



The MARCH-HPB project

Prognostic impact of HPV-associated p16-expression and smoking status on outcomes following radiotherapy for oropharyngeal cancer: The MARCH-HPV project



Pernille Lassen^a, Benjamin Lacas^{b,c}, Jean-Pierre Pignon^{b,c}, Andy Trotti^d, Bjorn Zackrisson^e, Qiang Zhang^f, Jens Overgaard^a, Pierre Blanchard^{g,c,*}, on behalf of the MARCH Collaborative Group

^a Department of Experimental Clinical Oncology, Aarhus University Hospital, Denmark; ^b Gustave-Roussy, Paris-Saclay University, Biostatistics and Epidemiology Department; ^c INSERM U1018, CESP, Université Paris-Sud, Université Paris-Saclay, Villejuif, France; ^d Moffitt Cancer Center, Department of Radiation Oncology, Tampa, USA; ^e Department of Radiation Sciences – Oncology, Umeå University, Umeå, Sweden; ^f NRG Oncology Statistics and Data Management Center (formerly RTOG), Philadelphia, USA; and ^g Gustave-Roussy, Paris-Saclay University, Radiotherapy Department, Villejuif, France

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ABSTRACT

Background and purpose: Evaluate the prognostic and predictive impact of HPV-associated p16-expression and assess the combined prognostic impact of p16 and smoking on altered fractionated radiotherapy (AFRT) for oropharyngeal cancer (OPC) within the frames of the update of the Meta-Analysis of Radiotherapy in Carcinomas of Head and neck (MARCH).

Materials and methods: Patients with OPC, known tumor p16-status and smoking history were identified from the MARCH update, resulting in a dataset of 815 patients from four randomized trials (RTOG9003, DAHANCA6&7, RTOG0129, ARTSCAN). Analysis was performed using a Cox model stratified by trial and adjusted on gender, age, T-stage, N-stage, type of radiotherapy fractionation, p16, smoking. Primary endpoint was progression-free survival (PFS).

Results: In total, 465 patients (57%) had p16-positive tumors and 350 (43%) p16-negative. Compared to p16-negative, p16-positive patients had significantly better PFS (HR = 0.42 [95% CI: 0.34–0.51], 28.9% absolute increase at 10 years) and OS (HR = 0.40 [0.32–0.49], 32.1% absolute increase at 10 years). No interaction between p16-status and fractionation schedule was detected. Smoking negatively impacted outcome; in the p16-positive subgroup, never smokers had significantly better PFS than former/current smokers (HR = 0.49 [0.33–0.75], 24.2% survival benefit at 10 years).

Conclusions: No predictive impact of p16-status on response to AFRT could be detected but the strong prognostic impact of p16-status was confirmed and especially p16-positive never smoking patients have superior outcome after RT.

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Human papillomavirus (HPV) is a well-established cause of oropharyngeal cancer (OPC) [1,2] and although the proportion of OPC attributable to HPV shows geographical variation [3], the incidence of HPV-associated OPC has increased at an epidemic rate over the past 40 years in many western countries [4–7]. HPV-positive OPCs are distinct in terms of epidemiological, clinical and molecular features when compared to HPV-negative OPC, and tumor HPV-status is recognized as the strongest independent prognostic factor for radiotherapy (RT) outcome in OPC, in favor of HPV-positivity [8–12]. These observations are explained in part by a higher sensitivity of HPV-positive tumors to RT [13,14] com-

bined with a different and more favorable risk factor profile and better general health status in the group of patients with HPV-positive disease [15].

Tobacco smoking, along with alcohol consumption, remains the main etiological factor in squamous cell carcinoma of the head and neck (HNSCC) worldwide and smoking independently affects treatment response and survival in a negative way for patients with OPC, regardless of tumor HPV-status [8,16,17]. Thus, smoking patients with HPV-positive disease have intermediate prognosis, and besides the excess co-morbidity [18] and risk of secondary cancers caused by significant lifetime exposure to smoking, the presumed dual HPV/tobacco etiology may result in different mutational profiles between HPV-positive never smokers and HPV-positive ever smokers [19]. Moreover, smoking during RT has been shown to compromise treatment outcome for patients with head

* Corresponding author at: Department of Radiation Oncology, Gustave Roussy Cancer Campus, 114 rue Edouard Vaillant, 94800 Villejuif, France.

E-mail address: pierre.blanchard@gustaveroussy.fr (P. Blanchard).

and neck cancer [20,21]. Although it has been shown repeatedly that the proportion of HPV-associated OPC is higher among never smokers than former/current smokers, still the majority of HPV-positive disease is found among patients with a history of either current or former smoking [22,23]. Thus, smoking continues to be of utmost clinical importance, modifying prognosis also for the group of patients with HPV-associated OPC, for whom clinical trials investigating de-intensified therapies are currently ongoing.

Altered fractionated RT (AFRT) regimens for the treatment of HNSCC have been investigated in numerous clinical trials, leading to the provision of conflicting results regarding tumor control and survival, mostly due to trial heterogeneity and limited sample size. However, the Meta-Analysis of Radiotherapy in Carcinomas of Head and neck (MARCH) has demonstrated that altered fractionation RT was associated with improved overall survival and progression-free survival when compared to conventional fractionation RT [24]. Moreover, altered fractionation significantly improved locoregional tumor control, predominantly by reducing the risk of local failure whereas the benefit on nodal control was less pronounced. These findings were recently confirmed in an update of the Meta-Analysis based on more patients and longer follow up [25]. Whether tumor HPV status is associated with a differentiated response to altered fractionation RT is less well investigated. During the update of the MARCH-analysis, data on HPV-associated p16-expression and smoking status have been collected from trials where this information was available. With this study, we aimed to evaluate the prognostic and predictive impact of p16-status and to assess the combined prognostic impact of p16 and smoking in altered fractionated RT of OPC within the frames of the update of the Meta-Analysis of Radiotherapy in Carcinomas of Head and neck.

Material and methods

Patients and trials

Search strategy, selection criteria and data collection for the update of the Meta-Analysis of Radiotherapy in HNSCC (MARCH) are described elsewhere, alongside with the checking procedure of individual patient data [25]. Among the 33 trials included only five collected p16-status [26–30]. Within those trials, p16-status was available for 999 OPC. The adjustment on smoking status led to the exclusion of one more trial (65 patients) [28] and additional 119 patients from the remaining trials due to missing values. Our analysis was restricted to trials where RT was given as the primary treatment modality excluding studies with postoperative RT, and only patients with OPC, known tumor HPV-status and smoking history were included, in turn yielding a dataset consisting of 815 patients from 4 different randomized trials (RTOG9003 [26], DAHANCA6&7 [27], RTOG0129 [29], ARTSCAN [30] (Fig. 1, [Supplementary Table 1](#)). In all trials HPV-association was assessed by use of p16-expression, an established surrogate for tumor HPV in OPC, and tumors were classified as p16-positive in case of strong and diffuse nuclear and cytoplasmic staining in >70% of tumor cells, evaluated by immunohistochemistry [31]. Smoking history was not reported uniformly between trials and no consistent information on lifetime exposure and pack-years was available. Thus, smoking was reported as never, former or current apart from in the ARTSCAN trial where smoking was collected as never/former or current smoking.

Endpoints

The primary endpoint was progression-free survival (PFS), defined as time from randomization to first failure (loco-regional or distant) or death from any cause. Secondary endpoints were

overall survival (OS), overall survival after first failure, loco-regional control (LRC), cancer and non-cancer mortality. OS was defined as time from randomization to death from any cause. Overall survival after first failure was defined as time from first failure to death from any cause, with the exclusion of patients without failure. Events considered for LRC were local failure, regional failure or synchronous regional and local failures. Since only the first event was collected, patients with distant failure were censored at that time. Patients alive without the events corresponding to each endpoint were censored at their date of last follow-up. Non-cancer mortality was defined as deaths without failure and resulting from known causes other than the treated head and neck cancer. Cancer mortality included deaths from any cause with previous failure and deaths from the treated head and neck cancer. Deaths without failure and from unknown cause were considered as cancer mortality if they occurred within 5 years after randomization and as non-cancer mortality [32] otherwise.

Statistical analysis

The prognostic effect of p16 was estimated on all endpoints. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated with a Cox model stratified by trial and adjusted on T-stage (T1–2, T3–4), N-stage (N0, N+), gender, age (<50, [50–60], [60–70], ≥70), treatment arm (standard or modified fractionation) and p16-status (positive, negative). In this model smoking status was coded as never/former vs current, in order to enable inclusion of ARTSCAN in the analysis. Survival rates were estimated for the control group every 3 months with the Kaplan–Meier method and calculated for the experimental group with the HR from the Cox model, based on the formula described by Stewart and Parmar [33], for PFS, OS and overall survival after first failure. For cancer and non-cancer mortality, the Fine & Gray model was used to estimate sub-distribution HRs (sub-HRs), adjusted on the same covariates than the Cox model. Survival rates were estimated with incidence curves [34]. LRC was analyzed using both methods. As recommended, estimations of HRs with cause-specific models were also performed [35] using the adjusted Cox model previously described. Adjusted and unadjusted cumulative incidences were calculated. All survival and cumulative incidence curves were truncated at five, eight or 12 years, depending on numbers of patients left at those times.

The predictive effect of p16-status was estimated only for PFS, OS and cancer mortality, since a lack of power was expected for the other endpoints. The same models, including smoking as never/former vs current were used for the evaluation of the prognostic effect of p16-status, with the addition of an interaction between p16-status and RT fractionation.

The prognostic effect of the combination between p16-status and smoking was studied for all endpoints. For this analysis, smoking status was classified as never smokers vs. former/current smokers since the group of never smokers is presumably distinct both in terms of tumor biology and mutational profile but also with regard to patient related co-morbidity and risk profile. Consequently, this led to the exclusion of the 166 patients from the ARTSCAN trial leaving 649 patients available for analysis. Otherwise, the same models were used, with an adjustment on the combination (p16-negative and never smokers, p16-negative and former/current smokers, p16-positive and never smokers, p16-positive and former/current smokers).

Chi² heterogeneity tests and I² statistic were used to investigate heterogeneity between trials [36]. In case of significant heterogeneity ($p < 0.10$), a random-effects model was used. Median follow-up time was estimated with the reverse Kaplan–Meier method [37]. Hazard proportionality was assessed with Schoenfeld residuals [38]. With 525 events, it would be possible to detect with

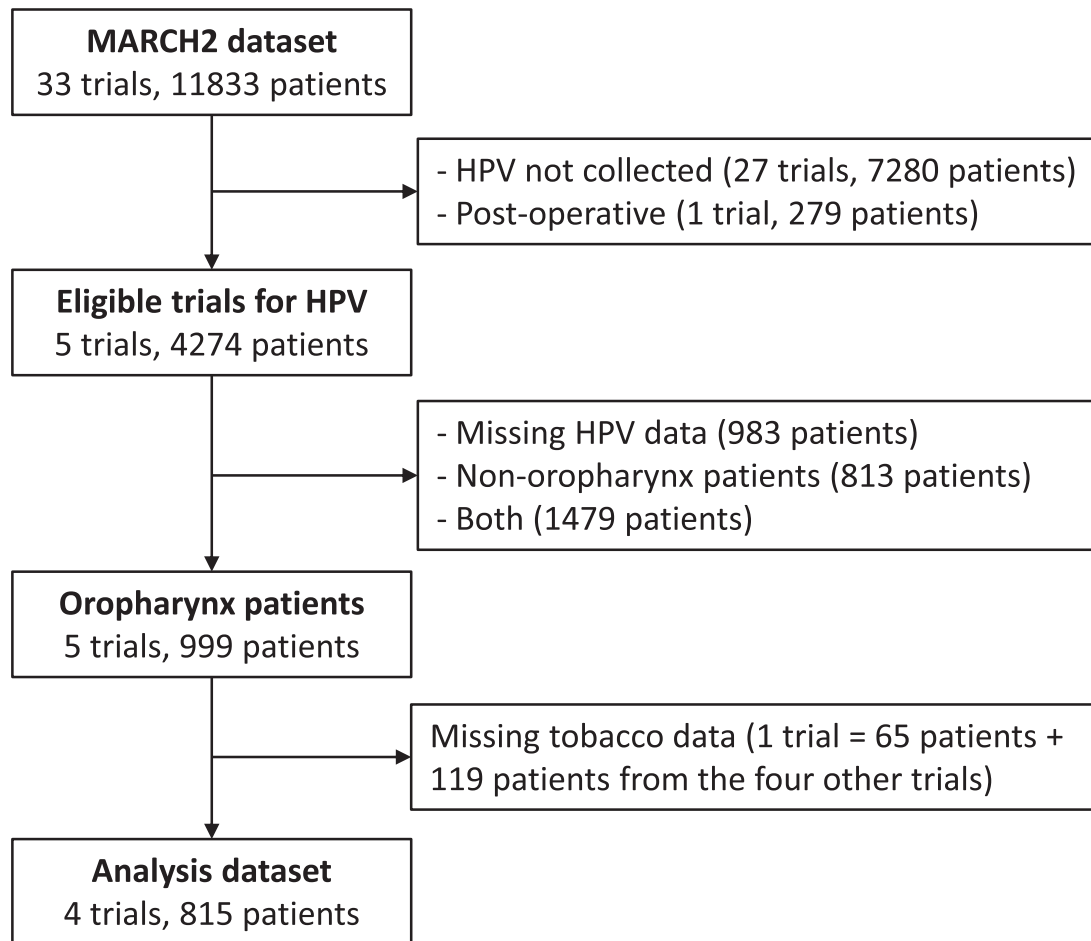


Fig. 1. Flowchart.

a power of 89% an absolute improvement in progression-free survival from 25% to 35% at 5-years (two-sided log-rank test, $\alpha = 5\%$).

All p -values were two-sided. Hazard proportionality was assessed with the package “Survival” of R software (version 3.3.2, R foundation for Statistical Computing, Vienna, Austria). All the other analyses were performed with SAS 9.4 (SAS Institute, Cary, NC). The adjusted cumulative incidences were calculated using the %CIFCOX macro. The article was written according to the PRISMA-IPD statement [39].

Results

Description

Four trials and 815 patients were available for the analysis of the prognostic and predictive effects of p16-status (Table 1, Fig. 1): 350 (43%) were p16-negative and 465 (57%) were p16-positive. Compared to p16-negative patients, p16-positive patients were younger, with better performance status (73% of PS0 versus 50%, $p < 0.0001$), had smaller tumors (47% of T1–2 versus 33%, $p < 0.0001$) but worse lymph node involvement (87% of N+ versus 76%, $p < 0.0001$) and worse clinical stage (77% of stage IV versus 66%, $p = 0.0006$). They also were more often never or former smokers (77% versus 36%, $p < 0.0001$). Median follow-up was 8.6 years [range: 7.9; 16.1].

Details on the population used for analysis of the combined prognostic effect of p16-status and smoking are shown in Supplementary Table 2. The vast majority of patients in both p16-groups

had a history of smoking, and only 6% and 18% were never smokers, in the p16 negative and positive groups respectively.

Prognostic effect of p16-status

Among the 815 patients, 493 died and 302 had a failure (Supplementary Table 3). For PFS (523 events), p16-positive patients had a significantly better outcome than p16-negative patients (HR = 0.42 [95% CI: 0.34; 0.51], $p < 0.0001$), with absolute benefits at 5 and 10 years of +31.0% and +28.9% respectively. Results were similar for OS (HR = 0.40 [0.32; 0.49], $p < 0.0001$), LRC (197 events, HR = 0.31 [0.22; 0.44], $p < 0.0001$), OS after first failure (272 deaths, HR = 0.64 [0.47; 0.87], $p = 0.003$), cancer mortality (323 deaths, HR = 0.34 [0.26; 0.44], $p < 0.0001$) and non-cancer mortality (170 deaths, HR = 0.54 [0.38; 0.75], $p = 0.0005$). There was heterogeneity between trials for PFS ($p = 0.03$, $I^2 = 67\%$), OS ($p = 0.02$, $I^2 = 69\%$), cancer and non-cancer mortality ($p = 0.10$, $I^2 = 53\%$ and $p = 0.07$; $I^2 = 57\%$) but the conclusions were similar with a random-effect model except for non-cancer mortality (Supplementary Fig. 1). Sub-HR were similar to HR for cancer mortality and LRC, but different for non-cancer mortality (sub-HR = 1.12 [0.79; 1.61], $p = 0.52$). Unadjusted cumulative incidences of LRC, cancer and non-cancer mortality are presented in Supplementary Fig. 2. Adjusted cumulative incidences were similar (not shown). Survival curves of the other endpoints are presented in Supplementary Fig. 3. Hazards were proportional for all endpoints except LRC and cancer mortality. Changing the coding format of age or T-stage allowed to resolve

Table 1

Baseline patient and tumor characteristics stratified by p16-status.

	p16-positive		p16-negative		p-Value (χ^2 test)	All	
	N	%	N	%		N	%
Sex					0.34		
Male	369	79.4	268	76.6		637	78.2
Female	96	20.6	82	23.4		178	21.8
Age					0.007		
<50	116	24.9	59	16.9		175	21.5
50–59	181	38.9	141	40.3		322	39.5
60–69	126	27.1	98	28.0		224	27.5
≥70	42	9.0	52	14.9		94	1.5
Median [range]	56 [31; 84]		58 [37; 84]			57 [31; 84]	
Performance status					<0.0001		
0	341	73.3	175	50.0		516	63.3
≥1	117	25.2	173	49.4		290	35.6
Missing/Unknown*	7	1.5	2	0.6		9	1.1
T-stage					<0.0001		
T1–2	216	46.5	114	32.6		330	40.5
T3–4	249	53.5	236	67.4		485	59.5
N-stage					<0.0001		
N0	62	13.3	84	24.0		146	17.9
N+	403	86.7	266	76.0		669	82.1
Stage**					0.0006		
I–II	19	4.1	33	9.4		52	6.4
III	90	19.4	87	24.9		177	21.7
IV	356	76.6	230	65.7		586	71.9
Smoking status					<0.0001		
Never/Former	360	77.4	125	35.7		485	59.5
Current	105	22.6	225	64.3		330	40.5
Treatment arm					0.22		
Conventional RT	206	25.2	140	17.2		346	42.4
Altered fractionated RT	259	31.8	210	25.8		469	57.6
Total	465	100	350	100		815	100

RT, Radiotherapy.

* Excluded from the p-value calculation.

** UICC7 TNM-classification.

this statistical issue without changing the estimation of the prognostic effect of p16 status.

Predictive effect of p16-status

Patients with p16-positive tumors had significantly better PFS, OS and decreased risk of cancer mortality than p16-negative patients in both arms of RT fractionation, with HRs of 0.38 [0.29; 0.52] ($p < 0.0001$), 0.37 [0.27; 0.51] ($p < 0.0001$), 0.30 [0.20; 0.44] ($p < 0.0001$) in standard RT arms, and 0.44 [0.35; 0.57] ($p < 0.0001$), 0.41 [0.32; 0.53] ($p < 0.0001$), 0.36 [0.26; 0.50] ($p < 0.0001$) in modified RT arms respectively. There were no prognostic effects of radiotherapy fractionation on PFS, OS and cancer mortality of patients, whatever p16-status, with HRs of 1.03 [0.81; 1.31] ($p = 0.80$), 1.11 [0.87; 1.41] ($p = 0.41$), 1.05 [0.78; 1.40] ($p = 0.76$) for p16-negative patients and 1.19 [0.89; 1.57] ($p = 0.24$), 1.23 [0.92; 1.66] ($p = 0.17$), 1.26 [0.86; 1.85] ($p = 0.24$) for p16-positive patients respectively. Thus, no predictive effects of p16-status could be detected (interaction tests: $p = 0.45$, $p = 0.58$, $p = 0.44$) (Table 2). There was heterogeneity between trials for OS ($p = 0.08$, $I^2 = 57\%$), but not for PFS ($p = 0.15$, $I^2 = 44\%$) or cancer mortality ($p = 0.11$, $I^2 = 51\%$) (Fig. 2).

Prognostic effect of the combination between p16-status and smoking

Among the 649 patients included in this analysis, 428 died and 259 had a failure (Table 3). Smoking was found to have a significant impact on outcome in the group of patients with p16-positive tumors where never smokers had significantly better PFS

than former/current smokers (HR = 0.49 [0.33; 0.75]), with survival benefits at 5 and 10 years of +18.7% and +24.2% respectively. PFS was also significantly better for the two categories of p16-positive patients compared to former/current smokers in the p16-negative subgroup: HR = 0.20 [0.14; 0.31] with survival benefits at 5 and 10 years of +51.3% and +52.3% respectively for never smokers, and +32.6% and +28.1% (HR = 0.41 [0.33; 0.51]) for former/current smokers (Figs. 3 and 4, Supplementary Table 4). In p16-negative patients, no significant survival difference were demonstrated between never smokers and former/current smokers (HR = 0.73 [0.44; 1.20]), although survival benefits at 5 and 10 years of +11.3% and +8.4%, respectively was found in favor of never smokers.

Results were similar for the other endpoints except for OS after first failure, where a non-significant survival difference between p16 positive never and former/current smokers was observed (HR = 1.02 [0.52; 2.03]) (Supplementary Figs. 4–6). There was heterogeneity for PFS and OS ($p = 0.05$, $I^2 = 53\%$, for both endpoints).

Discussion

The significant prognostic impact of p16-status on RT outcome was confirmed in this pooled analysis based on individual patient data selected from the main update of the MARCH analysis [25] and restricted to OPC-patients with known p16 and smoking status. The additional prognostic role of tobacco smoking on survival was also demonstrated. However there was no interaction between p16 status and the benefit of altered fractionation

Table 2
Predictive effect of p16-status.

	Altered fractionated RT (No. events/No. patients)	Conventional RT (No. events/No. patients)	HR [95% CI] Altered vs. conventional
<i>Progression-free survival</i>			
p16-negative	188/210	122/140	1.03 [0.81; 1.31] <i>p</i> = 0.80
p16-positive	130/259	83/206	1.19 [0.89; 1.57] <i>p</i> = 0.24
HR [95% CI] p16-positive vs. p16-negative	0.44 [0.35; 0.57] <i>p</i> < 0.0001	0.38 [0.29; 0.52] <i>p</i> < 0.0001	Test for interaction p16*RT <i>p</i> = 0.45
<i>Overall survival</i>			
p16-negative	185/210	115/140	1.11 [0.87; 1.41] <i>p</i> = 0.41
p16-positive	120/259	73/206	1.23 [0.92; 1.66] <i>p</i> = 0.17
HR [95% CI] p16-positive vs. p16-negative	0.41 [0.32; 0.53] <i>p</i> < 0.0001	0.37 [0.27; 0.51] <i>p</i> < 0.0001	Test for interaction p16*RT <i>p</i> = 0.58
<i>Cancer mortality</i>			
p16-negative	129/210	80/140	1.05 [0.78; 1.40] <i>p</i> = 0.76
p16-positive	70/259	44/206	1.26 [0.86; 1.85] <i>p</i> = 0.24
HR [95% CI] p16-positive vs. p16-negative	0.36 [0.26; 0.50] <i>p</i> < 0.0001	0.30 [0.20; 0.44] <i>p</i> < 0.0001	Test for interaction p16*RT <i>p</i> = 0.44

CI, confidence interval; HR, hazard ratio, RT, radiotherapy.

regimen, meaning that p16 status cannot be used to assign patients to a specific type of radiotherapy fractionation.

One unexpected result of this analysis was the difference between sub-HR from the Fine and Gray model and HR from the Cox model for cancer mortality. It could be explained by the fact that, due to survivorship bias, there was a non-significant increase in the cumulative incidence of non-cancer mortality in p16-positive patients. Indeed, the risk of cancer mortality was three times lower in p16-positive compared to p16-negative patients, leaving more p16-positive patients candidate to die from other causes. And despite the fact that the risk of non-cancer mortality was also divided by two among p16-positive patients, quantitatively more p16-positive than p16-negative patients died from non-cancer related causes.

The MARCH update confirmed that altered fractionation radiotherapy (AFRT) in HNSCC was found to be associated with a small but significant improvement in overall survival (+3.1% at 5 years) when compared with standard fractionation [25]. This improvement in survival was rather modest in the overall trial population, but more pronounced in the hyperfractionated RT group where the absolute benefit at 5 years was +8.1%. In the present analysis, restricted to OPC with known p16 and smoking status, we were not able to detect any predictive effect of p16-status on outcome after AFRT. A reasonable explanation to these findings is the three-step selection process we used to identify patients for inclusion in the present analysis. Firstly, only 4 of the 33 trials were suitable for inclusion. Most patients were randomized between standard and accelerated radiotherapy, and only a minority (55 patients) from one multi-arm trial were randomized to hyperfractionated radiotherapy [26]. Given that the effect size is smaller for accelerated than for hyperfractionated radiotherapy, the preponderance of accelerated radiotherapy might have masked an association between other forms of altered fractionation and p16 or smoking status. Moreover, the restriction of analysis to include only OPC with known p16 and smoking status within these 4 trials led to further selection of patients, resulting in a non-representative subgroup and possibly unbalanced covariates

between arms, as also indicated by the fact that study heterogeneity was observed when analyzing overall survival. We also attempted to look at the predictive effect of p16-status on loco-regional control, T-site control and N-site control, but due the low number of events, particularly at trial level, there was not enough statistical power to perform this analysis. Besides, in RTOG trials, a positive planned neck dissection following radiotherapy was not considered a failure and hence the incidence of regional relapses might be underestimated in these trials, although the definition was similar between arms. Consequently, these findings should be interpreted very cautiously and there is a need for collection of more data before firm conclusions can be drawn on the potential predictive impact of p16-status on response to AFRT in OPC.

Smoking patients with p16-positive tumors represent a clinical challenge due to their intermediate prognosis and significantly worse RT outcome compared to p16-positive patients with a history of never or less heavy smoking. The possible dual etiology (HPV/smoking) in p16-positive smokers results in a mutational profile [19] different from that of p16-positive never smokers, which might in turn affect the intrinsic radiosensitivity of the tumors, consequently contributing to the outcome differences observed. The underlying mechanisms are not yet fully understood, but it is a topic of ongoing investigation. The influence of smoking on RT-outcome, apart from its etiological role, is presumably the consequence of both accumulated lifetime exposure (pack years) and a reduction of treatment efficacy in case of smoking during RT [21]. Recently, a subset analysis of patients with OPC and known p16-status from the two RTOG trials (9003 and 0129) also included in the present analysis, demonstrated the impact of quantitative measures of tobacco smoking on survival outcomes [16]. It was found that the risk of progression and death from OPC increases both directly as a function of tobacco exposure at time of diagnosis and during therapy, and the profound influence of smoking was independent of tumor p16-status. Significant lifetime exposure to smoking is known to cause excess overall mortality [40] and this explains part of the outcome differences between

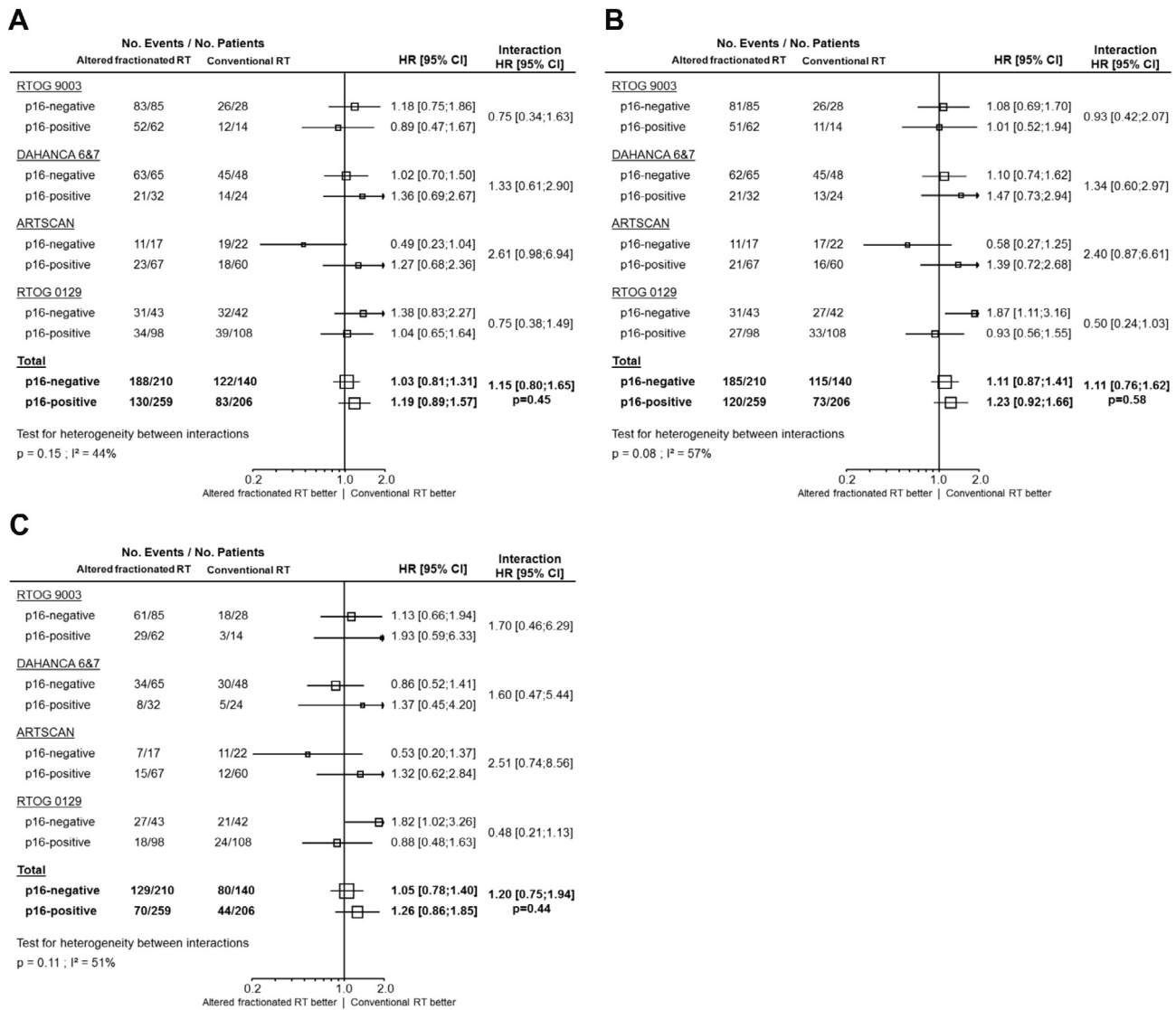


Fig. 2. Forest plot of the predictive effect of p16 status. (A) Progression-free survival; (B) overall survival; (C) cancer mortality. ARTSCAN, Accelerated RadioTherapy of Squamous cell Carcinomas in the head and Neck; CI, Confidence Interval; DAHANCA, Danish Head and Neck Cancer Group; HR, Hazard Ratio, RT, RadioTherapy; RTOG, Radiation Therapy Oncology Group.

Table 3
Cause of death and type of failure by p16-status and smoking status.

	p16-positive				p16-negative				All	
	Former/current smokers		Never smokers		Former/current smokers		Never smokers			
	N	%	N	%	N	%	N	%	N	%
Status at last follow-up										
Cancer related death	74	29.4	13	15.1	181	62.4	10	47.6	278	42.8
Non-cancer related death	56	22.2	13	15.1	75	25.9	6	28.6	150	23.1
Alive	122	48.4	60	69.8	34	11.7	5	23.8	221	34.1
Type of failure										
Locoregional failure	40	15.9	5	5.8	118	40.7	6	28.5	169	26.0
Distant failure	27	10.7	7	8.1	35	12.1	1	4.8	70	10.8
Locoregional and distant failures	9	3.6	0	–	10	3.4	1	4.8	20	3.1
No failure	176	69.8	74	86.1	127	43.8	13	61.9	390	60.1
Total	252	100	86	100	290	100	21	100	649	100

smokers and non-smokers also in p16-positive patients. The compromised RT-efficacy in case of actively smoking during treatment is probably the consequence of a combination of factors. One

explanation could be that smokers are known to have higher blood levels of carboxyhemoglobin levels [21] leading to reduced oxygen supply to tumors thereby enhancing the risk of hypoxia induced

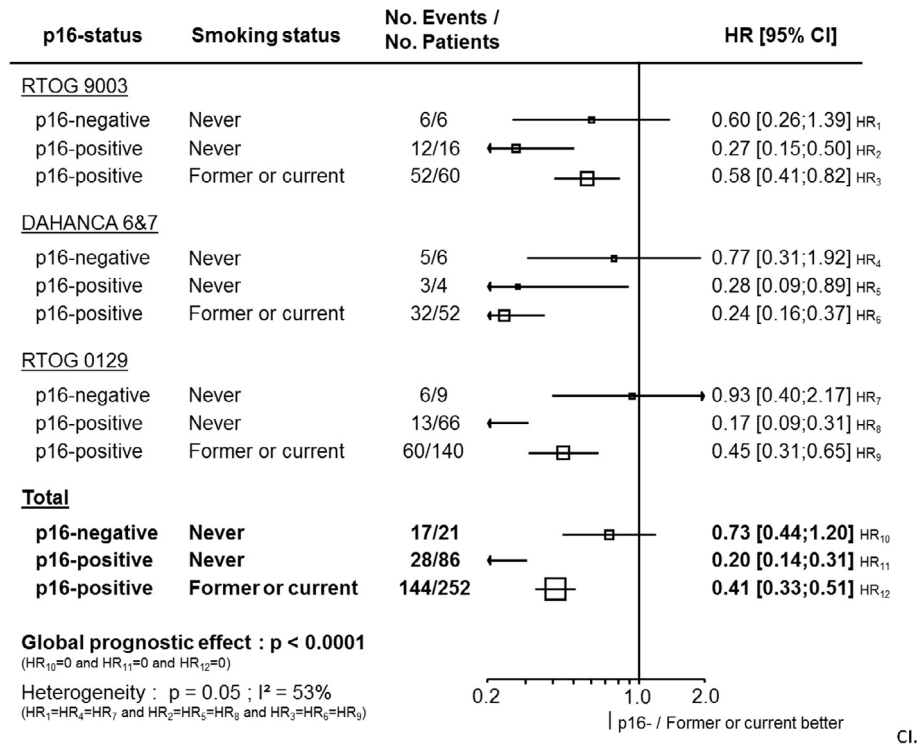


Fig. 3. Forest plot of the prognostic effect of the combination between p16-status and smoking status on progression-free survival. CI, Confidence Interval; DAHANCA, DANish Head and Neck CAncer Group; HR, Hazard Ratio; RT, RadioTherapy; RTOG, Radiation Therapy Oncology Group. Reference group is p16-negative patient, former or current smokers.

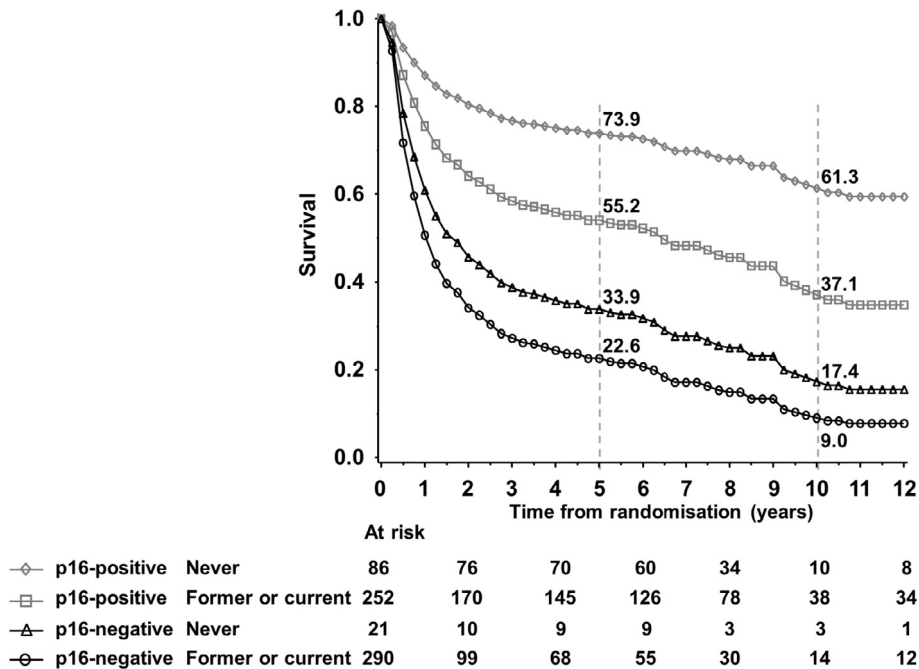


Fig. 4. Progression-free survival curves of the combination between p16-status and smoking status. Survival rates were estimated for the control group every 3 months with the Kaplan–Meier method and calculated for the experimental group with the HR from the Cox model, based on the formula described by Stewart and Parmar [33].

radioresistance and consequently poorer outcome [41]. Moreover, smokers may be at risk of more treatment breaks and consequently prolonged overall treatment time, due to increased acute toxicity and reduced treatment tolerance related to co-morbidity. In the present study, due to the way smoking data were collected in the individual trials, we were neither able to quantify lifetime expo-

sure (pack years) nor to distinguish between former or current smoking status and thus we are unable to elaborate on the above-mentioned potential causal relations between smoking and RT outcome.

In conclusion, our study confirms the profound prognostic impact of HPV-associated p16-expression on RT-outcome in OPC.

We did not observe a differentiated response to AFRT based on p16-status in this subgroup analysis, but for reasons outlined in the discussion, these findings should be interpreted very cautiously and more data are needed in order to make any firm conclusions in that regard. On the other hand, our data underscore the importance of smoking on RT-outcome regardless of p16-status and confirms that never smoking patients with p16-positive OPC have significant benefits both in terms of PFS, OS, LRC and cancer mortality compared with the subpopulation of p16-positive patients with a history of former or current smoking.

Conflict of interest statement

BL, JPP and PB report grants from Ligue National Contre le Cancer and grants from Institut National du Cancer during the conduct of the study. PL received grant from the Danish Cancer Society during the conduct of the study.

The other authors declared no conflicts of interest.

Role of the funding source

The funding sources had no role in study design, data collection, data analysis, data interpretation, or writing of the report. PB, BL and JPP had access to the raw data. The corresponding author had full access to all of the data and bears the final responsibility to submit for publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.radonc.2017.10.018>.

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