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ORIGINAL REPORT

Association of Human Papillomavirus and p16 Status With Outcomes in the IMCL-9815 Phase III Registration Trial for Patients With Locoregionally Advanced Oropharyngeal Squamous Cell Carcinoma of the Head and Neck Treated With Radiotherapy With or Without Cetuximab

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ABSTRACT

Purpose

We conducted a retrospective evaluation of the IMCL-9815 study to examine the association of human papillomavirus (HPV) and p16 protein expression status with outcomes in patients with oropharyngeal carcinoma (OPC) receiving radiotherapy (RT) plus cetuximab or RT alone.

Patients and Methods

In the IMCL-9815 study, patients were randomly allocated to receive RT plus weekly cetuximab or RT alone. A subpopulation of patients with p16-evaluable OPC was retrospectively evaluated on the basis of locoregional control (LRC), overall survival (OS), and progression-free survival (PFS). Evaluable samples from patients with p16-positive OPC were also tested for HPV DNA.

Results

Tumor p16 status was evaluable in 182 patients with OPC enrolled in the IMCL-9815 study; 41% were p16 positive. When treated with RT alone or RT plus cetuximab, p16-positive patients had a longer OS than p16-negative patients (hazard ratio, 0.40; 95% CI, 0.21 to 0.74 and hazard ratio, 0.16; 95% CI, 0.07 to 0.36, respectively). The addition of cetuximab to RT increased LRC, OS, and PFS in both patients with p16-positive OPC and those with p16-negative disease. Interaction tests for LRC, OS, and PFS did not demonstrate any significant interaction between p16 status and treatment effect ($P = .087$, .085, and .253, respectively). Similar trends were observed when patients with p16-positive/HPV-positive OPC (n = 49) and those with p16-positive/HPV-negative OPC (n = 14) were compared.

Conclusion

p16 status was strongly prognostic for patients with OPC. The data suggest that the addition of cetuximab to RT improved clinical outcomes regardless of p16 or HPV status versus RT alone.

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INTRODUCTION

Human papillomavirus (HPV) status is a significant risk factor for oropharyngeal carcinoma (OPC), with 45% to 90% of patients newly diagnosed with OPC positive for HPV infection.[1-4] Patients with HPV-positive disease are somewhat younger and have less tobacco exposure, more lifetime oral sex partners, and fewer comorbidities than patients with HPV-negative cancers.[5] p16 expression status is widely used as a surrogate marker of HPV infection in OPC.[5]

Several studies have demonstrated that patients with p16-positive/HPV-positive OPC treated with concurrent chemoradiotherapy (CRT) have improved locoregional control (LRC), overall survival (OS), and progression-free survival (PFS) compared with patients with HPV-negative OPC.[6,7] Given their longer life expectancy, patients with p16-positive/HPV-positive OPC are more likely to develop late cancer treatment–related toxicities. This is especially relevant for patients

with locally advanced squamous cell carcinoma of the head and neck (LA-SCCHN) receiving CRT, which improves LRC and survival at the cost of increased acute and late toxicities.[8-12]

Cetuximab, an anti–epidermal growth factor receptor (EGFR) monoclonal immunoglobulin G1 antibody, was approved by the US Food and Drug Administration in 2006 to treat LA-SCCHN in combination with radiation therapy (RT) and recurrent and/or metastatic SCCHN in combination with platinum-based therapy with fluorouracil or after progression during platinum-based therapy.[13-15] The IMCL-9815 registration trial and 5-year follow-up data indicated that cetuximab combined with RT increased LRC, OS, and PFS in patients with LA-SCCHN compared with RT alone.[15,16] This combined treatment did not increase grade 3 mucositis or dysphagia compared with RT alone. Importantly, the greatest gains were observed in patients with OPC, whose younger age, lower tumor stage, and higher performance score were characteristic of HPV-positive disease.[15] In the p16/HPV subanalysis of the EXTREME (Erbitux in First-Line Treatment of Recurrent or Metastatic Head and Neck Cancer) trial, the authors concluded that although the magnitude of survival benefit was most pronounced in the p16-negative population, interaction tests suggested that p16 status did not affect the efficacy of cetuximab.[17] Here, we evaluated p16/HPV status and the association with treatment outcomes with the addition of cetuximab to RT in patients with untreated OPC from the IMCL-9815 registration trial.

PATIENTS AND METHODS

Patients and Study Design

The study design of this phase III randomized trial has been previously described.[15] In brief, after approval by the institutional review boards (or equivalent) at participating institutions, medically suitable patients with stage III to IV nonmetastatic LA-SCCHN were randomly assigned to receive RT once daily (2.0 Gy per fraction; five fractions per week for 7 weeks), twice daily (1.2 Gy per fraction; 10 fractions per week for 6.0 to 6.5 weeks), or concomitant boost alone (72 Gy in 6 weeks, using twice-daily fractionation for the final 2.4 weeks) or RT with weekly cetuximab. The primary end point was duration of LRC; secondary end points included OS, PFS, response rate, quality of life, and safety. In our retrospective subanalyses, patients with sufficient tissue for p16 status evaluation were included (n = 311; Fig 1). The subanalysis described in this report focused on the 182 patients with p16-evaluable OPC. After determination of p16 status, patients with p16-positive OPC (n = 75) were evaluated for HPV status (n = 63). LRC, OS, and PFS were calculated as described previously.[15]

Determination of HPV Status

p16 status was evaluated through immunohistochemical (IHC) analysis using the CINtec Histology Kit (Ventana Medical Systems, Tucson, AZ); tumor tissue from some patients was not available for the analysis. Positive p16 status was defined as strong and diffuse nuclear and cytoplasmic staining in ≥ 70% of the tumor cells.[18]

Samples positive for p16 by this threshold were tested for HPV DNA using in situ hybridization, as previously published.[17] Briefly, formalin-fixed paraffin-embedded tumor specimens were evaluated for HPV DNA with the use

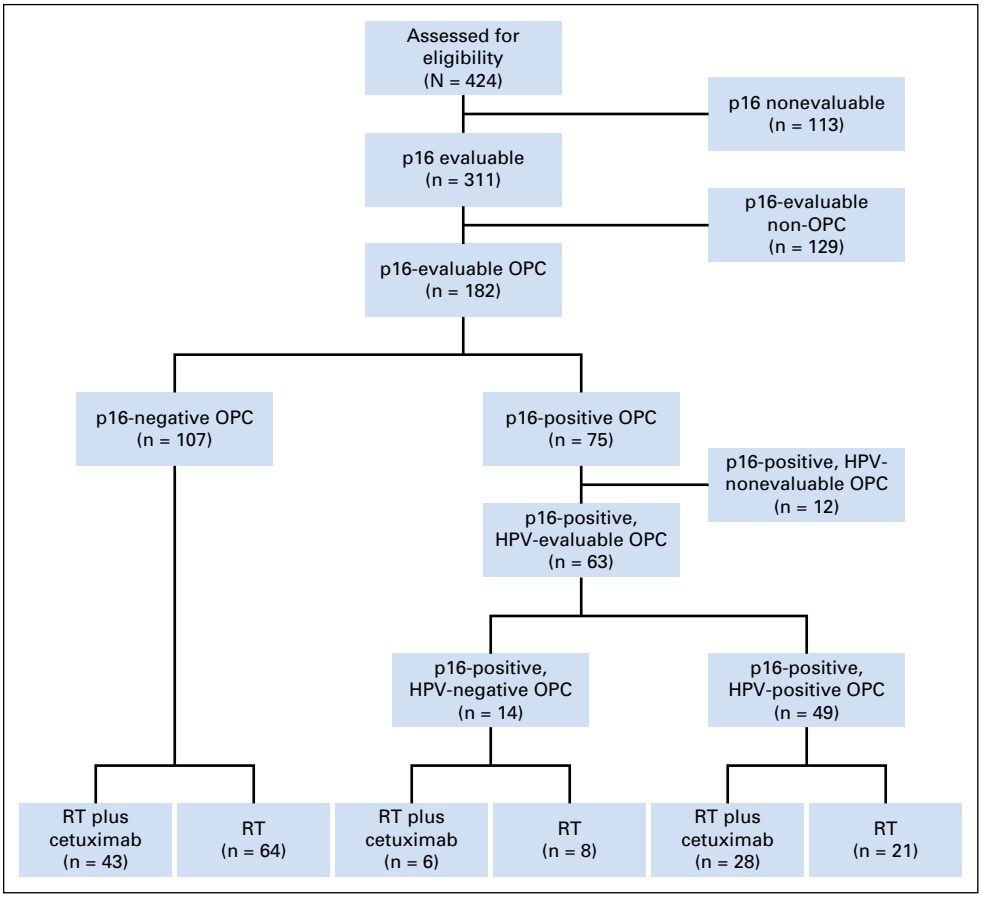

Fig 1. CONSORT diagram. HPV, human papillomavirus; OPC, oropharyngeal carcinoma; RT, radiotherapy.

of the in situ hybridization–catalyzed signal amplification method for biotinylated probes (Enzo Biochem, New York, NY). HPV positivity was defined as tumors in which specific staining of tumor cell nuclei for HPV was observed.

Statistical Methodology

Treatment and biomarker effects. The treatment effects of RT plus cetuximab versus RT alone in patients with OPC were investigated in the p16-evaluable, p16-positive, and p16-negative populations. Within the p16-positive OPC subgroup, the treatment effect of HPV status was also investigated. The number of patients, number of events, median time-to-event end points (LRC, OS, and PFS), and corresponding 95% CIs were evaluated per treatment arm; 95% CIs were calculated according to Brookmeyer and Crowley,[19] and hazard ratios (HRs; including 95% CIs) were calculated using the Cox proportional hazards model. The same methods were used to compare p16-positive and p16-negative patients with OPC for the biomarker effect.

Stratification for efficacy analysis. For intent-to-treat and p16-evaluable populations, HRs were stratified by performance score, N stage, T stage, and type of RT fractionation. Analyses in p16-positive and p16-negative patients and the treatment arms were unstratified.

Treatment–biomarker interaction test. The Cox proportional hazards model was fitted to the data from all p16-evaluable patients, with treatment arm and biomarker status as well as their interaction as explanatory variables. The interaction between the treatment effect and biomarker was tested with a two-sided Wald test.

RESULTS

Patients

The trial enrolled 253 patients with OPC; p16 subgroup analysis was performed for 182 of these patients (72%). Of the 182 patients with p16-evaluable OPC, 75 (41%) were p16 positive and 107 (59%) were p16 negative (Fig 1). In the US subgroup, 70 (60%) of 116 of the evaluable OPC samples were p16 positive. This percentage was consistent with the historical prevalence of HPV at the time this trial was performed.[7] Baseline characteristics of the p16 subgroups and the overall OPC population were broadly similar, with the exception that patients with p16-positive OPC had lower tumor and nodal stages, had higher performance status, and were predominantly from the United States (Table 1). As expected, the RT regimen to which patients were assigned was well balanced between treatment arms (Table 1). Of patients with p16-positive OPC, 75% received concomitant boost RT, 5% received once-daily RT, and 19% received twice-daily RT, as compared with patients with p16-negative disease (58%, 32%, and 9%, respectively).

p16 As a Prognostic and Predictive Biomarker

To evaluate the prognostic value of p16 status in patients with OPC, efficacy end points were determined for the p16-evaluable OPC subgroup. Because of the demonstrated treatment effect of cetuximab, the two treatment populations were examined separately. For patients treated with RT alone, statistical tests of the biomarker effect confirmed there was an improvement in LRC, OS, and PFS in patients who were p16 positive compared with those who were p16 negative (HR, 0.30; 95% CI, 0.16 to 0.58; HR, 0.40; 95% CI, 0.21 to 0.74; and HR, 0.30; 95% CI, 0.16 to 0.57, respectively). Among patients treated with RT plus cetuximab, the HRs for LRC, OS, and PFS also favored patients with p16-positive OPC compared with patients with p16-negative OPC (HR, 0.12; 95% CI, 0.05 to 0.30; HR, 0.16; 95% CI, 0.07 to 0.36; and HR, 0.18; 95% CI, 0.08 to 0.40, respectively; Table 2).

After showing the prognostic role of p16 status in this patient population, we evaluated a predictive role by examining the effect of p16 status on outcomes within a given treatment arm. The addition of cetuximab to RT improved LRC in patients with p16-evaluable OPC. Three-year LRC was greater for patients with p16-positive OPC who received RT plus cetuximab (87.0%) compared with those who received RT alone (65.4%; Table 2; Fig 2A). Similarly, patients with p16-negative OPC had a greater 3-year LRC when treated with RT plus cetuximab (31.6%) than those treated with RT alone (19.8%; Table 2; Fig 2A). HRs favored RT plus cetuximab for both the p16-positive and p16-negative subgroups (HR, 0.31; 95% CI, 0.11 to 0.88 and HR, 0.78; 95% CI, 0.49 to 1.25, respectively; Table 2). In this relatively small subset, no significant interaction between treatment group and p16 status could be shown ($P = .087$).

Comparably, gains in OS were observed in both p16-evaluable OPC subsets when cetuximab was added to RT.

Table 1. Characteristics of p16-Evaluable OPC Population

Characteristic	Total Patients With OPC (%) (n = 253)	p16 Evaluable (%)				
			Positive		Negative	
		Total (n = 182)	RT + Cetuximab (n = 41)	RT (n = 34)	RT + Cetuximab (n = 43)	RT (n = 64)
Male sex	81	79	83	82	77	77
Age < 65 years	77	75	81	74	81	67
Karnofsky performance score > 80	73	76	90	82	65	70
N0 nodal stage	11	13	7	9	14	17
T1-3 tumor stage	72	71	83	88	51	69
EGFR-positive cells, %						
≤ 50	46	59	71	62	51	55
> 50	32	40	27	38	49	44
EGFR status unknown	22	1	2	0	0	2
Concomitant boost RT regimen	58	65	78	71	56	59
Once daily RT regimen	23	21	2	9	35	30
Twice daily RT regimen	17	13	17	21	9	9
US region of origin	64	64	95	91	47	41

Abbreviations: EGFR, epidermal growth factor receptor; OPC, oropharyngeal carcinoma; RT, radiotherapy.

Table 2. Efficacy Outcomes by Treatment Arm and p16 Status in Patients With OPC

	3-Year Rate				HR (95% CI)			
	p16 Positive		p16 Negative		Treatment Effect*		Biomarker Effect†	
Outcome	RT + Cetuximab (n = 41)	RT (n = 34)	RT + Cetuximab (n = 43)	RT (n = 64)	p16 Positive (n = 75)	p16 Negative (n = 107)	RT + Cetuximab (n = 84)	RT (n = 98)
LRC	87.0	65.4	31.6	19.8	0.31 (0.11 to 0.88)	0.78 (0.49 to 1.25)	0.12 (0.05 to 0.30)	0.30 (0.16 to 0.58)
OS	87.8	72.3	41.9	33.5	0.38 (0.15 to 0.94)	0.93 (0.59 to 1.48)	0.16 (0.07 to 0.36)	0.40 (0.21 to 0.74)
PFS	82.1	64.7	29.1	15.6	0.46 (0.19 to 1.10)	0.76 (0.48 to 1.21)	0.18 (0.08 to 0.40)	0.30 (0.16 to 0.57)

NOTE. At the 3-year time point, there were a total of 33, 57, and 32 patients with p16-positive OPC and 9, 37, and 7 patients with p16-negative OPC still under observation for the calculation of LRC, OS, and PFS, respectively.
Abbreviations: HR, hazard ratio; LRC, locoregional control; OPC, oropharyngeal carcinoma; OS, overall survival; PFS, progression-free survival; RT, radiotherapy.
*Evaluation of the effect of treatment in the p16-positive and p16-negative subsets. HR < 1 favored RT plus cetuximab treatment.
†Evaluation of the effect of p16 status in the RT plus cetuximab and RT treatment arms. HR < 1 favored p16-positive status.

Patients with p16-positive OPC who received cetuximab in combination with RT had a greater 3-year OS rate than those receiving RT alone (87.8% and 72.3%, respectively; Table 2; Fig 2B). Similarly, the 3-year OS rates for patients with p16-negative OPC were 41.9% and 33.5%, respectively (Table 2; Fig 2B). HRs favored RT plus cetuximab in the p16-positive subgroup (HR, 0.38; 95% CI, 0.15 to 0.94). In the p16-negative OPC subset, the HR for OS was 0.93 (95% CI, 0.59 to 1.48; Table 2). Although this suggests a pronounced treatment effect only in patients with p16-positive OPC, no significant interaction between treatment group and p16 status could be shown ($P = .085$).

The 3-year PFS rate for patients with p16-positive OPC receiving RT plus cetuximab was 82.1% compared with 64.7% in patients who received RT alone (Table 2; Fig 2C). Among p16-negative patients treated with RT plus cetuximab or RT alone, the 3-year rates of PFS were 29.1% and 15.6%, respectively (Table 2; Fig 2C). RT plus cetuximab was associated with improved PFS in p16-positive and p16-negative subgroups (HR, 0.46; 95% CI, 0.19 to 1.10 and HR, 0.76; 95% CI, 0.48 to 1.21, respectively; Table 2). In this relatively small subset, no significant interaction between treatment group and p16 status could be shown ($P = .253$; Fig 2C).

HPV As a Predictive Biomarker in p16-Positive Tumors

The HPV status of p16-positive patients was further confirmed by testing for the presence of HPV DNA in tumor samples from patients with p16-positive OPC. Of the 75 patients with p16-positive OPC, 63 (84%) were evaluable for HPV DNA. There was 78% concordance (49 of 63) between p16-positive and HPV-positive tumors.

Although small sample sizes precluded conclusive tests of significance, 3-year LRC and OS rates for p16-positive patients with HPV-positive versus HPV-negative OPC broadly resembled findings from the p16 subgroup analysis. As summarized in Table 3, patients with HPV-positive OPC treated with RT plus cetuximab had a 3-year OS rate of 82.1% compared with 70.4% in patients who received RT alone (HR, 0.53; 95% CI, 0.18 to 1.52; Table 3; Fig 3A). Similarly, patients with HPV-negative disease treated with RT plus cetuximab had a 3-year OS rate of 100% compared with 85.7% in patients who received RT alone (Table 3; Fig 3B).

Similar trends were observed when LRC was evaluated. HPV-positive patients who received RT plus cetuximab had a 3-year LRC rate of 81.5% compared with 64.8% in patients who received RT alone (HR, 0.52; 95% CI, 0.16 to 1.63; Table 3; Fig 3C). In the HPV-negative subgroup, the 3-year LRC rate was 100% among patients who were treated with RT plus cetuximab versus 71.4% for patients treated with RT alone (Table 3; Fig 3D).

DISCUSSION

The addition of cetuximab to RT increases both the duration of locoregional disease control and survival in patients with LA-SCCHN, including OPC.[15] In this secondary analysis of the IMCL-9815 trial, we evaluated the impact of p16 protein and HPV DNA status on outcomes in patients with OPC. These data suggest that regardless of p16 status, patient outcomes were improved by the addition of cetuximab to RT compared with RT alone. Therefore, although p16 status is a strong prognostic biomarker, it does not seem to predict the effect of cetuximab in patients with LA-SCCHN.

This subgroup analysis suggested that a more pronounced benefit from cetuximab may be exhibited in the p16-positive population compared with the p16-negative population; however, no significant interaction between treatment group and p16 status could be shown. Thus, at least for this study, these data suggested that p16 status—although a strong prognostic biomarker—does not predict and is not a biomarker of the effect of cetuximab treatment. Furthermore, given the relatively small sample size of the subgroups in this analysis, additional studies with larger patient numbers in the primary treatment population would be required to reach a definitive conclusion. These findings provide valuable context for the Radiation Therapy Oncology Group (RTOG) 1016 trial, which has completed enrollment. In that trial, patients with p16-positive/HPV-positive OPC were allocated to receive RT in combination with either cisplatin or cetuximab. Results from RTOG 1016 will be valuable because each treatment arm comprises HPV-positive patients who are commonly somewhat younger and have higher performance status, lower tobacco exposure, and

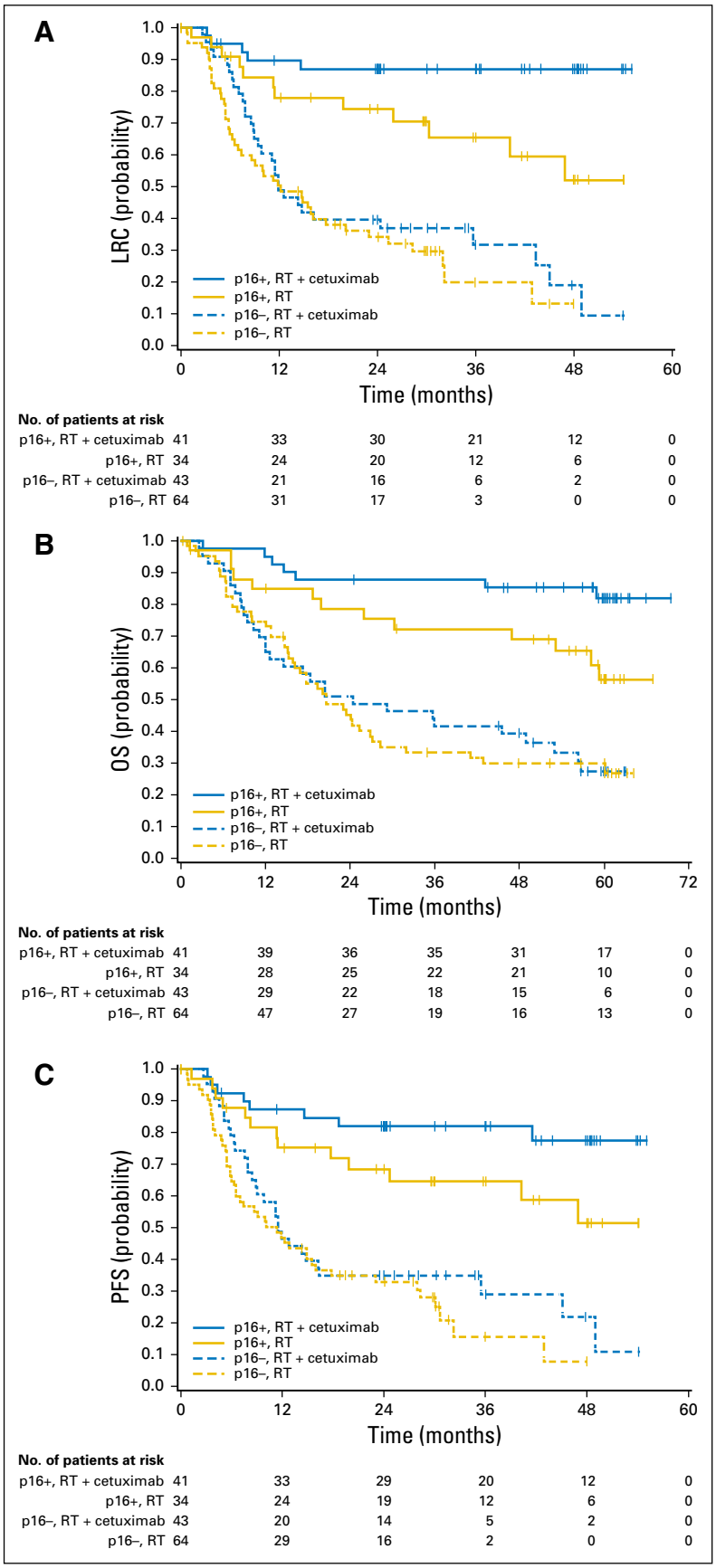

Fig 2. Kaplan-Meier plots of (A) locoregional control (LRC), (B) overall survival (OS), and (C) progression-free survival (PFS) in patients with p16-positive and p16-negative oropharyngeal carcinoma treated with radiotherapy (RT) plus cetuximab or RT alone. Because there was minor overlap of the Kaplan-Meier curves, which may have resulted from small sample size, additional statistical analyses were conducted to ensure there was no strong deviation from the original proportional hazards assumption. Both the log-log survival plots and time-dependent Cox models confirmed this assumption was not violated. p16+, p16 positive; p16−, p16 negative.

Table 3. Effect of RT Plus Cetuximab Versus RT Alone: Outcomes at 3 Years by HPV Status in Patients With p16-Positive OPC

Outcome	HPV Positive (%)		HPV Negative (%)	
	RT + Cetuximab (n = 28)	RT (n = 21)	RT + Cetuximab (n = 6)	RT (n = 8)
LRC	81.5	64.8	100	71.4
HR (95% CI)	0.52 (0.16 to 1.63)		NE	
OS	82.1	70.4	100	85.7
HR (95% CI)	0.53 (0.18 to 1.52)		NE	

Abbreviations: HPV, human papillomavirus; HR, hazard ratio; LRC, locoregional control; NE, not evaluated; OPC, oropharyngeal carcinoma; OS, overall survival; RT, radiotherapy.

fewer comorbidities than patients with HPV-negative LA-SCCHN.

Several recent trials have evaluated the effect of HPV status on the efficacy of anti-EGFR antibodies in patients with SCCHN. In the EXTREME (involving cetuximab) and SPECTRUM (Study of Panitumumab Efficacy in Patients With Recurrent and/or Metastatic Head and Neck Cancer; involving panitumumab) trials, EGFR inhibitors were evaluated for the treatment of recurrent and/or metastatic SCCHN. The EXTREME trial demonstrated that p16 status and HPV status have prognostic value independent of tumor p16 and HPV status.[20] Our results were largely consistent with these findings. In contrast, the SPECTRUM trial, which did not reach its primary end point of OS in the intent-to-treat population, showed that although panitumumab had efficacy in patients with p16-negative tumors, patients with p16-positive SCCHN derived no significant benefit from the addition of panitumumab to chemotherapy.[20] Furthermore, in the small phase II CONCERT-2 (Concomitant Chemotherapy and/or EGFR Inhibition With Radiation Therapy) trial, panitumumab plus radiotherapy was compared with chemoradiotherapy in patients with unresected, locally advanced squamous-cell carcinoma of the head and neck. Although there was no significant difference by treatment arm in the 2-year LRC for patients with p16-positive LA-SCCHN (n = 24), patients with p16-negative LA-SCCHN (n = 75) had a higher 2-year LRC when treated with CRT.[21]

There are several important considerations and possible explanations for the differences in our findings compared with the conclusions of the EXTREME, SPECTRUM, and CONCERT-2 trials. First, unlike cetuximab, panitumumab has not been approved for the treatment of SCCHN. Although the superiority of treatment with RT plus cetuximab compared with RT alone has been demonstrated,[15,16] an equivalent trial has not been performed with panitumumab. Second, although both panitumumab and cetuximab target EGFR, they are distinct antibodies and may not be identical in their actions; notably, induction of antibody-dependent cellular cytotoxicity is associated with cetuximab but not panitumumab.

Differences in study populations are also important to consider. The EXTREME and SPECTRUM trials were conducted in patients with recurrent and/or metastatic SCCHN, whereas the IMCL-9815 and CONCERT-2 trials were performed in previously untreated patients with LA-SCCHN, which may indicate different underlying biology in these settings. Finally, characterization of p16 status differed fundamentally among these trials; both EXTREME and IMCL-9815 set the threshold for p16 positivity at 70%, following expert recommendations,[15-17,20,21] whereas the threshold in SPECTRUM and CONCERT-2 was 10%. Thus, the p16-positive groups in the SPECTRUM and CONCERT-2 studies may have included patients who would have been classified as p16 negative in our analysis of IMCL-9815.[21]

The effect of p16 status on the efficacy of cetuximab monotherapy in second-line recurrent or metastatic SCCHN has not been fully investigated. Only retrospective analyses of small subsets from two phase II studies have examined the efficacy of cetuximab in p16-positive disease.[22,23] Larger prospective studies would be needed to fully understand the impact of HPV infection in second-line recurrent or metastatic SCCHN.

In addition to demonstrating that accelerated fractionation did not improve outcomes in patients with OPC who received concurrent cisplatin (the primary end point), the RTOG 0129 study provided important risk stratification data.[7] p16/HPV status was the most important prognostic factor, which was further modified by tobacco history; among patients with p16-positive/HPV-positive cancer, those with no or $<$ 10 pack-year smoking exposure had superior outcomes compared with patients with significant tobacco use. Our results confirm p16 as a prognostic factor in OPC, given that patients with p16-positive tumors had better outcomes in both treatment arms. A shortcoming of this trial is that tobacco history was not collected. However, $>$ 90% of the p16-positive patients in our trial were from the United States, which is characterized by a lower rate of tobacco use compared with other countries represented in the trial; also, concomitant boost was the predominant RT regimen used in the United States (data not shown).[24] Thus, these patients may represent a more homogeneous population.

p16 status is considered a valid surrogate for HPV in OPC. The level and localization of p16 protein expression in OPC reflect a number of distinct variables, including but not limited to the integration of high-risk HPV. Accordingly, the interpretation of p16 IHC staining as a surrogate marker of HPV infection must be informed by various histologic, anatomic, and clinical and technical considerations.[25] We found a 78% concordance between p16-positive and HPV-positive tumors, a rate that is commonly observed and may be ascribed to sample degradation during preparation and storage, as well as to the greater complexity of polymerase chain reaction compared with IHC.[25]

There were several limitations to this study. This was a retrospective analysis of p16 and HPV status in a previously unselected population and the number of patients in some of the subgroups was small. Therefore, differences in the baseline characteristics of p16-positive versus p16-negative subpopulations could not be controlled for in this analysis. Furthermore, the sample size of the p16-positive/HPV-negative subgroup was small, precluding statistical analysis of significance.

In conclusion, this unplanned, secondary analysis of the IMCL-9815 trial showed that the addition of cetuximab to RT benefited patients with OPC independent of p16 status. Although the magnitude of the gain seemed more pronounced in those with p16-positive tumors compared with those with p16-negative

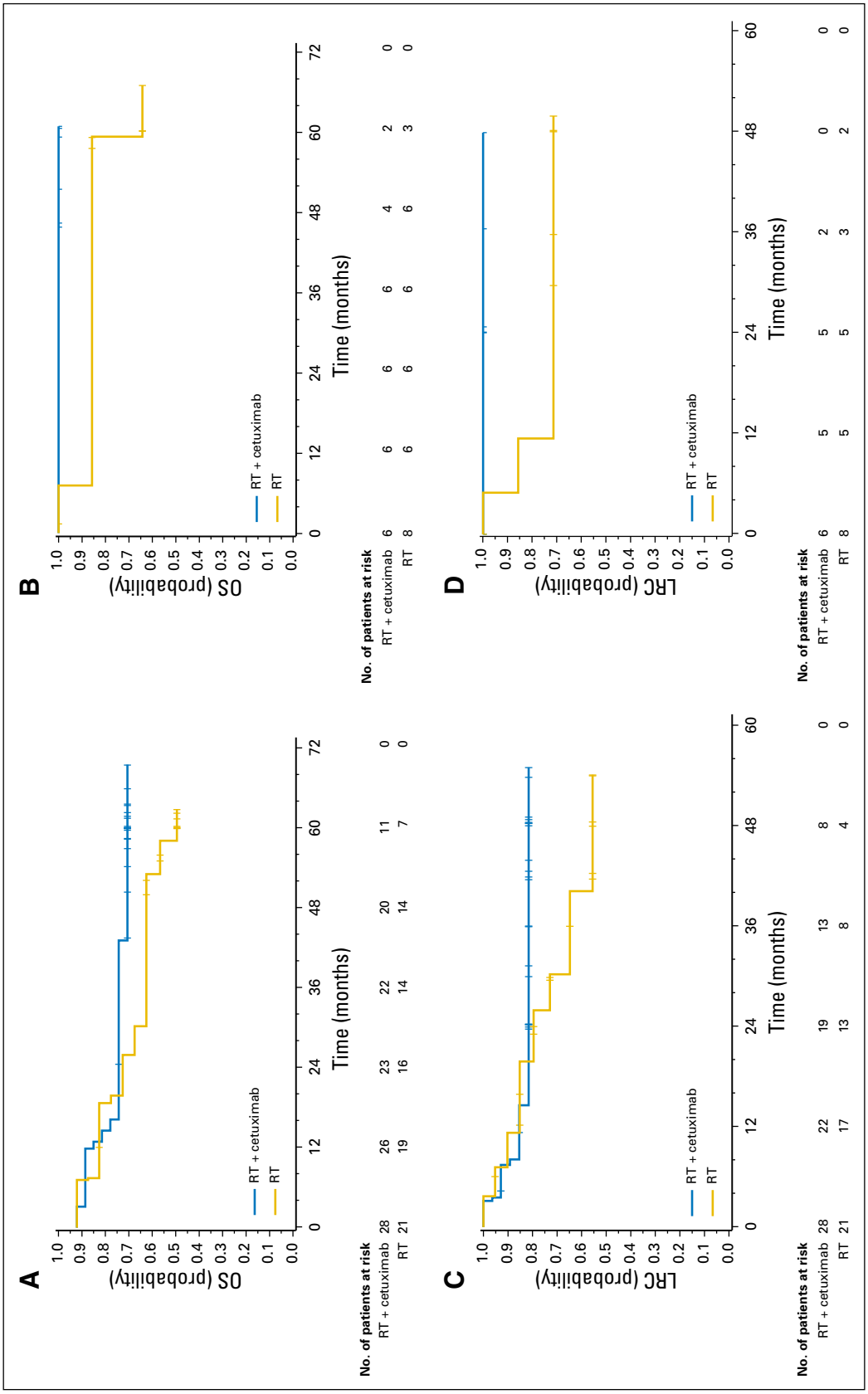

Fig 3. Kaplan-Meier plots of overall survival (OS) in (A) human papillomavirus (HPV)–positive and (B) HPV-negative disease and of locoregional control (LRC) in (C) HPV-positive and (D) HPV-negative disease in patients with p16-positive, HPV-evaluable oropharyngeal carcinoma treated with radiotherapy (RT) plus cetuximab or RT alone.

tumors, no significant interaction between treatment group and p16 status could be shown. Thus, this study suggested that p16 status is a prognostic biomarker for patients with OPC but does not predict response to cetuximab. The ongoing RTOG 1016 trial should provide valuable additional observations regarding the role of cetuximab in HPV-positive disease, given that trials thus far have included small p16-positive/HPV-positive sample sizes that complicate their interpretation. Given the expectation of better outcomes in patients with p16-positive disease, it may be years before a sufficient number of progression events or deaths occur to facilitate efficacy assessment in this population. However, data on acute toxicity are anticipated to be accessible much sooner. When available, the final data set—including efficacy and late toxicity end points—will provide important insight into differences in survival and quality of life in patients with p16-positive OPC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Gillison ML, Koch WM, Capone RB, et al: Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 92:709-720, 2000
2. Zandberg DP, Bhargava R, Badin S, et al: The role of human papillomavirus in nongenital cancers. CA Cancer J Clin 63:57-81, 2013
3. Chaturvedi AK, Engels EA, Pfeiffer RM, et al: Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 29:4294-4301, 2011
4. Mehanna H, Beech T, Nicholson T, et al: Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer: Systematic review and meta-analysis of trends by time and region. Head Neck 35:747-755, 2013
5. Gillison ML, D'Souza G, Westra W, et al: Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 100:407-420, 2008
6. Rischin D, Young RJ, Fisher R, et al: Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. J Clin Oncol 28:4142-4148, 2010
7. Ang KK, Harris J, Wheeler R, et al: Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 363:24-35, 2010
8. Brizel DM, Albers ME, Fisher SR, et al: Hyperfractionated irradiation with or without concurrent chemotherapy for locally advanced head and neck cancer. N Engl J Med 338:1798-1804, 1998
9. Bourhis J, Overgaard J, Audry H, et al: Meta-Analysis of Radiotherapy in Carcinomas of Head and Neck (MARCH) Collaborative Group: Hyperfractionated or accelerated radiotherapy in head and neck cancer: A meta-analysis. Lancet 368:843-854, 2006
10. Adelstein DJ, Li Y, Adams GL, et al: An intergroup phase III comparison of standard radiation therapy and two schedules of concurrent chemoradiotherapy in patients with unresectable squamous cell head and neck cancer. J Clin Oncol 21:92-98, 2003
11. Pignon JP, Syz N, Posner M, et al: Adjusting for patient selection suggests the addition of docetaxel to 5-fluorouracil-cisplatin induction therapy may offer survival benefit in squamous cell cancer of the head and neck. Anticancer Drugs 15:331-340, 2004
12. Pignon JP, Bourhis J, Domenge C, et al: Chemotherapy added to locoregional treatment for head and neck squamous-cell carcinoma: Three meta-analyses of updated individual data—MACH-NC Collaborative Group: Meta-Analysis of Chemotherapy on Head and Neck Cancer. Lancet 355:949-955, 2000
13. Gutowski MC, Briggs SL, Johnson DA: Epidermal growth factor receptor-reactive monoclonal antibodies: Xenograft antitumor activity alone and as drug immunoconjugates. Cancer Res 51:5471-5475, 1991
14. Huang SM, Harari PM: Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: Inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. Clin Cancer Res 6:2166-2174, 2000
15. Bonner JA, Harari PM, Giralt J, et al: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 354:567-578, 2006
16. Bonner JA, Harari PM, Giralt J, et al: Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 11:21-28, 2010
17. Vermorken JB, Psyrri A, Mesía R, et al: Impact of tumor HPV status on outcome in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck receiving chemotherapy with or without cetuximab: Retrospective analysis of the phase III EXTREME trial. Ann Oncol 25:801-807, 2014
18. Gillison ML, Zhang Q, Jordan R, et al: Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. J Clin Oncol 30:2102-2111, 2012
19. Brookmeyer R, Crowley J: A confidence interval for the median survival time. Biometrics 38:29-41, 1982
20. Vermorken JB, Stöhlmacher-Williams J, Davidenko I, et al: SPECTRUM investigators: Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): An open-label phase 3 randomised trial. Lancet Oncol 14:697-710, 2013
21. Giralt J, Trigo J, Nuyts S, et al: Panitumumab plus radiotherapy versus chemoradiotherapy in patients with unresected, locally advanced squamous-cell carcinoma of the head and neck (CONCERT-2): A randomised, controlled, open-label phase 2 trial. Lancet Oncol 16:221-232, 2015
22. Fayette J, Wirth LJ, Oprean C, et al: Randomized phase II study of MEHD7945A (MEHD) vs cetuximab (cet) in >= 2nd-line recurrent/metastatic squamous cell carcinoma of the head & neck (RMSCCHN) progressive on/after platinum-based chemotherapy (PtCT). Ann Oncol 25:iv340–iv356, 2014 (abstr 9860)
23. Vokes EE, Worden FP, Adkins D, et al: A randomized phase II trial of the MET inhibitor tivantinib + cetuximab versus cetuximab alone in patients with recurrent/metastatic head and neck cancer. J Clin Oncol 33, 2015 (suppl; abstr 6060)
24. World Health Organization: WHO Report on the Global Tobacco Epidemic, 2008: The MPOWER Package. http://www.who.int/tobacco/mpower/mpower_report_full_2008.pdf
25. El-Naggar AK, Westra WH: p16 expression as a surrogate marker for HPV-related oropharyngeal carcinoma: A guide for interpretative relevance and consistency. Head Neck 34:459-461, 2012

GLOSSARY TERMS

antibody-dependent cell-mediated cytotoxicity (ADCC): a mechanism of cell-mediated immunity whereby an effector cell of the immune system actively lyses a target cell that has been bound by specific antibodies.

biomarker: a functional biochemical or molecular indicator of a biologic or disease process that has predictive, diagnostic, and/or prognostic utility.

cetuximab: also called Erbitux or C225. Cetuximab is a monoclonal antibody that is designed to target the epidermal growth factor receptor and block its signaling activity by initiating receptor activation.

human papillomavirus (HPV): a double-stranded DNA virus from the papillomaviridae family. Human papillomavirus is a cause of cervical cancer as well as of a subset of cancers of the anus, oropharynx, penis, vagina, and vulva.

in situ hybridization: a method used to detect specific gene sequences in tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest.

predictive biomarkers: measurements associated with response to or lack of response to a particular therapy.

prognostic marker: a marker that predicts the prognosis of a patient (eg, the likelihood of relapse, progression, and/or death) independent of future treatment effects. A factor can be both prognostic and predictive.

p16: molecule that binds to cyclin-dependent kinase 4 and 6, thereby preventing their interaction with cyclin D. p16 (also known as $p16^{INK4}$) behaves as a negative regulator of proliferation and arrests cells in the G_0/G_1 phase of the cell cycle.

subgroup analysis: an analysis in which the intervention effect is evaluated in a defined subset of the participants in the trial, or in complementary subsets, such as by sex or in age categories. Sample sizes in subgroup analyses are often small and subgroup analyses therefore usually lack statistical power. Comparison of subgroups should be done by test of interaction rather than by comparison of *P* values. They are also subject to the multiple comparisons problem, which increases the probability of making a type I error (ie, attributing a difference to an intervention when chance is the more likely explanation).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Association of Human Papillomavirus and p16 Status With Outcomes in the IMCL-9815 Phase III Registration Trial for Patients With Locoregionally Advanced Oropharyngeal Squamous Cell Carcinoma of the Head and Neck Treated With Radiotherapy With or Without Cetuximab

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