Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24-35. DOI: 10.1056/NEJMoa0912217.

Supplemental Figure 1 is the CONSORT diagram for RTOG 0129; 361 of 372 (97%) and 360 of 371 (97%) patients from the SFX and AFX-C treatment arms, respectively, were included in analysis. On the SFX arm, only one patient included in analysis did not receive both radiation therapy and chemotherapy, compared to 6 on the AFX-C arm.

Supplemental Table 1 shows patient and tumor characteristics for oropharynx patients with and without HPV determination. In Supplemental Figure 2, Kaplan-Meier estimates for overall survival (top) and progression-free survival (bottom) are presented for oropharynx patients with and without HPV determination. No statistically significant differences were detected for baseline characteristics or outcome.

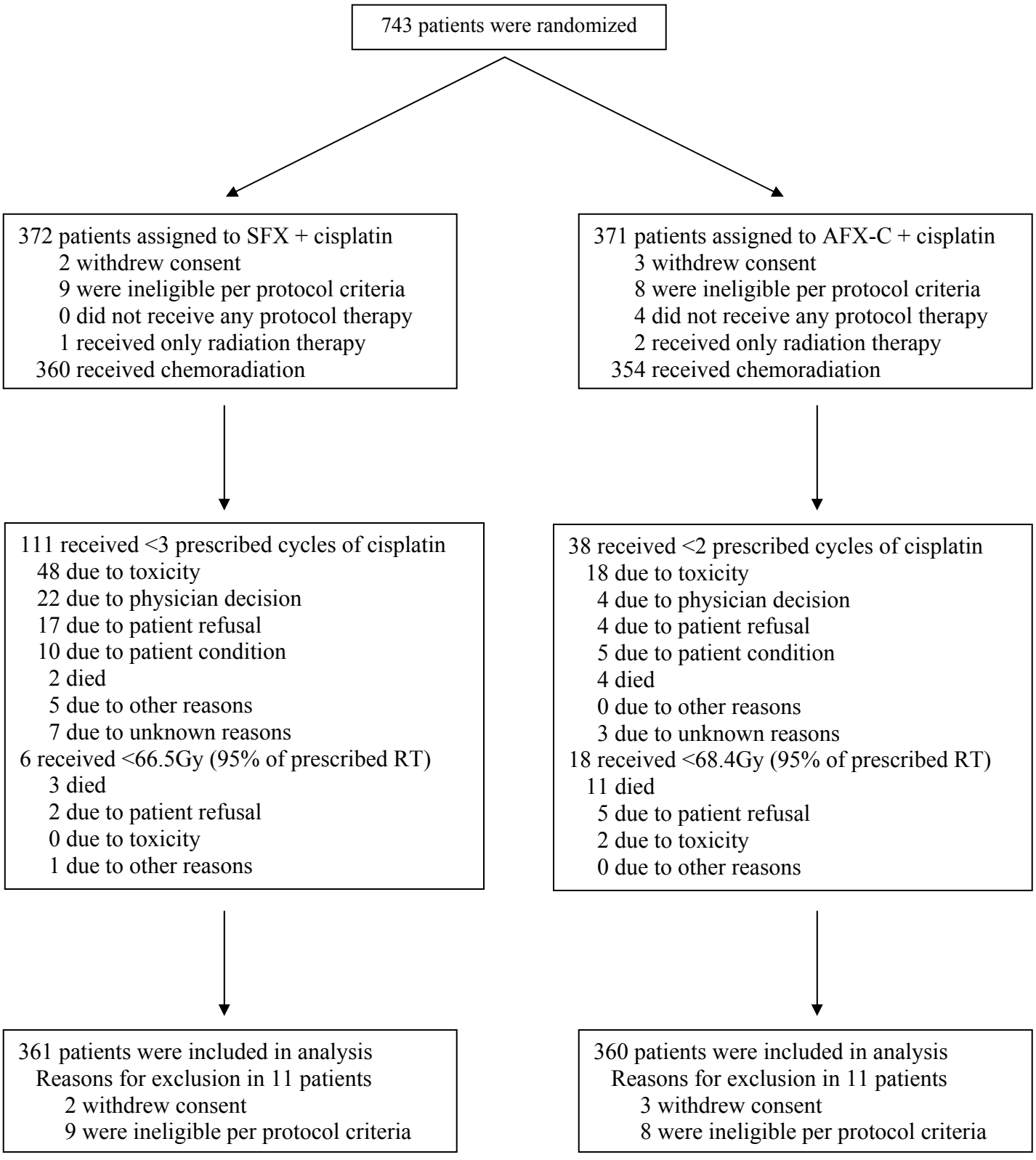
Supplemental Figure 3 shows representative cases of oropharynx cancer positive by HPV16 *in situ* hybridization and p16 immunohistochemistry. Panels show HPV16 *in situ* hybridization (A and C) and p16 (B and D) immunohistochemistry results for representative cases of HPV16-positive, invasive squamous cell carcinoma. HPV16 is visualized as multiple (Panel A) or single (Panel C) punctuate brown signals per tumor cell nucleus. Panel insets demonstrate positive controls performed in all assays derived from cell lines with ~ 500 (CaSki, inset Panel A) or 2 integrated copies of HPV16 (SiHa, inset Panel C). Strong and diffuse nuclear and cytoplasmic brown staining for p16 is specific to tumor cells (Panels B and D).

Supplemental Table 2 shows treatment delivery by assigned treatment and by HPV status. Compliance to prescribed radiation therapy was excellent for both arms. For patients assigned to the AFX-C arm, 91.1% received at least 10 of 12 planned BID fractionation days. Compliance to prescribed chemotherapy was better on the AFX-C arm with 87.8% receiving both cycles compared to 69.0% receiving all 3 cycles on the SFX arm. Treatment compliance was similar for HPV positive and negative patients.

Supplemental Table 3 shows toxicity and feeding tube use by assigned treatment. There were no differences detected for death within 30 days or for Grade 5 toxicity. Patients on the SFX arm had more Grade 3-4 hematologic toxicity (39.6% vs. 30.8%) and Grade 3-4 metabolic/laboratory toxicity (23.0% vs. 16.7%). Patients on the AFX-C arm had more late Grade 3-4 mucositis, but the overall incidence was very low for both arms (1.4% and 4.1%). Two-thirds of patients on each arm required a feeding tube at the end of treatment (68.7% and 67.1%) and approximately 10% still required a tube at 3 years from the start of treatment (8.3% and 10.1%).

Supplemental Table 4 shows all time-to-failure endpoints in addition to cause of death and site of first failure by assigned treatment. In Supplemental Figure 4, Kaplan-Meier estimates are presented for overall and progression-free survival, and cumulative incidence estimates for local-regional failure and distant metastasis, by assigned treatment. We detected no differences in overall survival (top left), progression-free survival (top right), local-regional failure (bottom left), or distant metastasis (bottom right) between the treatment arms.

Supplemental Figure 1



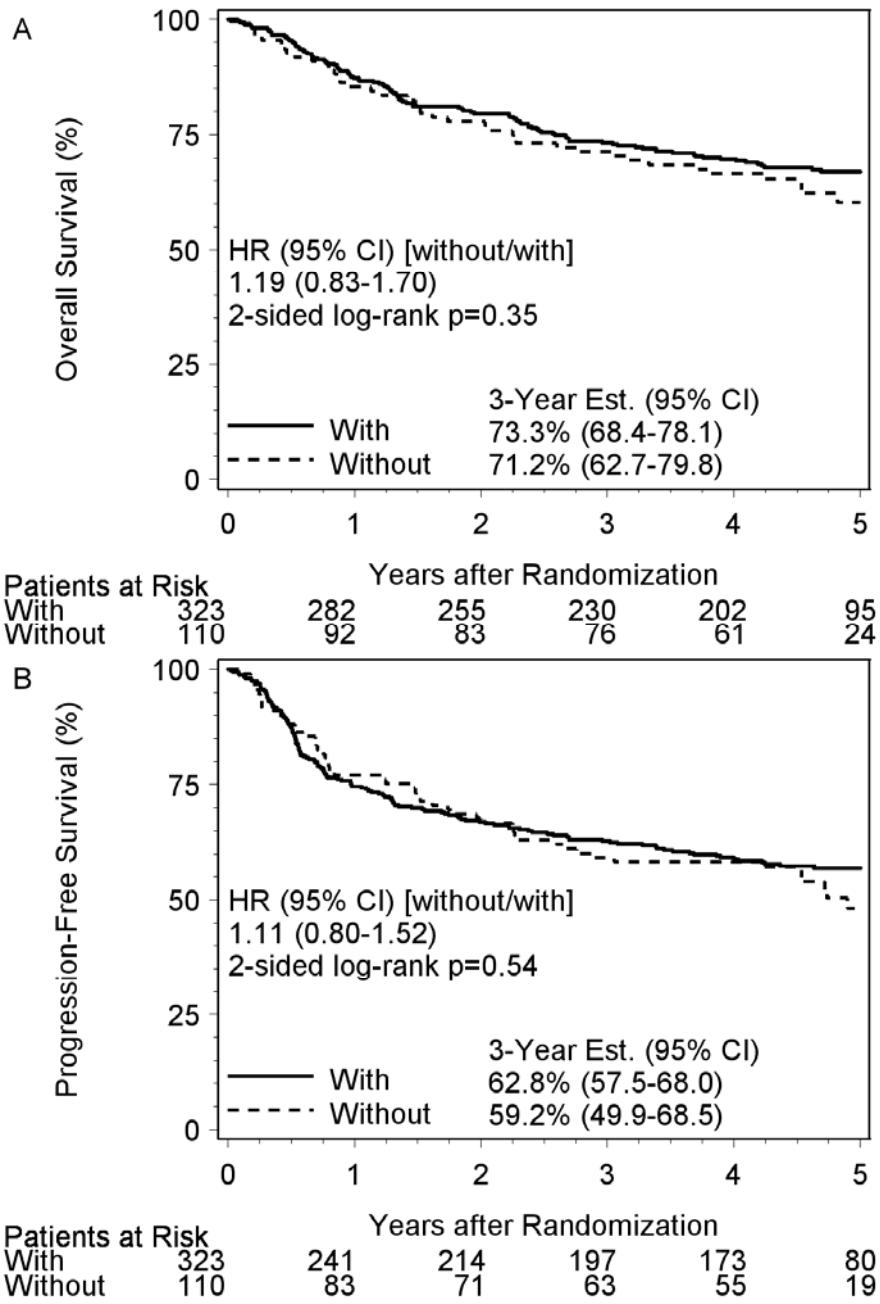
Supplemental Table 1. Patient and Tumor Characteristics by HPV Determination for Patients with an Oropharynx Primary.

	With HPV Determination (n=323)	Without HPV Determination (n=110)	p-value
Treatment Assignment			0.39 [1]
SFX + cisplatin	165 (51.1%)	51 (46.4%)	
AFX-C + cisplatin	158 (48.9%)	59 (53.6%)	
Age			0.12 [2]
Median	55	56.5	
Range	31 - 82	36 - 77	
Gender			0.54 [1]
Male	271 (83.9%)	95 (86.4%)	
Female	52 (16.1%)	15 (13.6%)	
Race			0.28 [1]
White	278 (86.1%)	90 (81.8%)	
Non-White	45 (13.9%)	20 (18.2%)	
Zubrod Performance Status			0.20 [1]
0	207 (64.1%)	63 (57.3%)	
1	116 (35.9%)	47 (42.7%)	
Anemia			0.66 [1]
No	236 (73.1%)	78 (70.9%)	
Yes	87 (26.9%)	32 (29.1%)	
T Stage			0.26 [3]
T2	99 (30.7%)	30 (27.3%)	
T3	127 (39.3%)	40 (36.4%)	
T4	97 (30.0%)	40 (36.4%)	
N Stage			0.40 [3]
N0	24 (7.4%)	13 (11.8%)	
N1	46 (14.2%)	10 (9.1%)	
N2a	37 (11.5%)	9 (8.2%)	
N2b	107 (33.1%)	32 (29.1%)	
N2c	78 (24.1%)	37 (33.6%)	
N3	31 (9.6%)	9 (8.2%)	
AJCC Stage			0.32 [1]
III	44 (13.6%)	11 (10.0%)	
IV	279 (86.4%)	99 (90.0%)	
Smoking History			0.65 [1]
Never smoked	73 (22.6%)	19 (17.3%)	
Former smoker	164 (50.8%)	57 (51.8%)	
Current smoker	56 (17.3%)	22 (20.0%)	
Unknown	30 (9.3%)	12 (10.9%)	

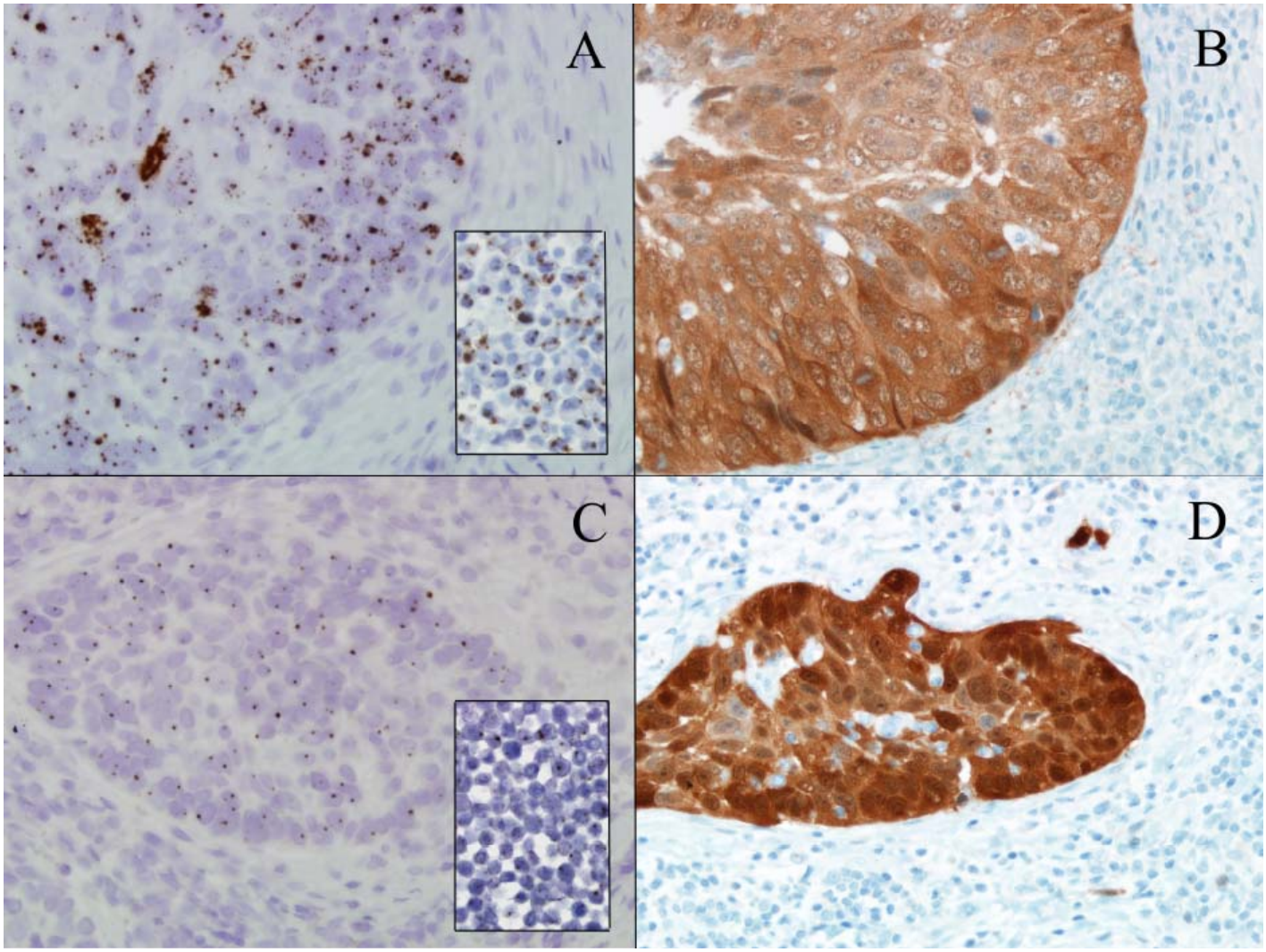
	With HPV Determination (n=323)	Without HPV Determination (n=110)	p-value
Pack Years			0.18 [2]
Median	20	30	
Range	0 - 152	0 - 110	

HPV = human papillomavirus.
SFX = standard fractionation radiotherapy.
AFX-C = accelerated fractionation with concomitant boost radiotherapy.
AJCC = American Joint Committee on Cancer.
[1] Pearson Chi-Square test.
[2] Kolmogorov-Smirnov test.
[3] Kruskal-Wallis test.
Anemia is defined as hemoglobin ≤ 13.5 for males and ≤ 12.5 for females.
A pack year is defined as the equivalent of smoking one pack of cigarettes per day for one year.
Pack years are missing for 57 (17.6%) and 23 (20.9%) patients with and without HPV determination.

Supplemental Figure 2



Supplemental Figure 3



Supplemental Table 2. Treatment Delivery by Assigned Treatment and HPV Status.

	All Patients		Oropharynx Only With HPV Determination	
	SFX+cisplatin (n=361)	AFX-C+cisplatin (n=360)	HPV Positive (n=206)	HPV Negative (n=117)
RT Dose Delivered (Gy)				
≥ 95% of protocol dose	355 (98.3%)	338 (93.9%)	199 (96.6%)	115 (98.3%)
≥ 90, < 95% of protocol dose	1 (0.3%)	1 (0.3%)	1 (0.5%)	0 (0.0%)
< 90% of protocol dose	5 (1.4%)	17 (4.7%)	5 (2.4%)	2 (1.7%)
No RT given	0 (0.0%)	4 (1.1%)	1 (0.5%)	0 (0.0%)
Twice-a-day (BID) RT				
RT included BID	1 (0.3%)	348 (96.7%)	99 (48.1%)	57 (48.7%)
RT did not include BID	357 (98.9%)	8 (2.2%)	106 (51.5%)	60 (51.3%)
No RT given	0 (0.0%)	4 (1.1%)	1 (0.5%)	0 (0.0%)
RT not evaluable	3 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Days BID RT Missed				
0	358 (99.2%)	266 (73.9%)	184 (89.3%)	105 (89.7%)
1-5	0 (0.0%)	71 (19.7%)	18 (8.7%)	10 (8.5%)
>5	0 (0.0%)	19 (5.3%)	3 (1.5%)	2 (1.7%)
No RT given	0 (0.0%)	4 (1.1%)	1 (0.5%)	0 (0.0%)
RT not evaluable	3 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
RT Elapsed Days				
< per protocol	14 (3.9%)	20 (5.6%)	11 (5.3%)	4 (3.4%)
Per protocol + 0-7	291 (80.6%)	300 (83.3%)	178 (86.4%)	100 (85.5%)
Per protocol + 8-14	35 (9.7%)	28 (7.8%)	12 (5.8%)	10 (8.5%)
Per protocol + 15 or more	21 (5.8%)	8 (2.2%)	4 (1.9%)	3 (2.6%)
No RT given	0 (0.0%)	4 (1.1%)	1 (0.5%)	0 (0.0%)
Cisplatin Delivery				
Per protocol	184 (51.0%)	225 (62.5%)	113 (54.9%)	67 (57.3%)
Variable, acceptable	128 (35.5%)	110 (30.6%)	72 (35.0%)	37 (31.6%)
Deviation, unacceptable	10 (2.8%)	3 (0.8%)	3 (1.5%)	3 (2.6%)
Incomplete chemotherapy	35 (9.7%)	13 (3.6%)	16 (7.8%)	7 (6.0%)
No chemotherapy given	1 (0.3%)	6 (1.7%)	1 (0.5%)	0 (0.0%)
Not evaluable	3 (0.8%)	3 (0.8%)	1 (0.5%)	3 (2.6%)
Cisplatin Cycles Delivered				
0	1 (0.3%)	6 (1.7%)	1 (0.5%)	1 (0.9%)
1	25 (6.9%)	38 (10.6%)	10 (4.9%)	11 (9.4%)
2	86 (23.8%)	316 (87.8%)	125 (60.7%)	66 (56.4%)
3	249 (69.0%)	0 (0.0%)	70 (34.0%)	39 (33.3%)
Cisplatin Dose Delivered (mg/m2)				
≥ 200	304 (84.2%)	225 (62.5%)	148 (71.8%)	84 (71.8%)
≥ 100, < 200 [1]	53 (14.7%)	120 (33.3%)	56 (27.2%)	30 (25.6%)
< 100	3 (0.8%)	9 (2.5%)	1 (0.5%)	2 (1.7%)
No chemotherapy given	1 (0.3%)	6 (1.7%)	1 (0.5%)	1 (0.9%)
Min	0.0	0.0	0.0	0.0
Q1	200.0	195.8	199.1	199.1

	All Patients		Oropharynx Only With HPV Determination	
	SFX+cisplatin (n=361)	AFX-C+cisplatin (n=360)	HPV Positive (n=206)	HPV Negative (n=117)
Median	298.4	200.0	200.0	200.0
Q3	300.0	200.0	297.2	289.6
Max	309.4	207.1	308.4	302.5

HPV = human papillomavirus.
SFX = standard fractionation radiotherapy.
AFX-C = accelerated fractionation with concomitant boost radiotherapy.
SFX: 70 Gy in 7 weeks and 3 cycles of cisplatin (100 mg/m2) on days 1, 22, and 43.
AFX-C: 72 Gy in 6 weeks and 2 cycles of cisplatin (100 mg/m2) on days 1 and 22.
RT = radiation therapy.
Q1 = first quartile.
Q3 = third quartile.
[1] For patients receiving ≥ 100 but < 200 mg/m2 of cisplatin, the median dose was 159.1 for SFX patients and 188.1 for AFX-C patients.

Supplemental Table 3. Toxicity and Feeding Tube Use by Assigned Treatment.

	SFX+cisplatin			AFX-C+cisplatin			
	Number/ Total	%	95% CI	Number/ Total	%	95% CI	p-value [1]
Deaths							
Within 30 days of treatment end, any cause	7/361	1.9	0.5, 3.4	12/360	3.3	1.5, 5.2	0.26
Grade 5 toxicity [2]	2/361	0.6	0.0, 1.3	3/360	0.8	0.0, 1.8	0.69
Grade 3-4 CTC Toxicities							
Neutropenia	96/361	26.6	22.0, 31.2	74/360	20.6	16.4, 24.7	0.07
Infection febrile neutropenia	34/361	9.4	6.4, 12.4	37/360	10.3	7.1, 13.4	0.71
Other hematologic	143/361	39.6	34.6, 44.7	111/360	30.8	26.1, 35.6	0.02
Metabolic/laboratory	83/361	23.0	18.7, 27.3	60/360	16.7	12.8, 20.5	0.04
Renal/genitourinary	22/361	6.1	3.6, 8.6	13/360	3.6	1.7, 5.5	0.16
Auditory/hearing	10/361	2.8	1.1, 4.5	6/360	1.7	0.3, 3.0	0.45
Dermatology/skin	31/361	8.6	5.7, 11.5	35/360	9.7	6.7, 12.8	0.61
Mucositis/stomatitis	143/361	39.6	34.6, 44.7	119/360	33.1	28.2, 37.9	0.07
Other gastrointestinal	189/361	52.4	47.2, 57.5	187/360	51.9	46.8, 57.1	0.94
All others	100/361	27.7	23.1, 32.3	90/360	25.0	20.5, 29.5	0.45
Overall	302/361	83.7	79.8, 87.5	288/360	80.0	75.9, 84.1	0.21
Grade 3-4 Late RTOG/EORTC Toxicities							
Mucous membrane	5/351	1.4	0.2, 2.7	14/343	4.1	2.0, 6.2	0.04
Esophagus	25/351	7.1	4.4, 9.8	33/343	9.6	6.5, 12.7	0.27
Larynx	13/351	3.7	1.7, 5.7	15/343	4.4	2.2, 6.5	0.70
Salivary gland	13/351	3.7	1.7, 5.7	18/343	5.2	2.9, 7.6	0.36
Skin	5/351	1.4	0.2, 2.7	2/343	0.6	0.0, 1.4	0.45
Subcutaneous tissue	9/351	2.6	0.9, 4.2	8/343	2.3	0.7, 3.9	1.00
Bone	10/351	2.8	1.1, 4.6	15/343	4.4	2.2, 6.5	0.31
All others	32/351	9.1	6.1, 12.1	29/343	8.5	5.5, 11.4	0.79
Overall	74/351	21.1	16.8, 25.3	88/343	25.7	21.0, 30.3	0.18
Nutritional Support via Feeding Tube							
Pretreatment	89/361	24.7	20.2, 29.1	79/360	21.9	17.7, 26.2	0.43
End of treatment	248/361	68.7	63.9, 73.5	239/356	67.1	62.3, 72.0	0.80
1 year from start of treatment	88/301	29.2	24.1, 34.4	84/302	27.8	22.8, 32.9	0.72
2 years from start of treatment	28/242	11.6	7.5, 15.6	38/242	15.7	11.1, 20.3	0.23
3 years from start of treatment	16/192	8.3	4.4, 12.2	20/199	10.1	5.9, 14.2	0.60

SFX = standard fractionation radiotherapy.
AFX-C = accelerated fractionation with concomitant boost radiotherapy.
CI = confidence interval.
CTC = Common Toxicity Criteria, version 2.0.
RTOG = Radiation Therapy Oncology Group.
EORTC = European Organisation for Research and Treatment of Cancer.
[1] Fisher's exact test.
[2] SFX: 1-hyponatremia, 1-hemorrhage; AFX-C: 2-febrile neutropenia; 1-hemorrhagic stroke.

Supplemental Table 4. Survival Estimates, Causes of Death, and Patterns of Failure by Assigned Treatment.

	SFX + cisplatin (n=361)	AFX-C + cisplatin (n=360)	p-value
Overall Survival			0.18 [1]
3-year estimate (95% CI)	64.3% (59.3-69.2)	70.3% (65.6-75.1)	
Cause of Death	(n=157)	(n=146)	0.54 [2]
Index cancer	97 (61.8%)	80 (54.8%)	
Second primary tumor	12 (7.6%)	11 (7.5%)	
Protocol treatment	2 (1.3%)	3 (2.1%)	
Non-protocol treatment	2 (1.3%)	1 (0.7%)	
Unrelated to cancer or treatment	22 (14.0%)	22 (15.1%)	
Unknown	22 (14.0%)	29 (19.9%)	
Progression-Free Survival			0.50 [1]
3-year estimate (95% CI)	55.8% (50.6-60.9)	57.0% (51.8-62.1)	
Local-Regional Failure			0.80 [3]
3-year estimate (95% CI)	25.6% (21.1-30.1)	28.2% (23.6-32.9)	
Distant Metastasis			0.14 [3]
3-year estimate (95% CI)	13.1% (9.6-16.6)	10.4% (7.2-13.5)	
Site of First Failure	(n=183)	(n=182)	0.42 [4]
Local-regional	70 (38.3%)	80 (44.0%)	
Distant metastasis	52 (28.4%)	42 (23.1%)	
Dead, no documented progression	61 (33.3%)	60 (33.0%)	
Second Primary Tumor			0.99 [5]
3-year estimate (95% CI)	9.2% (6.2-12.2)	9.0% (6.0-12.0)	
Site of Second Primary Tumor	(n=45)	(n=45)	<0.001 [6]
Head & Neck	7 (15.6%)	9 (20.0%)	
Lung	21 (46.7%)	22 (48.9%)	
Esophagus	2 (4.4%)	1 (2.2%)	
Bladder	1 (2.2%)	0 (0.0%)	
Cervix	1 (2.2%)	0 (0.0%)	
Prostate	2 (4.4%)	3 (6.7%)	
Colon	2 (4.4%)	0 (0.0%)	
Rectum	1 (2.2%)	1 (2.2%)	
Kidney	1 (2.2%)	1 (2.2%)	
Cecum	0 (0.0%)	1 (2.2%)	
Peritoneum	1 (2.2%)	0 (0.0%)	
Breast	0 (0.0%)	2 (4.4%)	
Skin	4 (8.9%)	4 (8.9%)	
Unknown	2 (4.4%)	1 (2.2%)	

	SFX + cisplatin (n=361)	AFX-C + cisplatin (n=360)	p-value

SFX = standard fractionation radiotherapy.
AFX-C = accelerated fractionation with concomitant boost radiotherapy.
CI = confidence interval.
[1] 1-sided Log-Rank test.
[2] Pearson Chi-Square test; index cancer, protocol treatment, and non-protocol treatment were combined.
[3] 1-sided Gray's test.
[4] Pearson Chi-Square test.
[5] 2-sided Gray's test.
[6] Pearson Chi-Square test; smoking-related cancers (head & neck, lung) vs. others.

Supplemental Figure 4