; LA SCCHN, locoregionally advanced squamous cell carcinoma of the head and neck; LRC, locoregional control; OPC, oropharyngeal carcinoma; OS, overall survival; PFS, progression-free survival; R/M SCCHN, recurrent and/or metastatic squamous cell carcinoma of the head and neck; RT, radiotherapy.

Materials and Methods

In developing this nonsystematic review, we queried PubMed, as well as American Society of Clinical Oncology and European Society for Medical Oncology annual meeting abstracts, to identify studies and review articles relevant to the prognostic and potentially predictive characteristics of HPV infection in patients with SCCHN. While there were no formal inclusion or exclusion criteria, priority was granted to clinical studies that were phase III or utilized a randomized study design. Outputs of the search results were hand-curated. No unpublished material is included in this review.

Available Assays for the Detection of HPV Status in SCCHN

At present, there is no consensus regarding the optimal methodology for assessment of HPV status in patients with SCCHN. As mentioned earlier, p16 is commonly deployed as a surrogate biomarker for HPV status [9–11]. The biological rationale underlying this surrogacy stems from the fact that the HPV E7 viral protein triggers degradation of the retinoblastoma tumor suppressor protein in infected cells, which in turn initiates a feedback loop that results in the activation of senescence-promoting pathways that include increased expression of p16. Hence, p16 status directly provides a general readout of retinoblastoma protein (RB) activity, leading to the possibility of discordance between p16 status and HPV status in cases in which RB is inactivated via HPV-independent mechanisms (i.e., p16-positive but HPV-negative tumors).

p16 status is typically assessed via immunohistochemistry (IHC), a strategy that affords the advantages of a relatively technically and analytically straightforward assay that possesses high sensitivity [46]. Although there is a widely accepted cutoff

Table 2. Efficacy outcomes of the IMCL-9815 trial by p16 status

Population	Parameter	p16: efficacy outcomes (3-year rate, %)				p16: HR [95% CI]			
		p16+		p16−		Biomarker effect (prognostic)		Treatment effect (predictive)	
		Cetuximab + RT (*n* = 44)	RT (*n* = 39)	Cetuximab + RT (*n* = 107)	RT (*n* = 121)	Cetuximab + RT (*n* = 151)	RT (*n* = 160)	p16+ (*n* = 83)	p16− (*n* = 228)
ITT	LRC	85.5	64.9	31.0	21.4	0.12 [0.05–0.27]	0.29 [0.16–0.51]	0.35 [0.13–0.90]	0.79 [0.58–1.08]
	OS	86.4	73.3	40.6	35.5	0.17 [0.08–0.35]	0.35 [0.20–0.62]	0.45 [0.19–1.06]	0.92 [0.67–1.25]
	PFS	81.0	64.2	28.5	16.7	0.17 [0.09–0.35]	0.29 [0.17–0.51]	0.47 [0.21–1.08]	0.77 [0.57–1.04]
		Cetuximab + RT (*n* = 41)	RT (*n* = 34)	Cetuximab + RT (*n* = 43)	RT (*n* = 64)	Cetuximab + RT (*n* = 84)	RT (*n* = 98)	p16+ (*n* = 75)	p16− (*n* = 107)
OPC	LRC	87.0	65.4	31.6	19.8	0.12 [0.05–0.30]	0.30 [0.16–0.58]	0.31 [0.11–0.88]	0.78 [0.49–1.25]
	OS	87.8	72.3	41.9	33.5	0.16 [0.07–0.36]	0.40 [0.21–0.74]	0.38 [0.15–0.94]	0.93 [0.59–1.48]
	PFS	82.1	64.7	29.1	15.6	0.18 [0.08–0.40]	0.30 [0.16–0.57]	0.46 [0.19–1.10]	0.76 [0.48–1.21]

Abbreviations: CI, confidence interval; HR, hazard ratio; ITT, intention to treat; LRC, locoregional control; OPC, oropharyngeal carcinoma; OS, overall survival; PFS, progression-free survival; RT, radiotherapy.

of 70% for p16-positive tumor cells when determining p16 status, not all studies adhere to it [31, 42, 47]. Furthermore, as with any surrogate biomarker, there is a risk of discordance between p16 status and the actual HPV status, which can be exacerbated by a failure to use a stringent cutoff (50%–70%) for percentage p16-positive tumor cells. Current estimates posit that the discordance rate between p16 IHC and direct detection of HPV DNA/RNA may approach 25%, with p16-positive but HPV-negative tumors constituting the majority of discordant cases (perhaps for the reasons outlined above) [48, 49]. This discordance is generally lower for OPC SCCHN. For example, studies have shown that although the positive predictive value of p16 IHC as a surrogate for HPV status was 92.7% in patients with OPC, this value dropped to 41.3% in patients with non-OPC SCCHN [50–52]. Accordingly, whereas utilization of p16 as a surrogate biomarker for HPV status is less of a valid approach in patients with non-OPC, it is more valid in OPC SCCHN [53].

Because determination of p16 status does not differentiate between HPV16 and non-HPV16 subtypes [54], several other methods for the determination of HPV status are also available. HPV DNA detection in tumors does not directly prove causal association between the viral infection and SCCHN, as HPV is ubiquitously present in humans. Therefore, HPV DNA polymerase chain reaction (PCR) is a sensitive, but not specific, method for determination of HPV status. p16 immunohistochemistry followed by PCR for HPV DNA has been proposed as a reliable algorithm for detection of HPV in fresh or paraffin-embedded OPC specimens, combining both the specificity of p16 IHC with the sensitivity of HPV DNA PCR, and therefore helping detect higher-risk cases by identifying the causal relationship between presence of HPV DNA and an active infection [55].

Another option is HPV DNA or RNA in situ hybridization (ISH), which can differentiate between integrated and episomal forms of HPV in tumors but also lacks sensitivity. HPV RNA-ISH specifically affords the advantages of tumor-specific expression of the target mRNA and the temporal advantage of preneoplastic expression of viral E6 mRNA [56]. However, the ISH protocol itself is not always feasible given limited sample availability and the necessity for fresh, and not frozen or paraffin-embedded, tissue samples. Furthermore, the HPV-RNA ISH protocol is currently being used in clinical trials to determine HPV-positive versus negative status only [57].

We believe that the most informative method for determining HPV status involves the direct detection of viral E6/E7 mRNA in fresh tissue samples via PCR. A drawback of this method is the potential for decreased sensitivity in lower-quality clinical samples or samples with low E6/E7 expression [58, 59]. On the other hand, advantages of this method include generally high sensitivity, specificity, and tumor-specific expression of the mRNA/DNA target [56]. Moreover, this assay can be deployed not only on fresh tumor samples, but also, when necessary, on formalin-fixed, paraffin-embedded tissue blocks (although the samples require additional quality-control evaluation, adding a layer of complexity to the protocol) [13, 60]. Thus, we believe that HPV E6/E7 mRNA detection via PCR could evolve as a new standard for assessing HPV status in patients with SCCHN due to its overall superiority and practicality. This technology is especially important for non-OPC SCCHN, in which the concordance between p16 and HPV status is less clear.

Overview of p16 and HPV Subgroup Analyses From the IMCL-9815 and EXTREME Trials

The overall designs and subgroup distributions of the IMCL-9815 and EXTREME trials are summarized in Table 1 [24, 25, 29, 30,

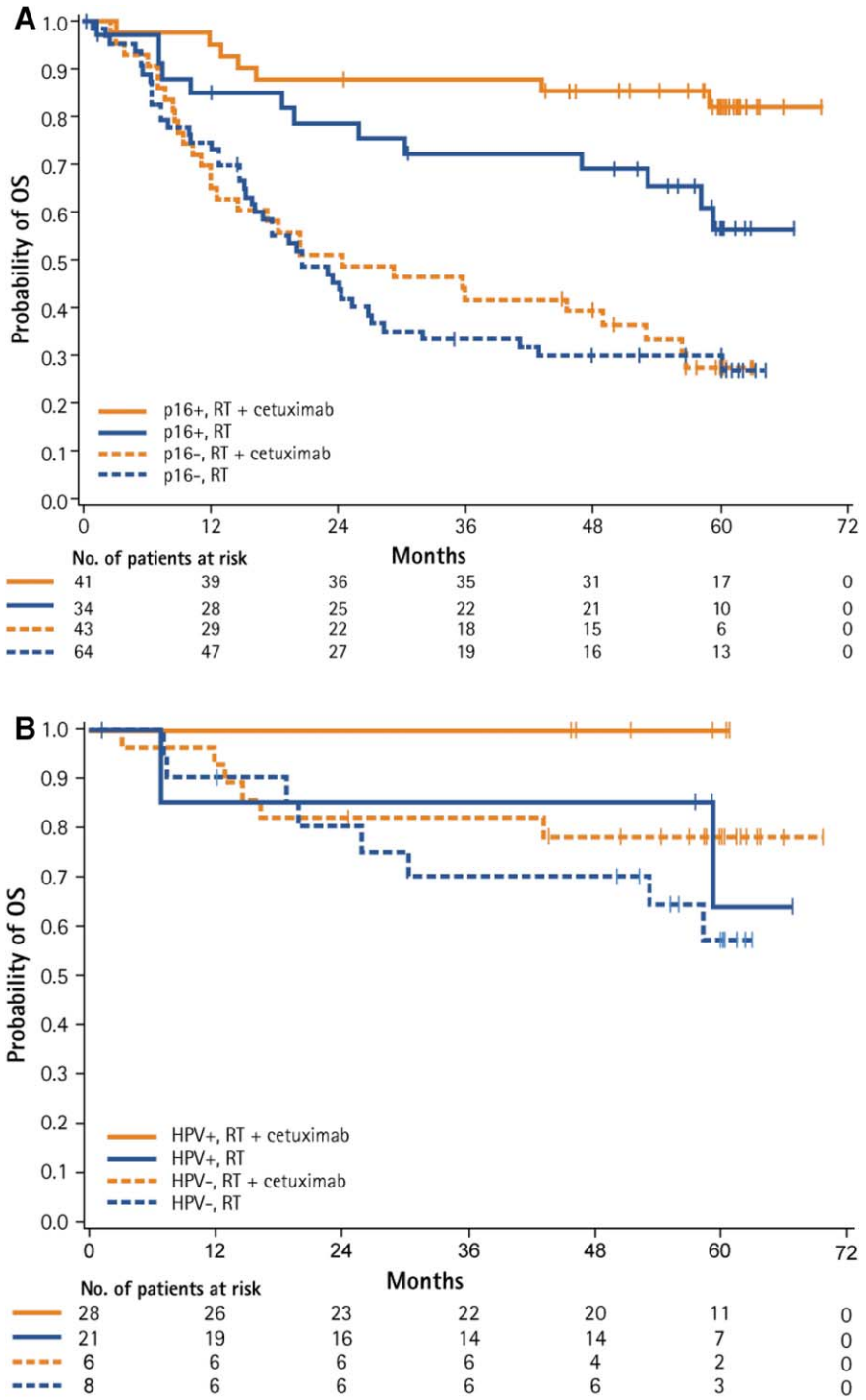

Figure 1. Effect of p16 **(A)** and HPV **(B)** status on OS in patients with locoregionally advanced squamous-cell carcinoma of the head and neck treated with RT ± cetuximab in the oropharyngeal carcinoma subgroup. Reprinted from [24] with permission © 2016 American Society of Clinical Oncology. All rights reserved.

Abbreviations: HPV, human papillomavirus; OS, overall survival; RT, radiotherapy.

32, 33, 61–63]. Many North American patients were included in the IMCL-9815 trial, whereas EXTREME included many patients from southern Europe; distinctions between these populations could account for any differences in p16/HPV status between the two trials. Additionally, although we believe that HPV E6/E7 mRNA detection via PCR is the most informative method for HPV status determination, the analyses in the IMCL-9815 and EXTREME trials were performed using the most scientifically recognized methods available at the time.

p16 and HPV as Potential Prognostic Biomarkers

p16 in LA SCCHN

Within the IMCL-9815 intention-to-treat (ITT) population, patients with p16-positive tumors had superior LRC, OS, and PFS than those with p16-negative tumors in both the cetuximab plus RT and RT alone treatment arms. The same observation was made for the OPC subgroup (Table 2, Fig. 1) [24, 61–63].

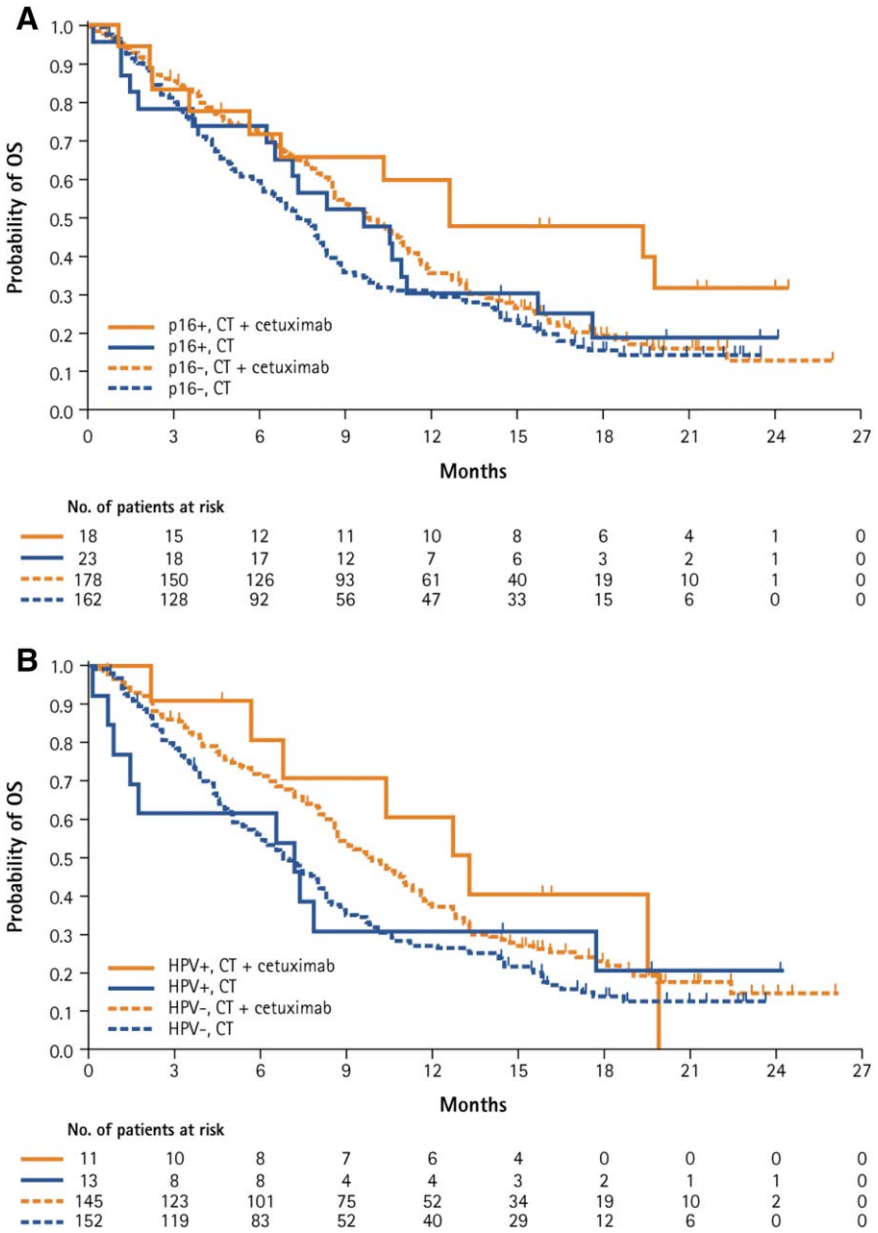

Figure 2. Effect of p16 **(A)** and HPV **(B)** status on OS in patients with recurrent and/or metastatic squamous-cell carcinoma of the head and neck treated with platinum + 5-fluorouracil ± cetuximab in the intention-to-treat population. Reprinted from [25] by permission of Oxford University Press and the European Society for Medical Oncology.

Abbreviations: CT, chemotherapy; HPV, human papillomavirus; OS, overall survival.

p16 in R/M SCCHN

Analogously, in both the ITT population and the OPC subgroup of EXTREME, p16-positive status was associated with better OS in both the cetuximab plus platinum plus 5-FU and platinum plus 5-FU treatment arms. In the ITT population, PFS and response rate favored p16-positive status in the platinum plus 5-FU arm, but did not unambiguously differ based on p16 status in the cetuximab plus platinum plus 5-FU arm. Therefore, no clear and consistent prognostic role for p16 status in terms of its influence on PFS and response rate in the ITT population could be established. Due to the small number of patients with p16-positive OPC in this trial, these data are insufficient for a definitive conclusion to be drawn (Table 4, Fig. 2) [25, 63].

HPV in LA SCCHN

Given the small number of patients with p16-positive but HPV-negative tumors, it is difficult to draw firm conclusions regarding the putative prognostic role of HPV status in this group regarding the endpoints of LRC, OS, and PFS from either the IMCL-9815 trial ITT population or OPC subgroup (Table 3, Fig. 1) [24, 61–63].

HPV in R/M SCCHN

There was a trend toward longer OS in the HPV-positive versus HPV-negative subgroup of the EXTREME ITT population in both the cetuximab plus platinum plus 5-FU and platinum plus 5-FU

Table 3. Efficacy outcomes of the IMCL-9815 trial by HPV status.

		HPV: efficacy outcomes (3-year rate)				HPV: HR [95% CI]			
		HPV+		HPV−		Biomarker effect (prognostic)		Treatment effect (predictive)	
Population	Parameter	Cetuximab + RT ($n = 30$)	RT ($n = 24$)	Cetuximab + RT ($n = 6$)	RT ($n = 9$)	Cetuximab + RT ($n = 36$)	RT ($n = 33$)	HPV+ ($n = 54$)	HPV− ($n = 15$)
ITT	LRC	82.8	60.9	100	75.0	N/A	1.67 [0.36–7.74]	0.42 [0.14–1.26]	N/A
	OS	83.3	69.8	100	87.5	N/A	1.78 [0.38–8.27]	0.50 [0.18–1.41]	N/A
	PFS	79.9	60.2	80.0	75.0	1.21 [0.15–9.94]	1.88 [0.41–8.58]	0.51 [0.20–1.35]	0.69 [0.06–7.68]
		Cetuximab + RT ($n = 28$)	RT ($n = 21$)	Cetuximab + RT ($n = 6$)	RT ($n = 8$)	Cetuximab + RT ($n = 34$)	RT ($n = 29$)	HPV+ ($n = 49$)	HPV− ($n = 14$)
OPC	LRC	81.5	64.8	100	71.4	N/A	1.28 [0.27–6.18]	0.52 [0.16–1.63]	N/A
	OS	82.1	70.4	100	85.7	N/A	1.56 [0.33–7.39]	0.53 [0.18–1.52]	N/A
	PFS	78.4	63.8	80.0	71.4	1.31 [0.16–10.75]	1.45 [0.31–6.82]	0.63 [0.23–1.74]	0.59 [0.05–6.57]

Abbreviations: CI, confidence interval; HPV, human papillomavirus; HR, hazard ratio; ITT, intention-to-treat; LRC, locoregional control; N/A, not applicable; OPC, oropharyngeal carcinoma; OS, overall survival; PFS, progression-free survival; RT, radiotherapy.

Table 4. Efficacy outcomes of the EXTREME trial by p16 status.

		p16: efficacy outcomes				p16: HR/odds ratio [95% CI]			
		p16+		p16−		Biomarker effect (prognostic)		Treatment effect (predictive)	
Population	Parameter	Cetuximab + platinum + 5-FU ($n = 18$)	Platinum + 5-FU ($n = 23$)	Cetuximab + platinum + 5-FU ($n = 178$)	Platinum + 5-FU ($n = 162$)	Cetuximab + platinum + 5-FU ($n = 196$)	Platinum + 5-FU ($n = 185$)	p16+ ($n = 41$)	p16− ($n = 340$)
ITT	OS, mo	12.6	9.6	9.7	7.3	0.59 [0.32–1.10]	0.83 [0.50–1.36]	0.63 [0.30–1.34]	0.82 [0.65–1.04]
	PFS, mo	5.6	3.6	5.7	3.1	1.17 [0.69–2.01]	0.87 [0.53–1.43]	0.73 [0.36–1.47]	0.49 [0.38–0.63]
	Response rate, %	50	22	37	17	1.74 [0.66–4.60]	1.33 [0.46–3.88]	3.60 [0.93–13.95]	2.75 [1.66–4.58]
		Cetuximab + platinum + 5-FU ($n = 8$)	Platinum + 5-FU ($n = 16$)	Cetuximab + platinum + 5-FU ($n = 65$)	Platinum + 5-FU ($n = 47$)	Cetuximab + platinum + 5-FU ($n = 73$)	Platinum + 5-FU ($n = 63$)	p16+ ($n = 24$)	p16− ($n = 112$)
OPC	OS, mo	19.4	9.5	10.8	7.9	0.40 [0.14–1.12]	0.76 [0.39–1.50]	0.51 [0.16–1.61]	0.86 [0.56–1.33]
	PFS, mo	7.5	4.3	5.9	3.2	0.79 [0.36–1.75]	0.98 [0.50–1.91]	0.53 [0.20–1.37]	0.51 [0.32–0.80]
	Response rate, %	75	13	32	21	6.29 [1.17–33.82]	0.53 [0.10–2.72]	21.00 [2.37–185.93]	1.77 [0.74–4.22]

HRs are presented for OS and PFS, whereas odds ratios are presented for response rate.
Abbreviations: 5-FU, 5-fluorouracil; CI, confidence interval; HR, hazard ratio; ITT, intention to treat; mo, months; OPC, oropharyngeal carcinoma; OS, overall survival; PFS, progression-free survival.

Table 5. Efficacy outcomes of the EXTREME trial by HPV status.

Population	Parameter	HPV: efficacy outcomes				HPV: HR/odds ratio [95% CI]			
		HPV+		HPV−		Biomarker effect (prognostic)		Treatment effect (predictive)	
		Cetuximab + platinum + 5-FU ($n = 11$)	Platinum + 5-FU ($n = 13$)	Cetuximab + platinum + 5-FU ($n = 145$)	Platinum + 5-FU ($n = 152$)	Cetuximab + platinum + 5-FU ($n = 156$)	Platinum + 5-FU ($n = 165$)	HPV+ ($n = 24$)	HPV− ($n = 297$)
ITT	OS, mo	13.2	7.1	9.7	6.7	0.80 [0.39–1.63]	0.92 [0.48–1.77]	0.72 [0.28–1.83]	0.73 [0.56–0.94]
	PFS, mo	4.8	4.3	5.6	3.0	1.18 [0.62–2.27]	1.12 [0.60–2.08]	0.48 [0.19–1.21]	0.50 [0.38–0.66]
	Response rate, %	64	8	34	20	3.43 [0.96–12.28]	0.33 [0.04–2.60]	21.00 [1.94–227.21]	1.99 [1.18–3.63]
		Cetuximab + platinum + 5-FU ($n = 8$)	Platinum + 5-FU ($n = 10$)	Cetuximab + platinum + 5-FU ($n = 48$)	Platinum + 5-FU ($n = 44$)	Cetuximab + platinum + 5-FU ($n = 56$)	Platinum + 5-FU ($n = 54$)	HPV+ ($n = 18$)	HPV− ($n = 92$)
OPC	OS, mo	19.4	7.2	10.9	7.3	0.65 [0.25–1.69]	1.09 [0.50–2.37]	0.47 [0.15–1.47]	0.74 [0.46–1.20]
	PFS, mo	5.8	4.3	5.9	2.9	1.11 [0.49–2.52]	1.34 [0.60–2.97]	0.32 [0.10–1.02]	0.54 [0.33–0.87]
	Response rate, %	75	0	31	25	6.60 [1.19–36.59]	N/A	N/A	1.36 [0.55–3.41]

HRs are presented for OS and PFS, whereas odds ratios are presented for response rate.

Abbreviations: 5-FU, 5-fluorouracil; CI, confidence interval; HPV, human papillomavirus; HR, hazard ratio; ITT, intention to treat; mo, months; OPC, oropharyngeal carcinoma; OS, overall survival; PFS, progression-free survival.

treatment arms. This observation persisted in the cetuximab plus platinum plus 5-FU arm, but not in the platinum plus 5-FU arm of the OPC subgroup. Consistent with the p16 analysis, there was no clear and consistent prognostic effect on PFS and response rate for HPV status in the cetuximab plus platinum plus 5-FU and platinum plus 5-FU arms of the ITT population. An analogous conclusion was reached for the OPC subgroup (Table 5, Fig. 2) [25, 63].

p16 and HPV as Potential Predictive Biomarkers

p16 in LA SCCHN

Within the IMCL-9815 ITT population, adding cetuximab to RT resulted in superior 3-year LRC, OS, and PFS in patients with both p16-positive and p16-negative tumors. The addition of cetuximab to RT also increased 3-year LRC, OS, and PFS in patients with both p16-positive and p16-negative tumors within the OPC subgroup of IMCL-9815. Although the treatment effects were stronger in the p16-positive subgroup, interaction tests for LRC, OS, and PFS revealed no significant interaction between p16 status and treatment both in the ITT population ($p = .098$, $p = .134$, and $p = .252$, respectively) and the OPC subgroup ($p = .087$, $p = .085$, and $p = .253$, respectively; Table 2, Fig. 1) [24, 61–63].

> Within the IMCL-9815 ITT population, adding cetuximab to RT resulted in superior 3-year LRC, OS, and PFS in patients with both p16-positive and p16-negative tumors. The addition of cetuximab to RT also increased 3-year LRC, OS, and PFS in patients with both p16-positive and p16-negative tumors within the OPC subgroup of IMCL-9815.

p16 in R/M SCCHN

OS and PFS were numerically improved in patients treated with cetuximab plus platinum plus 5-FU as compared with patients treated with platinum plus 5-FU in both the p16-positive and p16-negative subgroups of the EXTREME trial ITT population. Interaction tests for OS ($p = .482$) and PFS ($p = .430$) further underlined that the treatment effect persisted regardless of p16 status. In addition, adding cetuximab to platinum plus 5-FU improved the response rate in both patients with p16-positive and p16-negative disease. Similarly, within the OPC subgroup of EXTREME, there was a trend toward improved OS and PFS in cetuximab-treated patients in both the p16-positive and p16-negative subgroups, and the addition of cetuximab to platinum plus 5-FU improved—at least numerically—the response rate in both subgroups of patients with OPC. Interaction tests for OPC subgroups were not performed due to the very small sample sizes (Table 4, Fig. 2) [25, 63].

HPV in LA SCCHN

Although the number of patients in the p16-positive, HPV-evaluable ITT subgroup from the IMCL-9815 trial was small, 3-

year LRC, OS, and PFS data appeared to be consistent with those previously obtained during the p16 subgroup analysis for the HPV-positive subgroup. The small size of the HPV-negative subgroup precluded drawing meaningful conclusions. While similar statistical considerations apply to the IMCL-9815 p16-positive HPV-evaluable OPC subgroup, 3-year LRC, OS, and PFS again seemed similar to the findings reported in the p16 subgroup analysis for the HPV-positive subgroup. The small size of the HPV-negative subgroup did not permit drawing meaningful conclusions (Table 3, Fig. 1) [24, 61–63].

HPV in R/M SCCHN

In consonance with the findings of the p16 subgroup analysis—although the OS and PFS difference between treatment arms only reached a p value smaller than .05 in the HPV-negative subgroup—OS and PFS were longer in cetuximab-treated patients regardless of HPV status. Furthermore, no clear interaction was suggested between HPV status and treatment for either OS ($p = .824$) or PFS ($p = .975$) in the ITT population. Analogously, the addition of cetuximab to platinum plus 5-FU resulted in increased response rate in both the HPV-positive and HPV-negative subgroups of the ITT population. In the OPC subgroup of EXTREME, OS was numerically better and PFS was improved in patients receiving cetuximab in both the HPV-positive and HPV-negative subgroups. Additionally, adding cetuximab to platinum plus 5-FU numerically improved the response rate in patients with HPV-negative tumors; drawing meaningful conclusions regarding response rate in the HPV-positive subgroup was not possible in light of the small number of patients. Interaction tests were not performed due to the very small sample size (Table 5, Fig. 2) [25, 63].

Key Conclusions From the p16 and HPV Subgroup Analyses of the IMCL-9815 and EXTREME Trials

These subgroup analyses of the IMCL-9815 and EXTREME trials evaluated the roles of p16 and HPV as potential prognostic and predictive biomarkers in patients with SCCHN (LA SCCHN and R/M SCCHN, respectively) [24, 25, 61–63]. In both trials, p16 was found to be a valid surrogate for HPV in OPC. Based on observations made in the EXTREME trial and the available literature, this may not be the case in non-OPC SCCHN, although it should be noted that the high concordance between p16-positivity and HPV-positivity in the six-patient non-OPC subgroup of the IMCL-9815 trial was not in line with these conclusions. Both studies suggested that p16 and HPV are prognostic biomarkers, with biomarker positivity associated with increased survival, particularly for OPC [24, 25, 61–63]. Additionally, both studies reported efficacy gains upon the addition of cetuximab to the control regimen (RT and platinum plus 5-FU, respectively) and looked at the biomarker subgroups of p16-positive, p16-negative, HPV-positive, and HPV-negative OPC; interaction tests did not show a significant interaction between biomarker status and treatment effect [24, 25, 61–63]. Taken together, these observations suggest that, although p16 and HPV are prognostic biomarkers in patients with LA SCCHN and R/M SCCHN, it could not be shown that they are predictive for the response to the described cetuximab-containing regimens in either indication [64]; consequently, the data suggest that the addition of cetuximab appears to provide benefit over the control arm regardless of p16 and HPV status in both LA SCCHN and R/M SCCHN.

Current Controversies and Future Outlook

Currently controversial is the extent to which the findings from the p16 and HPV subgroup analyses of IMCL-9815 and EXTREME can be extrapolated to patients receiving cetuximab monotherapy. Although this topic lies beyond the scope of the present review, which is focused on combination regimens involving cetuximab plus either RT or platinum plus 5-FU, it should be noted that very little information is presently available [44]. Our conclusions are derived from retrospective analyses of the two cetuximab registration trials, because HPV became relevant after the study completions. Further prospective validation is needed for definitive conclusions to be made.

Additionally, though further confirming the prognostic value of p16 and HPV status, ostensibly divergent results concerning the potential predictive impact of p16 and HPV status have been obtained from two studies involving the anti-EGFR monoclonal antibody panitumumab. First, the CONCERT-2 trial compared panitumumab plus RT with chemoradiotherapy (CRT) in patients with LA SCCHN. There was no significant difference between treatment arms in terms of 2-year LRC in patients with p16-positive disease, whereas 2-year LRC favored the CRT arm in patients with p16-negative tumors; the effect of HPV was very low, and outcomes favoring CRT were largely driven by patients with p16-negative LA SCCHN [47]. Second, in the SPECTRUM trial, which investigated the effect of adding panitumumab to chemotherapy in patients with R/M SCCHN, panitumumab was more active in patients with p16-negative tumors, and no benefit was observed upon the addition of panitumumab to chemotherapy in patients with p16-positive disease [42]. However, neither CONCERT-2 nor SPECTRUM met their primary endpoints in the ITT population, rendering biomarker-defined subgroup analyses from these trials difficult to interpret. An added confounding variable when interpreting CONCERT-2 and SPECTRUM is that both trials used a different p16 cutoff for positivity (10%) than did EXTREME and IMCL-9815 (70%) [24, 42, 47]. Finally, it should be reiterated that cetuximab and panitumumab are not biologically identical; indeed, their different affinities for EGFR, as well as the distinct characteristics of cetuximab-induced ADCC [39], may account for the observed apparent differences.

Because of their more favorable prognosis, a consideration for patients with HPV-positive OPC concerns the extent to which it may be possible to reduce the collateral toxicities of anticancer treatments in this subgroup while maintaining treatment [14]. Indeed, treatment deintensification for patients with LA SCCHN represents a topic of major current clinical research interest, in light of the fact that current standard-of-care treatment with high-dose CRT is associated with significant acute and late toxicities [65–69]. Accordingly, treatment regimens that reduce treatment-related toxicities and, in particular, life-threatening late side effects without compromising efficacy are urgently needed. This is particularly the case for patients with HPV-positive OPC, who are likely to experience longer durations of treatment [11]. Strategies currently under study in patients with HPV-positive SCCHN involve, but are not limited to, reducing the dose of RT and the use of bioradiation with cetuximab instead of CRT (RTOG 1016 [NCT01302834], De-EscaLate [NCT 01874171], and TROG 12.01 [NCT 01855451]). As grade 3–4 mucositis and radiation dermatitis were not found to have significantly increased with cetuximab/RT

compared with RT alone, and quality of life was not adversely affected in IMCL-9815, cetuximab/RT could be a viable replacement for CRT in patients with HPV-positive SCCHN, in the event of a positive outcome in the above-mentioned trials [31, 70]. Furthermore, there is interest in using induction chemotherapy to differentiate between patients who will need more aggressive locoregional therapy and those for whom a lower RT dose approach (reduced RT dose from 69.3 to 54 Gy) may be an option based on patient responses to induction therapy (ECOG 1308 [NCT01084083]). Other studies are focused on evaluating the use of reduced-dose CRT versus accelerated but lower-dose RT alone (NRG-002 [NCT02254278]). Finally, the ECOG 3311 trial (NCT01898494) employs transoral robotic surgery for eligible patients and uses a risk-based adjuvant therapy approach to minimize RT application, RT dose, and the concurrency of chemotherapy. Finally, approximately 20% of patients with HPV-positive SCCHN will experience disease recurrence [27]; more studies are needed to shed light on how patients with increased risk of relapse can be identified during the diagnosis and treatment of their first disease occurrence. It should also be noted that p16 continues to be widely accepted as a surrogate marker for HPV in OPC, including in scenarios such as during patient selection for enrollment into treatment de-escalation trials. While the p16 assay is not 100% specific for HPV association, and approximately 10% of OPC tumors test as p16-positive/HPV DNA-negative, this assay remains an informative and practical tool for identifying patients with OPC with a good versus poor prognosis [50].

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Conclusion

In conclusion, available data from retrospective analyses suggest that, while p16 and HPV are prognostic biomarkers in patients with LA SCCHN and R/M SCCHN, it could not be shown that they are predictive for the described cetuximab-containing regimens in either indication; consequently, although HPV testing provides important prognostic information, it is not a requirement for treating patients with SCCHN with cetuximab plus RT or platinum-based chemotherapy. Additionally, the available evidence suggests that while p16 is a valid surrogate for HPV in OPC, this may not be the case in non-OPC SCCHN. Collectively, the topics reviewed herein hold key implications for the clinical management of SCCHN and should be reviewed by oncologists before deciding how (and how not) to incorporate p16 and HPV testing into their practices. Data from ongoing prospective studies are anticipated to help resolve any remaining open questions (NCT01302834, NCT 01874171, NCT 01855451).

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Disclosures

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