Secondary analysis of the MARCH database - looking for an interaction between HPV status, smoking status, and the effect of altered fractionation radiotherapy – the MARCH-HPV initiative

A joint project supported by MARCH secretariat and DAHANCA (represented by Prof Overgaard and Dr Lassen)

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Background

1. The role of HPV has been demonstrated in the pathogenesis of a subgroup of oropharyngeal cancer (OPC). Currently it is accepted that HPV-induced OPC have a better prognosis, both in terms of overall survival and disease control, compared to classical HNSCC that are tobacco-alcohol related[1,2].
2. Whether HPV status is associated with a higher sensitivity to treatment modifications such as the use of concomitant chemoradiotherapy or altered fractionation radiotherapy remains controversial, and most of the published evidence is currently against this hypothesis[3,4].
3. However the statistical interaction between the benefits of altered fractionation radiotherapy and HPV status has currently been looked for only in individual studies, in which the power is *de facto* limited.
4. During the update of the MARCH meta-analysis, data on HPV and smoking status have been collected from trials where this information was available[5]. These data represent a unique opportunity to look for an interaction between HPV status and altered fractionation radiotherapy.

Description of data available and methods used for HPV status determination

HPV status is available for 6 trials: DAHANCA 6-7, ARTSCAN, ORO 9301, pCAIR, RTOG 0129 and RTOG 9003. However, pCAIR is a post-operative trial and will be excluded. Finally, 5 trials and 1 812 are eligible.

Table 1 describes tumor localization among these patients. In order to analyze a population as homogeneous as possible, only patients with oropharynx, larynx or hypopharynx will be included.

Table 1: Tumor localization

	Human Papilloma Virus		**TOTAL**
	Negative	Positive	
Oral cavity	132	9	141
Oropharynx	475	524	999
Larynx	473	49	522
Hypopharynx	113	11	124
Others	20	5	25
Missing / Unknown	1	0	1
TOTAL	**1214**	**598**	**1812**

One thousand six hundred and fourty-five (1 645) patients will be included in all HPV analyses. Among these patients, 999 had oropharynx tumors and 646 had larynx or hypopharynx tumors.

Table 2 describes the number of patients for which the information on tobacco status is available.

Table 2: Amount of data (patients) available regarding smoking and HPV status

Smoking status	Human Papilloma Virus		TOTAL
	Negative	Positive	
	All trials		
Never	37	195	**232**
Former	220	185	**405**
Current	422	132	**554**
Missing / Unknown	382	72	**454**
TOTAL	**1061**	**584**	**1645**
	DAHANCA 6-7		
Never	8	4	
Former	74	25	
Current	210	48	
Missing / Unknown	273	27	
	ARTSCAN		
Never	9	107	
Former	0	0	
Current	30	20	
Missing / Unknown	15	29	
	ORO 9301		
Never	0	0	
Former	0	0	
Current	0	0	
Missing / Unknown	61	4	
	RTOG 0129		
Never	11	66	
Former	85	123	
Current	45	25	
Missing / Unknown	30	12	
	RTOG 9003		
Never	9	18	
Former	61	37	
Current	137	39	
Missing / Unknown	3	0	

Smoking status is not available in ORO 9301. In the ARTSCAN trial, it is not possible to know if patients were former smokers, or if they had never smoked.

Smoking status will be analyzed as two categories (current, former/never) in order to include as many patients as possible. The number of patients with both tobacco status and HPV status is 1 191, 815 with oropharynx tumor and 376 with larynx or hypopharynx tumors (Table 3).

Table 3: Amount of data available for both tobacco and HPV status for all patients and within OPC and non OPC

Smoking status	Human Papilloma Virus		TOTAL
	Negative	Positive	
	All localizations		
Never	37	195	**232**
Former	220	185	**405**
Current	422	132	**554**
Missing / Unknown	382	72	**454**
TOTAL	**1061**	**584**	**1645**
	Oropharynx		
Never	30	193	**223**
Former	95	167	**262**
Current	225	105	**330**
Missing / Unknown	125	59	**184**
TOTAL	**475**	**524**	**999**
	Non-oropharynx		
Never	7	2	**9**
Former	125	18	**143**
Current	197	27	**224**
Missing / Unknown	257	13	**270**
TOTAL	**586**	**60**	**646**

The patient data come from 5 trials (DAHANCA 6-7, ARTSCAN, ORO 9301, RTOG 0129 and RTOG 9003), which have mostly tested mild acceleration. The ORO 9301 trial included only patients with oropharynx cancer. Table 4 gives the results according to HPV status and tumor localization (oropharynx vs. larynx/hypopharynx). Only the RTOG and DAHANCA trials included both oropharyngeal cancers and other localization. In the six trials HPV, positivity was assessed using p16 immunohistochemistry, and the methods used in each of these trials are depicted in Table 5. In patients with oropharynx cancer, the rate of HPV positivity ranged from 6.2% to 74.3%. Quantity of tobacco smoked was not available across trials.

Table 4: Description of HPV status information according to tumor location for all trials with HPV data

Tumor localization	**Human Papilloma Virus**		**TOTAL**
	Negative	Positive	
	All trials		
Oropharynx	475	524	**999**
Non-oropharynx	586	60	**646**
TOTAL	**1061**	**584**	**1645**
	DAHANCA 6-7		
Oropharynx	142	70	
Non-oropharynx	423	34	
	ARTSCAN		
Oropharynx	54	156	
Non-oropharynx	0	0	
	ORO 9301		
Oropharynx	61	4	
Non-oropharynx	0	0	
	RTOG 0129		
Oropharynx	102	218	
Non-oropharynx	69	8	
	RTOG 9003		
Oropharynx	116	76	
Non-oropharynx	94	18	

Table 5: Amount of data available and description of the HPV marker used as well as threshold chosen

Abbreviations: H, hypopharynx; L, larynx; OPC, oropharynx

Trial name (ref for methods used for HPV)	n. of pts with HPV data (% of total trial pts n.)	n. of OPC pts (with HPV data)	n. of L/HP pts (with HPV data)	HPV diagnostic marker	Positivity threshold (% tumor cells)	Methods used
RTOG 9003[6]	336 (30%)	192	112	p16	≥70%	mouse monoclonal antibody (MTM Laboratories, Heidelberg, Germany)
DAHANCA 6-7[7]	792 (53%)	212	457	p16	>70% (strong diffuse)	JC8(sc-56330; Santa Cruz Biotechnology Inc., Santa Cruz, CA)
ARTSCAN[8]	210 (28%)	210	0	p16	>70%	JC8 (sc-56330; Santa Cruz Biotechnology Inc., Santa Cruz, CA) OR CINtec® p16 Histology (805–4713), (Ventana Medical SystemsInc., Arizona, USA)
RTOG 0129[4]	409 (55%)	320	77	HPV-16 DNA (ISH)	Positive staining in tumor nuclei	HPV-16 then 12additional HPV subtypes (GenPoint, Dako)
				p16	≥70%	mouse monoclonal antibody (MTM Laboratories, Heidelberg, Germany)
ORO 9310	65 (34%)	65	0	?		?

Another part of this study is the overall survival after the first failure. The failure distribution is presented in Table 6.

Table 6: Failure distribution

Failure	Human Papilloma Virus				TOTAL	
	Negative		Positive			
	N	***%***	**N**	***%***	**N**	***%***
	Overall					
Loco-regional	373	*35.2*	92	*15.8*	**465**	***28.3***
Distant	82	*7.7*	54	*9.3*	**136**	***8.3***
Both	34	*3.2*	16	*2.7*	**50**	***3.0***
Unknown	1	*0.1*	3	*0.5*	**4**	***0.2***
No failure	571	*53.8*	419	*71.8*	**990**	***60.2***
TOTAL	**1061**	***100***	**584**	***100***	**1645**	***100***
	[0;1[year					
Loco-regional	251	*23.7*	55	*9.4*	**306**	
Distant	41	*3.9*	18	*3.1*	**59**	
Both	27	*2.5*	11	*1.9*	**38**	
	[1;2[year					
Loco-regional	71	*6.7*	19	*3.3*	**90**	
Distant	26	*2.5*	16	*2.7*	**42**	
Both	5	*0.5*	4	*0.7*	**9**	
	≥ 2 years					
Loco-regional	51	*4.8*	18	*3.1*	**69**	
Distant	15	*1.4*	20	*3.4*	**35**	
Both	2	*0.2*	1	*0.2*	**3**	

Study Objectives

This study aims at evaluating smoking status and HPV status as predictive factors for radiotherapy fractionation modification in HNSCC. To look for an interaction between HPV status, smoking status, and the conjunction of HPV and smoking status and the effect of altered fractionation radiotherapy on

- Progression-free survival
- Secondary objectives:
    - Overall survival
    - Cancer specific mortality
    - Loco-regional control
    - Overall survival after first failure

As mild acceleration was not associated with an increased overall survival in the entire meta-analysis we have chosen progression-free survival as a primary endpoint. Indeed choosing overall survival in this analysis would lead for sure to a negative result as treatment effect on overall survival was not important.

Statistical analysis plan

A marker is considered prognostic if survival is different in marker positive and negative patients. A marker is considered predictive if the effect of treatment is different in marker positive and negative patients. As HPV status was consistently reported as a prognostic factor, this analysis will be centered on its predictive value.

Endpoints: The primary endpoint is progression-free survival, events are loco-regional or distant recurrence, deaths whatever the cause. Overall survival will be a secondary endpoint. According to the number of events (see power calculation paragraph and the amount of event in each secondary endpoint), other endpoints such as those listed above will be considered for analysis. Each endpoint is computed from date of randomization.

The following tables give an idea of the power considerations based on different sample sizes that are in the same range as in the present study.

Table 7: Power variation for prognostic studies with 1000 patients*

Survival difference	% of marker + (M+) patients			
	10%	25%	40%	50%
10%**	43%	53%	67%	70%
20%***	90%	99%	99%	99%

* 2 sided test; alpha = 1%
** 5-year S: 55% in M+ patients vs. 45% in M- patients
*** 5-year S: 60% in M+ patients vs. 40% in M- patients

Table 8: Power variation for predictive studies with 1000 patients*

Treatment effect difference between M+ and M- patients				
	10%	25%	40%	50%
10%**	5%	12%	16%	17%
20%***	27%	59%	73%	75%

* 2 sided test; alpha = 1%
** 5-year S difference between treatment + (T+) and T- patients: +5% in M+ patients vs. -5% in M- patients
*** 5-year S difference between T+ and T- patients: +15% in M+ vs. -5% in M- patients

Analyses will be restricted to OPC patients.
The main analysis will be prognostic and predictive effects of HPV status on progression-free survival. Then, the interaction between HPV status and smoking status will be studied. In the absence of predictive effect between HPV and treatment ($p<0.10$), this analysis will be performed in all type of radiotherapy fractionation or only in conventional fractionation otherwise.
Analyses involving smoking status will be performed twice:

- Without the ARTSCAN trial. Smoking status will be coded "Never" and "Former / Current"
- With the ARTSCAN trial. Smoking status will be coded "Never / Former" and "Current"

Analyses will be performed using a Cox model stratified by trial and adjusted on T-stage (T1-2 vs T3-4), N-stage (N0 vs N+), gender and age. Hazards proportionality of the Cox model will be checked and the performance of the model will be evaluated.

All analyses will be performed according to the intention-to-treat principle. Survival rates will be estimated using the Kaplan-Meier method. Heterogeneity of the prognostic and predictive analyses according to trial will be studied. Hazard ratio will be reported with their 95% confidence interval. Stratified survival curves will be estimated for control and experimental groups using annual death rates and HRs, according to the Peto method[10]. All reported p-values will be two-sided. A p-value of 0.05 will be considered significant. All analyses will be performed using SAS software, version 9.3.

A sensitivity analysis will be performed with the pCAIR trial, which included only 17 patients with oropharynx tumor and available HPV status.

The tumor stage developed by O'Sullivan was supposed to be tested on this dataset. But number of pack-year is required to calculate this stage, data available for few patients. Only 247 HPV-positive patients with oropharynx tumor and available pack-year information are in the dataset.
The validation will not be performed.

Repartition of the work among different entities (MARCH secretariat vs. DAHANCA representatives)

The study was proposed to DAHANCA in recognition of their valuable input to HNC meta-analyses. IT was accepted by Prof Overgaard and the work was handed to Dr P. Lassen, a world renowned expert in the role HPV in HNSCC.

The study protocol was drafted by MARCH Secretariat (PB, JPP, BL), discussed with the DAHANCA team and then circulated among the MARCH steering committee.

The statistical analysis will be performed by the Gustave Roussy team.

The results will be discussed among the working group, and within the meta-analysis steering committee. If timely possible they will be presented at an international meeting prior or concomitantly to manuscript submission. The manuscript will be drafted by Dr Lassen, who will then be the first author. Dr Lassen will also be responsible for the submission of the paper to a peer reviewed journal.

Publication strategy and authorship

Dr Lassen, who will be the first author of the publication. Second author will be M. Lacas and third author Dr Pignon. Dr Blanchard will be the last author and Pr Overgaard the penultimate author. All

groups who provided HPV data will be included in the authors of the manuscript, with RTOG authors signing as 4th and 5th authors.

The publication will be presented on behalf of the MARCH cooperative group.

References

1. Gillison, M. L., Chaturvedi, A. K., Anderson, W. F. & Fakhry, C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* (2015). doi:10.1200/JCO.2015.61.6995
2. Dillon, M. T. & Harrington, K. J. Human Papillomavirus-Negative Pharyngeal Cancer. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* (2015). doi:10.1200/JCO.2015.60.7804
3. Lassen, P. *et al.* The influence of HPV-associated p16-expression on accelerated fractionated radiotherapy in head and neck cancer: evaluation of the randomised DAHANCA 6&7 trial. *Radiother. Oncol. J. Eur. Soc. Ther. Radiol. Oncol.* **100,** (2011).
4. Ang, K. K. *et al.* Human papillomavirus and survival of patients with oropharyngeal cancer. *N. Engl. J. Med.* **363,** 24–35 (2010).
5. Blanchard, P., Lacas, B. & Bourhis, J. Meta-analysis of radiotherapy in head and neck carcinomas: an update. *Eur J Cancer 2013 Vol. 49 Suppl. 2 S1-S1028 LBA 26*
6. Gillison, M. L. *et al.* Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **30,** 2102–2111 (2012).
7. Lassen, P. *et al.* The influence of HPV-associated p16-expression on accelerated fractionated radiotherapy in head and neck cancer: evaluation of the randomised DAHANCA 6&7 trial. *Radiother. Oncol. J. Eur. Soc. Ther. Radiol. Oncol.* **100,** 49–55 (2011).
8. Söderström, K., Nilsson, P., Dalianis, T., Kjellén, E. & Zackrisson, B. Regional recurrence of oropharyngeal cancer after definitive radiotherapy: a case control study. *Radiat. Oncol. Lond. Engl.* **10,** 117 (2015).

9. Snietura, M. *et al.* Impact of HPV infection on the clinical outcome of p-CAIR trial in head and neck cancer. *Eur. Arch. Oto-Rhino-Laryngol. Off. J. Eur. Fed. Oto-Rhino-Laryngol. Soc. EUFOS Affil. Ger. Soc. Oto-Rhino-Laryngol. - Head Neck Surg.* **268,** 721–726 (2011).

10. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Early Breast Cancer Trialists' Collaborative Group. *Lancet* **339,** 1–15 (1992).