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Phase III randomised trial

Mature results from a Swedish comparison study of conventional versus accelerated radiotherapy in head and neck squamous cell carcinoma – The ARTSCAN trial

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ABSTRACT

Background and purpose: This report contains the mature five-year data from the Swedish ARTSCAN trial including information on the influence of p16 positivity (p16+) for oropharyngeal cancers.
Material and methods: Patients with previously untreated squamous cell carcinoma without distant metastases of the oral cavity, oropharynx, larynx (except T1–2, N0 glottic cancers) and hypopharynx were included. Patients were randomised between accelerated fractionation (AF) (1.1 Gy + 2 Gy per day, 5 days/week for 4.5 weeks, total dose 68 Gy) and conventional fractionation (CF) (2 Gy per day, 5 days/week for 7 weeks, total dose 68 Gy). Human papillomavirus (HPV)-associated p16-expression was assessed retrospectively in tumour tissues from patients with oropharyngeal carcinoma.
Results: There was no significant difference in loco-regional control (LRC) between AF and CF (log-rank test $p = 0.75$). LRC at 5 years was 65.5% for AF and 64.9% for CF. Overall survival (OS) was similar in both arms ($p = 0.99$). The estimated cancer specific survival (CSS) at 5 years was 62.2% (AF) and 63.3% (CF) ($p = 0.99$). 206 specimens were analysed for p16 with 153 specimens (74%) identified as p16+. P16 status did not discriminate for response to AF vs. CF with regard to LRC, OS or CSS. Patients with p16+ tumours had a statistically significant better overall prognosis compared with p16− tumours.
Conclusion: This update confirms the results of the 2-year report. We failed to identify a positive effect resulting from AF with regards to LRC, OS and CSS. The addition of information on the HPV-associated p16 overexpression did not explain this lack of effect.
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The role of accelerated fractionation without total dose reduction (AF) in head and neck cancer has been the focus of several studies [1–12]. A number of different treatment schedules have been applied and most of them show an improved loco-regional control (LRC) [3–6,10,11]. In [2,3], the increased LRC is gained at the expense of greater late morbidity. Altered fractionation, both AF and hyperfractionated (HF), has been the focus of meta-analyses [13,14] where the effect of AF versus conventional fractionation (CF) on overall survival (OS) is estimated to be +2% at 5 years. The benefit of altered fractionation is greater in patients with younger age and advanced primary tumours [14]. It is also reported that AF has a greater efficiency for primary tumours compared with node metastases [11]. In a recent report on the final results of RTOG 9003 [12] it was concluded that HF, but not AF, improved loco-regional control (LRC) and OS without increasing the risk for late side effects.

In oropharyngeal cancers, the presence of human papilloma virus (HPV) is shown to have an independent prognostic significance [15]. Although HPV may be present in other tumour sites of the head and neck region, it seems to have a prognostic influence only on the oropharyngeal cancer patients [16]. To explore the effects of HPV association in oropharyngeal cancer on the results of the present study, immunohistochemical detection of HPV-associated p16-expression was performed.

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The preliminary two-year follow up of the present study [1] failed to show any statistically significant benefit from AF concerning LRC and OS. The acute toxicity was more severe in the AF group while there was no evidence for a difference in late complications. The present report contains the mature five-year data from the Swedish ARTSCAN trial including information on the influence of HPV measured as p16 positivity (p16+) for oropharyngeal cancers. Possible differential effects from fractionation on the patterns of recurrence for primary tumours vs. nodal and distant are investigated.

Materials and method

Objectives and end points

The primary objective of the present report was to compare the five-year LRC of radiotherapy (RT) given as AF, delivered in 4.5–5 weeks versus CF in 7 weeks with a prescribed tumour dose of 68 Gy in both treatment arms. Secondary objectives were: OS, cancer-specific survival (CSS), treatment-related morbidity, variations in outcome in different sub-sites and stages, and differences in patterns of loco-regional recurrences with respect to treatment type. During the follow up period, the HPV association with oropharyngeal cancers and the related beneficial influence on prognosis has been recognised. Therefore a new objective was to analyse the impact of HPV association on the outcome of AF.

Patients, trial design and radiotherapy

The patient characteristics and the methods of the study have been described in detail previously [1,17]. In brief, the study originally included 750 patients with squamous cell carcinoma of the oral cavity, oropharynx, larynx (except T1–2, N0 glottic cancers) and hypopharynx. Patients with distant metastases, prior surgery and/or chemotherapy were excluded. Patients were randomised between AF (1.1 Gy + 2 Gy per day, 5 days/week for 4.5 weeks, total dose 68 Gy) and CF (2 Gy per day, 5 days/week for 7 weeks, total dose 68 Gy). In AF the two daily fractions were separated by at least 7 h. The use of CT-based three-dimensional conformal radiotherapy (3DCRT) and/or intensity-modulated radiotherapy (IMRT) was mandatory. Dose prescriptions were made according to ICRU specifications [18,19]. About 10% of all patients received IMRT. They were included during the last years of the recruitment period. A comprehensive QA-programme was performed to monitor the treatment [17].

Oropharyngeal cancers and p16 analysis

Three hundred patients with oropharyngeal cancer from the four largest centres in Sweden were selected for retrieval of original paraffin embedded tumour material. The retrieved tumour blocks were processed as follows: tumour biopsy sections (4–5 μm) were de-paraffinised and rehydrated, with antigen retrieval in citrate buffer (pH 6) and unspecific binding sites blocked with 1.5% horse serum in PBS. The sections were then stained with mAb p16INKA4a (clone: JC8, dilution 1:100, Santa Cruz Biotech, Santa Cruz, CA, USA) at +8 °C overnight, before incubation for 45 min. with biotinylated anti-mouse antibody (dilution 1:200, Vector Laboratories, Burlingame, CA, USA). Alternatively, the slides were stained with the CINtec® p16 Histology (805-4713), Ventana Medical Systems Inc., Arizona, USA by following the same protocol with the exception of incubation with the antibody for 1 h at room temperature. For antigen detection, the avidin–biotin–peroxidase complex (ABC) kit (Vectastain, Vector Laboratories, Burlingame, CA, USA) was used. Slides developed in chromogen 3′-diaminobenzydine (DAB) (Vector Laboratories, Burlingame, CA, USA) and counterstained with haematoxylin were then washed and dehydrated, and then cover mounted using VectaMount permanent mounting media (Vector Laboratories, Burlingame, CA, USA). The p16 staining was regarded as positive if >70% of the tumour cells were strongly p16-positive [12].

Statistical considerations

Similar statistical considerations as previously reported in the two-year follow up [1] were made in this analysis of the five-year data. However, to conform to the literature, all follow up is calculated from the date of randomisation instead of the treatment start. Loss of LRC was defined as clinical and/or pathological persistence or recurrence of tumour (either locally or regionally) after the primary treatment. If the patient was never considered to be free of tumour the time to loss of LRC was set to zero.

Differences between groups were evaluated by the chi-squared test or Fisher's exact test for categorical variables and the Mann–Whitney test for continuous variables. LRC, OS and CSS were estimated by the Kaplan–Meier method and compared by the log-rank test and hazard ratios (HR) with [95% CI]. HR were defined so that HR <1 indicates a lower risk for the AF arm. All tests were two-sided and p-value of less than 0.05 was considered statistically significant.

Results

Seven hundred fifty patients were included in the study between November 1998 and June 2006. Seventeen patients were considered non-eligible (8 in CF and 9 in AF, respectively) leaving 733 patients for evaluation [1]. When the ARTSCAN database was closed in August 2012 all patients had been followed for 5 years after the end of radiotherapy. Thereafter, only survival and cause of death data were obtained from The Swedish Health and Welfare Statistical Database on Cause of Death. Median follow-up times for LRC and OS were 5.3 years and 9.1 years, respectively.

At the time of analysis 300 (150 in each treatment arm) of the 733 eligible patients were alive. Two hundred seventy patients (134 in AF group and 136 in CF) had died from the disease (loco-regional failure and/or metastatic). Four patients died from treatment related toxicity (two in each arm). Death from secondary cancer was encountered in 67 cases (36 AF and 31 CF). In 87 cases (41 AF and 46 CF) there were other known causes while the cause of death could not be retrieved in 5 cases (3 AF and 2 CF).

Patient characteristics

Demographics of the patients are described in [1]. The treatment arms were well balanced with regard to age, sex, performance status, initial haemoglobin concentration (Hb), T stage, N stage and tumour site. The stage distribution was as follows: stage I $n = 31$ (4.2%), II $n = 94$ (12.8%), III $n = 203$ (27.7%) and IV $n = 405$ (55.3%).

Tumour response and survival

All eligible patients

There was no significant difference in LRC between the two trial arms ($p = 0.75$). LRC at 5 years estimated by the Kaplan–Meier method was 65.5% in the AF treatment arm and 64.9% in the CF treatment arm (Fig. 1). In a separate analysis LRC was also estimated considering death as a competing risk. The correction had only a very small and non-significant effect on the estimates and not at all on the relation between outcomes. Data from this analysis is therefore not presented.

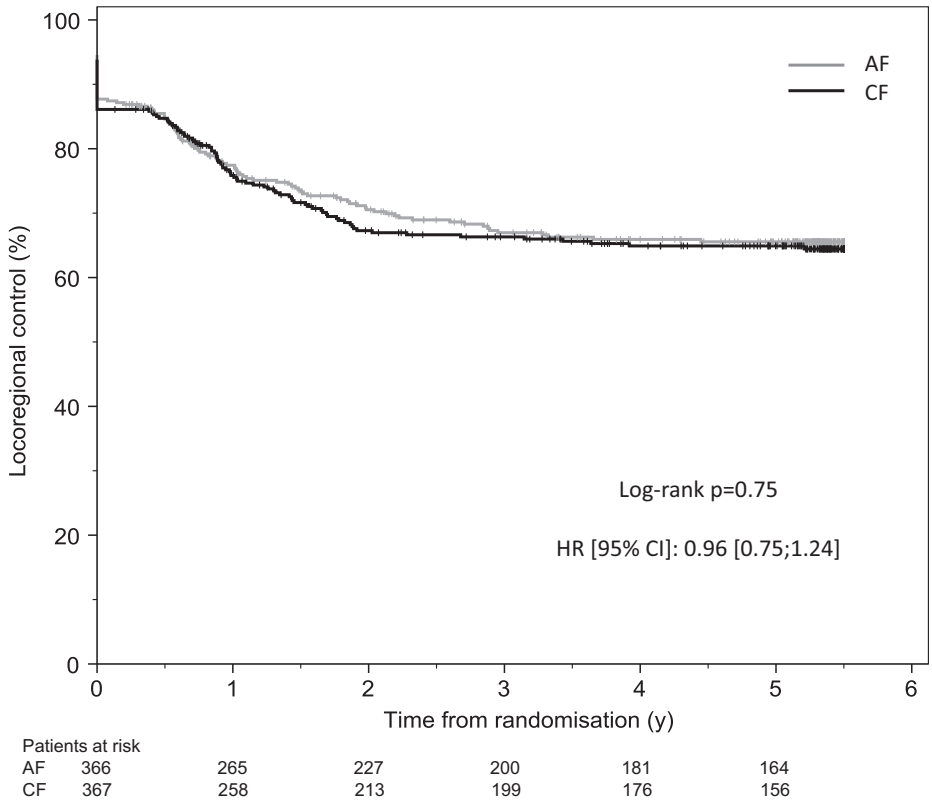

Fig. 1. Loco-regional control as function of fractionation schedule.

The major prognostic factors for LRC identified by univariate Kaplan–Meier estimation in the two-year analysis remained, i.e. age, KPS, Hb, T stage, smoking and tumour site. Neither gender nor presence of lymph-node metastases (N0 vs. N+) showed any statistically significant impact on LRC (Table 1).

Overall survival did not differ between the treatment groups ($p = 0.99$). Median OS (95% CI) was 5.1 y (3.3–6.9 y) and 5.4 y (3.7–7.1 y) in the AF and CF group, respectively (Fig. 2).

Estimated CSS at 5 years was 62.2% (AF) and 63.3% (CF) ($p = 0.99$).

There was no statistically significant difference in the patterns of failure (T, N, M) between the treatment arms ($p = 0.20$) (Table 2).

We performed multivariate Cox regression analyses with LRC and OS as dependent variables including the prognostic factors in Table 1 and treatment arm as covariates. All univariate statistically significant variables contributed significantly to the model while treatment arm did not (corrected HR = 0.83 [0.64;1.08], $p = 0.17$ for LRC and HR = 0.89 [0.73;1.08], $p = 0.23$ for OS).

Sub-group analyses

The outcome with respect to LRC for different tumour sub-sites are reported in Table 3 and shown in Fig. 3a–d. There were no significant differences in LRC between AF and CF within the different patient subgroups of Table 1. In the previous report [1] a non-significant trend for better LRC in oral cancers after AF vs. CF was noted. The trend remained in the present update ($p = 0.10$).

For laryngeal cancers the estimated median (95% CI) OS time was 4.3 (2.7–5.8) years for AF vs. 3.9 (1.6–6.2) years for CF. The corresponding figures for hypopharyngeal cancers were 1.5 (1.0–2.0) vs. 1.5 (1.1–1.9) years, and for cancers of the oral cavity 3.1 (0.1–6.3) vs. 2.2 (0.7–3.6) years (Table 3). For oropharyngeal tumours, the Kaplan–Meier estimated OS at 5 and 10 years were 66% and 58% for AF versus 64% and 57% for CF, respectively. CSS showed a pattern similar to that of LRC and OS without any statistically significant difference between AF and CF (Table 3).

The response of T1–2 tumours was similar for AF and CF with an estimated LRC at 5 years of 75.7% and 78.1%, respectively ($p = 0.57$). Previously, we reported a trend towards better LRC for AF at 2 years for T3–4 tumours. This trend was reduced in this update; LRC 55.7% for AF vs. 51.7% for CF at 5 years ($p = 0.29$). There was no significant difference in LRC between AF vs. CF for patients with N0 ($p = 0.10$) and N+ ($p = 0.38$) tumours.

Analysis of any presence of p16 overexpression was performed for oropharyngeal cancers. Paraffin embedded tumour specimens could be retrieved and analysed from 206 of the 357 evaluable patients with oropharyngeal cancer. For assessment of possible selection bias in the group whose tumour material was analysed, the groups with known p16 status vs. unknown were compared with respect to LRC and OS. No difference in LRC (HR = 0.86 [0.55;1.34], $p = 0.51$) or OS (HR = 0.97 [0.70;1.34], $p = 0.86$) could be detected. Updated demographics for oropharyngeal cancer patients with known p16 status are shown in Table 4. The patients' characteristics differ significantly between patients with p16 overexpression vs. without. The p16-overexpressing patients had lower T stage, higher Karnofsky performance score (KPS), younger age and were less frequently smoking.

The 206 patients with oropharyngeal cancer for whom we were able to retrospectively analyse p16 status were assessed with regard to differential response to AF vs. CF. The p16 status of the tumours did not change the response to AF vs. CF with regard to LRC, OS or CSS (Table 3). A statistically significant better overall prognosis was found, however, for p16+ compared with p16− tumours (Table 5).

Morbidity

The acute morbidity was reported in [1] and was significantly worse in the AF group. Tube feeding was more common in the

Table 1
Patient and disease characteristics versus LRC.

Variables	Grouping	Kaplan–Meier-estimated LRC at 5 yrs (%)	Log-rank *p*-value	HR [95% CI]
Patient characteristics				
Age	⩽60 y	74.6	<0.0001	0.53 [0.41;0.69]
	>60 y	56.7		
Gender	Females	65.9	0.67	0.94 [0.71;1.26]
	Males	65.1		
Karnofsky score	100	78.4	<0.0001	0.37 [0.28;0.48]
	<100	51.9		
Haemoglobin*	>140 g/l	74.1	<0.0001	0.56 [0.42;0.75]
	⩽140 g/l	57.6		
Smoking at start of RT*	No	71.2	0.0004	0.59 [0.44;0.80]
	Yes	57.1		
Disease characteristics				
T stage	T1	90.1	0.0005	0.31 [0.16;0.63]
	T2	72.1		
	T2	72.1	0.058	0.73 [0.52;1.02]
	T3	63.5		
	T3	63.5	<0.0001	0.53 [0.39;0.73]
	T4	43.0		
Nodal status	N0	63.6	0.68	1.05 [0.82;1.36]
	N+	66.3		
Tumour site	Oropharynx	76.7	0.016	0.87 [0.77;0.98]
	Larynx	66.8		
	Larynx	66.8	0.003	0.75 [0.61;0.91]
	Oral cavity	48.9		
	Oral cavity	48.9	0.29	0.83 [0.57;1.20]
	Hypopharynx	41.4		
	Larynx	66.8	<0.0001	0.46 [0.32;0.67]
	Hypopharynx	41.4		

* Incomplete data: Hb $n = 623$ and smoking status $n = 531$.

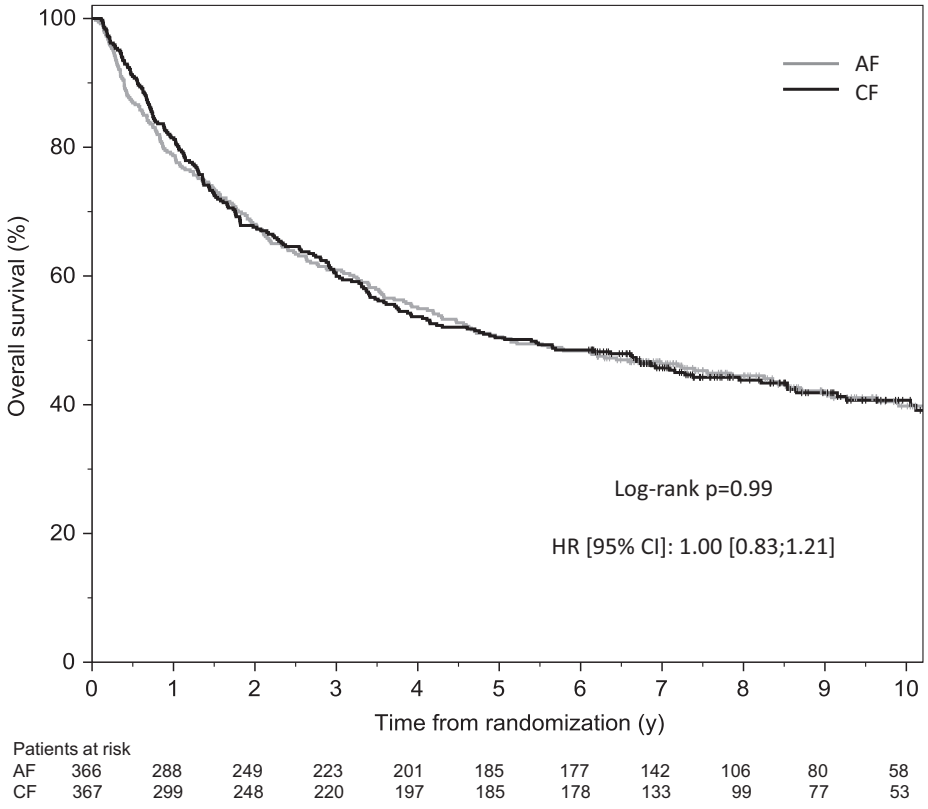

Fig. 2. Overall survival as function of fractionation schedule.

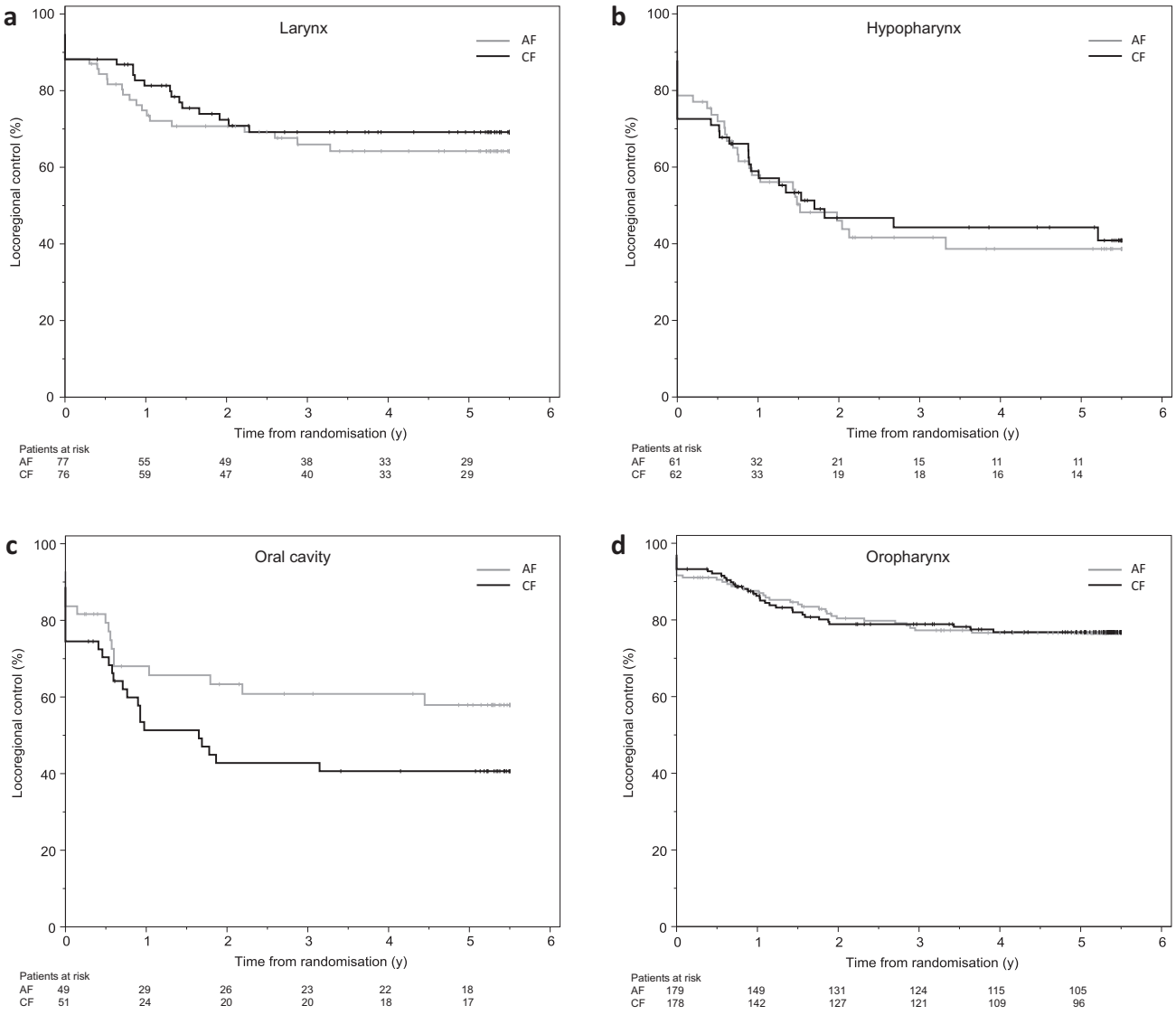

Fig. 3. Loco-regional control as function of fractionation schedule for the different tumour subgroups: (a) larynx, (b) hypopharynx, (c) oral cavity, and (d) oropharynx.

Table 2
Patterns of first failure.

Site of failure	AF $N = 366$		CF $N = 367$	
	n	%	*n*	%
T	42	(11.5)	54	(14.7)
N	16	(4.4)	21	(5.7)
T + N	48	(13.1)	40	(10.9)
T + M	2	(0.5)	3	(0.8)
N + M	5	(1.4)	0	(0.0)
T + N + M	3	(0.8)	3	(0.8)
M	26	(7.1)	21	(5.7)
(no failure)	224	(61.2)	225	(61.3)

AF group but there was no difference in weight loss observed between the study groups [20]. Up to 5 years of follow-up showed no significant difference in severe late reactions observed between the treatments. The number of patients with exposed bone or bone necrosis was 7.8% in the AF and 5.6% in the CF group ($p = 0.38$). No case of severe fibrosis was reported. In the CF group 4 patients had a tracheostomy due to side effects versus none in AF. No case of unexpected spinal injury was observed. The incidence of trismus showed a dependence of dose to mastication structures but was not significantly different between the fractionation schedules [21].

Discussion

Compared with the two-year report of the present study [1], the results here are very similar. No statistically significant difference between AF and CF concerning LRC, OS and CSS could be identified. Neither was there any significant impact of the fractionation on the incidence or severity in the assessed late morbidity. Factors like age, T-stage, N-stage or tumour site did not show any influence on the response to AF vs. CF. The only exception was a strong but non-significant trend towards a higher LRC for cancers in the oral cavity. This finding is within the scope of an ongoing clinical trial (ISRCTN00608410). In previous reports, a statistically significant improved LRC with AF was established [3–5,8,10,11]. This was also the result of a meta-analysis by Bourhis et al. [13]. In all studies except one [5], no statistically significant difference in OS was detected. In [13] an improvement of OS at 5 years of 2% was estimated. More recently, the result of the RTOG 9003 study was

Table 3
LRC, OS and CSS per tumour site.

Tumour site	Kaplan–Meier estimated LRC/OS/CSS at 5 yrs (%)		Log-rank *p*-value	HR [95% CI] AF vs. CF
	AF	CF		
LRC				
Larynx	64.2	69.2	0.51	1.21 [0.68;2.13]
Hypopharynx	38.7	44.3	0.83	1.05 [0.65;1.70]
Oral cavity	57.9	40.6	0.10	0.63 [0.36;1.13]
Oropharynx	76.6	76.8	0.98	1.00 [0.65;1.56]
P16 pos	82.5	83.6	0.98	0.99 [0.45;2.17]
P16 neg	72.0	46.4	0.23	0.57 [0.22;1.49]
OS				
Larynx	45.5	47.4	0.87	1.03 [0.70;1.52]
Hypopharynx	18.0	27.4	0.31	1.21 [0.83;1.78]
Oral cavity	42.9	35.3	0.86	0.96 [0.60;1.52]
Oropharynx	65.9	64.0	0.63	0.93 [0.67;1.27]
P16 pos	78.5	73.0	0.72	0.90 [0.51;1.58]
P16 neg	36.0	32.1	0.28	0.70 [0.36;1.34]
CSS				
Larynx	63.4	69.0	0.65	1.14 [0.65;2.00]
Hypopharynx	28.8	37.4	0.47	1.18 [0.75;1.83]
Oral cavity	57.3	45.5	0.28	0.72 [0.41;1.29]
Oropharynx	73.1	74.5	0.93	1.02 [0.68;1.52]
P16 pos	84.2	76.6	0.30	0.70 [0.35;1.39]
P16 neg	53.3	48.8	0.69	0.85 [0.37;1.92]

Table 4
Patient characteristics for the subjects with known p16 status.

	p16 positive		p16 negative		*p*-value
	n = 153	(%)	*n* = 53	(%)	
Age at randomisation					
Median (range)	57	(35–79)	60	(37–83)	0.0076
Gender					0.36
Male	118	(77.1)	37	(69.8)	
Female	35	(22.9)	16	(30.2)	
Total	153	(100)	53	(100.0)	
Karnofsky index					0.0002
<100	47	(32.2)	32	(62.7)	
100	99	(67.8)	19	(37.3)	
Total	146	(100.0)	51	(100.0)	
Haemoglobin conc.					1.00
⩽140	68	(52.3)	23	(51.1)	
>140	62	(47.7)	22	(48.9)	
Total	130	(100.0)	45	(100.0)	
Smoker*					<0.0001
Yes	19	(15.2)	30	(76.9)	
No	106	(84.8)	9	(23.1)	
Total	125	(100.0)	39	(100.0)	
T stage					0.016
T1	30	(19.6)	5	(9.4)	
T2	66	(43.1)	15	(28.3)	
T3	31	(20.3)	19	(35.8)	
T4	26	(17.0)	14	(26.4)	
Total	153	(100.0)	53	(100.0)	
Nodal status					0.23
N0	26	(17.0)	14	(26.4)	
N1-N2B	96	(62.7)	32	(60.4)	
N2C-N3	31	(20.3)	7	(13.2)	
Total	153	(100.0)	53	(100.0)	
Distant metastases					1.00
No	136	(88.9)	47	(88.7)	
Yes	17	(11.1)	6	(11.3)	
Total	153	(100.0)	53	(100.0)	
RT treatment arm					0.63
CF	74	(48.4)	28	(52.8)	
AF	79	(51.6)	25	(47.2)	
Total	153	(100.0)	53	(100.0)	

* Active smoker at start of RT (patient reported).

Table 5
LRC, OS and CSS for oropharynx cancer patients with known p16 status.

Tumour site	Kaplan–Meier estimated LRC/OS/CSS at 5 yrs (%)		Log-rank *p*-value	HR [95% CI] P16+ vs. p16−
	p16+	p16−		
LRC	83.0	61.4	0.0008	0.37 [0.20;0.68]
OS	75.8	34.0	<0.0001	0.32 [0.21;0.49]
CSS	80.4	51.2	<0.0001	0.36 [0.21;0.61]

reported by Beitler et al. [12]. The initially reported favourable outcome of continuous AF was no longer statistically significant. In addition to this, Bourhis et al. [22] found no difference in effectiveness between AF and CF when combined with concomitant cisplatinum. The results from the present study as well as [12,22] thus question the value of AF. In the present study, the LRC at 5 years of 64.9% in the CF arm was comparatively high to those reported by many other studies. This might have led to a smaller relative difference between AF and CF that would perhaps not then be detected. The varying results between the mentioned studies of AF are not explained by obvious differences in the patient characteristics in the respective materials. In recent years, HPV associated oropharyngeal cancer has been identified as a disease with a better prognosis compared with oropharyngeal cancers without this association [16,23]. In concordance with many other studies, oropharyngeal carcinoma patients constitute a large proportion of the material in this report. It is well known that the proportion of HPV association in oropharyngeal cancers may vary between different populations, e.g. [24,25]. For that reason we collected original diagnostic material from the oropharyngeal cancer patients. From the original 357 evaluable patients material could be retrieved and analysed from 206 (58%). Immunohistochemical evaluation of p16 was performed, as p16 is a prognostic factor in oropharyngeal carcinoma [26] and has a strong association to HPV, which makes it a suitable surrogate marker [27]. The proportion of p16+ patients was 74% among the 206 analysed cases. To assess possible selection bias, the 206 patients with known p16 status were compared with the 151 patients with unknown p16 status and were found to have similar LRC, OS and CSS ($p = 0.51$, $p = 0.86$ and $p = 0.98$, respectively). The p16+ patients had a significantly better prognosis with regard to LRC, OS and CSS than the p16− patients. There was a statistically non-significant trend towards a higher proportion of mainly LRC with AF than CF in p16− patients. However, the total number of p16− patients was small ($n = 53$) and probably insufficient to detect any true difference in response between AF and CF. One hypothesis that might be raised is that other studies with a smaller proportion of p16+ patients may have detected a difference between AF and CF in such cases, while this study fails to do so. In this respect the present study is inconclusive. Lassen et al. [28] concluded that the beneficial effect from AF was independent of p16 status, which speaks against our finding of a trend for p16− patients only.

In conclusion this update confirms the results of the previous report [1]. We failed to identify a positive effect from AF regarding LRC, OS and CSS. The addition of information on the HPV associated p16 overexpression did not explain this lack of effect. Small effects may be obscured by the fact that the overall outcome is comparatively good in the present study. This may hamper the statistical inference and demand for even larger studies or meta-analyses. Furthermore, a low proportion of p16− oropharyngeal tumours prohibits conclusions on this sub-group.

Conflict of interest statement

The authors have nothing to disclose.

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