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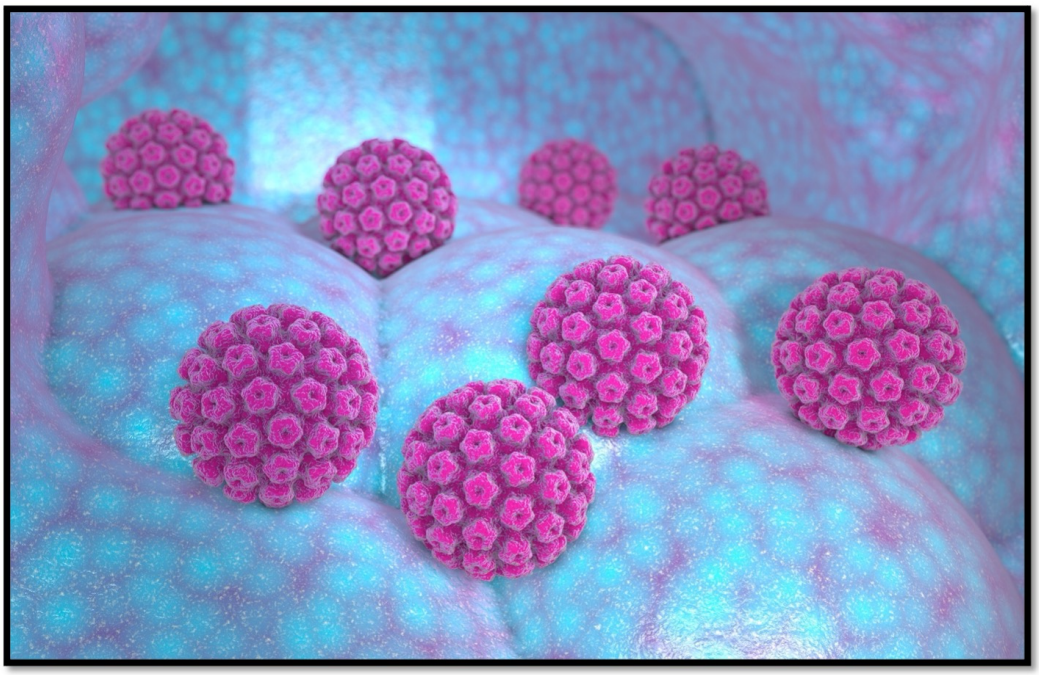

Doctoral Dissertation

Christian Grønhøj

B.Sc., M.D., PhD, ass. prof.

Clinical, epidemiological, and tumor-specific investigations in human papillomavirus (HPV) associated oropharyngeal cancer

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B.Sc., M.D., PhD, ass. prof.

Department of Head and Neck Surgery, Otorhinolaryngology and Audiology University Hospital, Rigshospitalet
Copenhagen, Denmark

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The Faculty of Health and Medical Sciences at the University of Copenhagen has accepted this dissertation for public defence for the doctoral degree in Medicine. Copenhagen, 5 October 2023.

Bente Merete Stallknecht, Head of Faculty

Officially appointed opponents:
Professor Antti Mäkitie, Department of Otorhinolaryngology – Head and Neck Surgery, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
Professor Christian Godballe, Department of ORL - Head & Neck Surgery and Audiology, Odense University Hospital and Department of Clinical Research, University of Southern Denmark

Chair of the assessment committee:
Professor Ulrik Lassen, Dept. Of Oncology, Copenhagen University Hospital, Rigshospitalet, chair

Chair of the defence ceremony:
Professor Thomas Benfield, University of Copenhagen

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Correlation between human papillomavirus and p16 overexpression in oropharyngeal tumours: a systematic review

Grønhøj Larsen C, Gyldenløve M, Jensen DH, Therkildsen MH, Kiss K, Norrild B, et al. Correlation between human papillomavirus and p16 overexpression in oropharyngeal tumours: a systematic review. Br J Cancer. 2014 Feb 11;110(6):1587–94. (1)

Study 2

Impact of p16-overexpression on overall and progression-free survival outcomes in oral cavity squamous cell carcinomas: A semi-national, population-based study

Schneider K, Jakobsen KK, Jensen JS, Wessel I, Christensen A, Specht L, et al. Impact of p16-overexpression on overall and progression-free survival outcomes in oral cavity squamous cell carcinomas: A semi-national, population-based study. Oral Oncol. 2020 Dec 1;111(105031). (2)

Study 3

Pattern of and survival following loco-regional and distant recurrence in patients with HPV+ and HPV- oropharyngeal squamous cell carcinoma: A population-based study

Grønhøj C, Jakobsen KK, Jensen DH, Rasmussen J, Andersen E, Friborg J, et al. Pattern of and survival following loco-regional and distant recurrence in patients with HPV+ and HPV− oropharyngeal squamous cell carcinoma: A population-based study. Oral Oncol. 2018;83:127–33. (3)

Study 4

Systematic review on location and timing of distant progression in human papillomavirus-positive and human papillomavirus-negative oropharyngeal squamous cell carcinomas

Tiedemann D, Jakobsen KK, von Buchwald C, Grønhøj C. Systematic review on location and timing of distant progression in human papillomavirus-positive and human papillomavirus-negative oropharyngeal squamous cell carcinomas. Head Neck. 2019 Mar 23;41(3):793–8. (4)

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Grønhøj C, Jensen JS, Wagner S, Dehlendorff C, Friborg J, Andersen E, et al. Impact on survival of tobacco smoking for cases with oropharyngeal squamous cell carcinoma and known human papillomavirus and p16-status: a multicenter retrospective study. Oncotarget 2019 Jul. 10(45):4655–63 (5)

Study 6

Association between head and neck cancer and sexually transmitted diseases: a Danish nationwide, case-control study

Grønhøj C, Jakobsen KK, Wingstrand VL, Jensen D, Iachina M, Egeberg A, et al. Association between head and neck cancer and sexually transmitted diseases: a Danish nationwide, case-control study. Acta ORL. 2020 Jul 2;140(7):615–9. (6)

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Comorbidity in HPV+ and HPV− oropharyngeal cancer patients: A population-based, case-control study

Grønhøj C, Kronberg Jakobsen K, Kjær E, Friborg J, von Buchwald C. Comorbidity in HPV+ and HPV- oropharyngeal cancer patients: A population-based, case-control study. Oral Oncol. 2019;96:1–6. (7)

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The impact of comorbidities on survival in oral cancer patients: a population-based, case-control study

Ghanizada M, Jakobsen KK, Jensen JS, Wessel I, Filtenborg Tvedskov J, Grønhøj C, et al. The impact of comorbidities on survival in oral cancer patients: a population-based, case-control study. Acta Oncol (Madr). 2021;60(2):173–9. (8)

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Impact of comorbidity on survival in patients with head and neck squamous cell carcinoma: A nationwide case-control study spanning 35 years

Ruud Kjær EK, Jensen JS, Jakobsen KK, Lelkaitis G, Wessel I, von Buchwald C, et al. The Impact of Comorbidity on Survival in Patients With Head and Neck Squamous Cell Carcinoma: A Nationwide Case-Control Study Spanning 35 Years. Front Oncol. 2021 Feb 17;10. (9)

Acknowledgements

My engagement with head and neck cancer research, specifically oropharyngeal cancer, began in 2012. Here, I was working at the Department of Cardio-thoracic surgery in Rigshospitalet in the hope of qualifying for a position at the Department of Otorhinolaryngology-Head and Neck Surgery. I made contact with my later mentor and supervisor, Christian von Buchwald, and shortly after, I was involved in a large systematic review on oropharyngeal cancer. With the priceless help of co-authors, including Mette Gyldenløve MD, PhD, we refined this project into the first part of this doctoral thesis. Following my introductory position at the Department of ORL-Head and Neck surgery, I was allowed to work for 3 years as a full-time PhD-student working with head and neck cancer, namely oropharyngeal cancer. This opportunity laid the grounds for the content of this thesis and paved the way to working with the excellent co-authors and collaborators included in this work. I would like to give specific thanks to professor Christian von Buchwald, an outstanding supervisor, mentor, and colleague, all along encouraging, helpful, and visionary in his management of our research group.

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Christian Grønhøj
Rigshospitalet (2022)

Aims of this thesis

The thesis aims to elucidate our understanding of patients treated for a human papilloma positive (HPV) oropharyngeal squamous carcinoma (OPSCC) and provide perspective and comparison to head and neck cancer patients without HPV-associated carcinogenesis.

The focus of this thesis is:

1. Correlation between the p16-marker and HPV infection in OPSCC patients.
2. The impact of p16-overexpression in head and neck cancer patients (HNC) patients.
3. Association to sexually transmitted diseases in HNC patients.
4. The impact of tobacco exposure on OPSCC patients.
5. The impact of comorbidities in HNC patients and associations between HPV+ and HPV- patients.

The specific aims are:

- **In paper 1**: Assess means of p16 and HPV diagnostics and quantify overexpression of p16 in HPV-positive and -negative OPSCCs by mode of immunohistochemical staining of carcinomas.
- **In paper 2:** Determine the prevalence of p16-overexpression in patients treated for an oral cavity squamous cell and evaluate the potential prognostic role of p16-overexpression in patients with OSCC.
- **In paper 3:** Report predictive factors, pattern, the timing of loco-regional recurrence and distant recurrence, as well as survival outcomes following recurrence in patients diagnosed with OPSCC.
- **In paper 4:** Systematically review the literature on studies reporting location and timing of distant recurrence after HPV+ or HPV− OPSCCs.
- **In paper 5:** Evaluate the impact of tobacco smoking on survival for cases with OPSCC with known HPV and p16-status.
- **In paper 6:** Determine the association between selected sexual transmitted diseases (syphilis, gonorrhea, HIV) and head and neck cancer patients.
- **In paper 7:** Examine differences in comorbidities in patients with OPSCC stratified on HPV-DNA status.

- **In paper 8:** Examine the comorbidities among oral cavity squamous cell carcinoma (OSCC) patients and investigated the impact of comorbidities on overall survival and recurrence-free survival.
- **In paper 9:** Characterize the comorbidity burden of HNSCC patients and investigate the relation to overall survival and cancer-specific mortality.

The Papilloma Virus: a historical perspective

More than 87 million different viruses exist on planet Earth and is without comparison the most abundant microbes (10). The number of viruses are estimated to be 10 million times more than the number of stars in the Universe, and in the oceans alone, the number is estimated to 10^{30} virus particles. It is shown that more than a trillion virus fall on every square meter of the face of Earth every day, and viruses have directly impacted our evolution (11). An example of this is the *arc gene* which is a central component in upholding our consciousness; this specific gene is created through viral advancement(12).

A number of cancers are attributable to infections (13). In the Western part of the world roughly 5% of cancers are attributable to infectious diseases, whereas in areas such as Eastern and Southern Asia, the proportion of cancers related to infections range up to 25% and even higher in sub-Saharan Africa with proportions up to one third. In perspective, one out of six cancers are related to an acute or chronic infection, and one out of eight cancers are attributable to a viral infection. The virus that has caused most cancers is by far the human papilloma virus which estimated causes around 700,000 cancers worldwide annually of which the abundance of these are cervical cancers being most prevalent in the third world where screening and vaccine strategies have not been employed or are working poorly (13).

The distinct appearance of warts was documented as far as the classical Greek era and the Roman times, but the biological understanding was not initiated until the 1890s where Joseph Payne described the development and contagious mode of transmission of warts on a young boy's hand (14). In folklore mythology, the jackalope is a rabbit with horns similar to a deer (*Figure 1*). This image resembles true pathology in rabbits as anatomy similar to horns exist and are well-described. These horn-like structures are neoplasms caused by a papillomavirus infection and historically one of the first demonstrations of a virus to be the cause of a neoplasm (*Figure 2*). Parsons and Kidd reported in 1943 that oral papillomatosis in rabbits could be

Figure 1 Picture of the mysterious jackalope. No copyrights reserved.

transmitted via saliva as a viral disease and may be latent and activated upon relevant stimuli (15). They also described sites in the mucosa for infection and tumor development. To this day, no similar study in humans has been published.

In the late 1940s, the first reports of cervix cancer associated with HPV infection were published. The following two decades provided multiple publications with descriptions on precancerous, HPV-related lesions in the cervix and the morphology of these, but the pioneering publications were published in the late 1970s. Two separate groups described the appearance of koilocytotic cells in Pap smears from epithelial lesions, and described the morphological characteristics of a viral etiology when viewed under a light microscope (16,17). These findings were key promoters for the era of HPV discoveries in the head and neck region. The pioneer in HPV research, Dr. Harald zur Hausen, first characterized HPV1-4, also addressing the differences between these HPV-subtypes, then characterized the virus as the etiological agent of classical warts (HPV6), leading to the finding of HPV11 from a laryngeal papilloma (18,19). His work led to the acknowledgment of a Nobel prize in Medicine and Physiology in 2008.

Dürst and colleagues reported a keystone finding within HPV research: the characterization of HPV16 from a cervical tumor (20). In the late 1970s, Quick *et al.* described two clinically familiar diseases, juvenile laryngeal papillomas and adult papillomas, and the research group provided evidence of the relationship between genital condylomas and laryngeal papillomas (21).

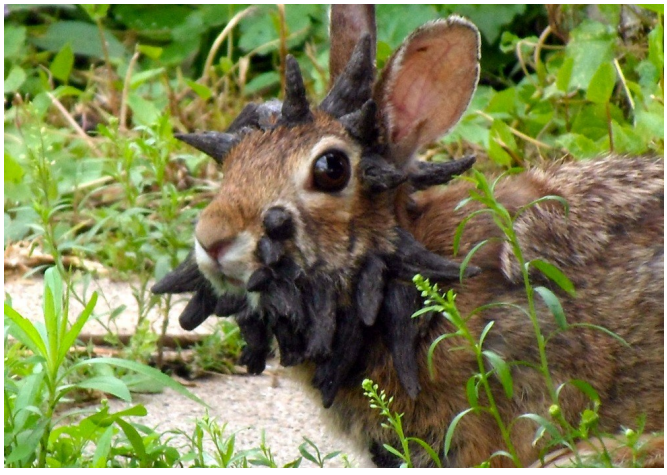

Figure 2 Rabbit with neoplasms caused by a papillomavirus infection (Sylvilagus floridanus). Photo by Gunnar Boettcher / AP

At the beginning of the 1980s, Syrjänen *et al.* was the first group to report the association between HPV infection and tumor development in laryngeal squamous cell carcinomas (22). Via HPV antigen testing through immunohistochemistry, this group also reported the HPV association to inverted papillomas from the nasal cavity and sinuses being clinically relevant due to the possible risk of malignant transformation. The Syrjänen group concomitantly to another research group (Jenson *et al.*) provided evidence of oral (likely

oropharyngeal) squamous cell carcinomas (23). In the early 1990s, the next major breakthrough in HPV research was performed by Kirnbauer *et al* (24). Here, the L1 major capsid proteins were expressed in insect cells with virions similar to the native papillomavirus. These findings were the base for the development of vaccines administered today, e.g., Gardasil ® and Cervarix ®.

Conferring data from WHO, HPV is today the most common sexually transmitted infection worldwide, and it is estimated that up to 75% of the US reproductive-age population at some point are exposed to HPV(25). Most of these cases go unreported, are asymptomatic, and likely spontaneously cleared, but at any time in the US, nearly 7% of individuals harbor HPV in the oral cavity or oropharynx (25).

The first study to characterize the subtype HPV16 in oropharyngeal squamous cell carcinoma was published in 1989 by Brandsma and Abramson, identifying two out of seven tonsillar squamous cell carcinomas as HPV+ (26), and in the early 2000s, two pioneering works were published. First, from the Mellin/Dalianis group, a series of 84 tonsillar carcinomas (46% HPV+) where the episomal state of the virus was investigated; and from the Gillison group, 52 tonsillar carcinomas were analyzed (62% HPV+) (27,28). The results from these groups also indicated that the patients with HPV+ tumors had improved prognosis as survival outcomes were reported demonstrating that smoking, alcohol drinking, and tumor morphology correlated to treatment response. These studies were at the forefront in understanding how HPV status impacts survival and epidemiology for patients with oropharyngeal cancer.

Clinical identity of HPV+ OPSCC

Head and neck squamous cell carcinoma (HNSCC) is the sixth most frequent cancer globally, with 890,000 new cases in 2018, including cancers of the oral cavity, sinuses, larynx, rhinopharynx, hypopharynx, and oropharynx (29,30). The oropharynx comprises the soft palate, uvula, the lingual tonsils, and the base of tongue. Similar to other HNSCCs, the carcinogenesis of oropharyngeal squamous cell carcinomas (OPSCC)s is historically related to exposure to betel chewing, tobacco and alcohol consumption, but within the previous two decades a new phenotype of OPSSC patients is introduced. These patients are diagnosed with HPV-driven tumors in the oropharynx and present superior prognosis and different tumor

biology compared to the alcohol and tobacco driven tumors. Patients with HPV+ OPSCCs are typically younger with healthier lifestyles including higher socioeconomic status stressing the paradigm-shift in the cause of carcinogenesis from the 'conventional' older patient with a long(er) history of tobacco and alcohol abuse to a healthy mid-age male with few or no comorbidities. Table 1 provides an overview of HNSCC patients.

The diagnostic work-up and treatment in the HPV-era

The evolution of HPV+ tumors in the oropharynx has altered the diagnostic work-up and to a certain extend the treatment modalities of patients with HNSCC. A specialized reticulated, lymphoepithelial mucosal tissue is located in the oropharynx with lengthy so-called crypts residing in the palatine and lingual tonsils. Although it is not fully understood, these sites are especially prone to HPV infection. Patients with HPV+ OPSCC most often present with a mass on the neck that may be explained by the epithelium in the oropharynx being characterized by a disjointed basal membrane and with intraepithelial blood vessels that may allow for the early metastasis to lymph nodes. Prior to the HPV-era, a cystic mass in level 2 or 3 in the neck was considered a benign branchial cleft cyst, but unlike the HPV- tumors, a HPV+ tumor will often metastasize to a lymph node in level 2 or 3 highly resembling a branchial cleft cyst. Clinically and ultrasonically, it can be difficult to differentiate the two diseases apart as the primary oropharyngeal tumor may be unidentifiable, hence the only finding is a cystic mass on the neck. To differentiate between malignancy and a benign condition, fine needle aspiration is performed which can be used to test HPV DNA in the smear (31). The identification of HPV in the smear will result in an diagnostic work-up in search of the primary tumor. If the primary tumor is not identifiable, the patient is categorized as HPV+ cancer of unknown primary (CUP). In this case, the patient should be planned for a pan-endoscopy under general anesthesia following MRI and FDG-PET-CT scans. If the tumor is still not identified, transoral robotic surgery (TORS) may be employed in resecting the superficial part of the tongue base, e.g., lingual mucosectomy (32,33). TORS is also in use for the curative intended treatment protocol of early-stage OPSCC, and this fairly new treatment option (approved by the FDA in 2009) has now gained use in most surgical oncological

Disease entity	**Human papilloma virus positive oropharyngeal cancer**	**Alcohol and tobacco related cancers of the oral cavity, oropharynx, larynx and hypopharynx**
Etiology	Integration of nine types oncogenic HPV into host keratinocytes' DNA History of sexual activity	Exposure to tobacco, alcohol, betel nut chewing. DNA repair deficits Genetic mutations to growth regulatory proteins
Age	Younger (late 50's)	Older (early 60's)
Gender	3:1 men	3:1 men
Social status	Higher	Lower
Clinical presentation	Small primary tumor Early, cystic, enlarged regional lymph node	Large primary tumor Lymph node involvement typically later
Secondary malignancies	Rare	Common
Distant progression	Rare	Common
Treatment response	Multi-modal treatment depending on (surgery, radiation, chemotherapy).	For oral cavity cancers: primarily surgery; for high stage tumors adjuvant radiotherapy. For pharyngeal tumors: primarily radiotherapy +/- chemotherapy
Prognosis	Highly dependent on tumor cell responsiveness. Approx. 70-85% survival.	Contingent upon clinical stage, tumor size and patient comorbidities. Approx. 50% survival.
Biological characteristics		
Molecular Pathogenesis	HPV E6 and E7 proteins silence p53 and *Rb* tumor suppressor genes, respectively	Heterogeneous often involving p53, FHIT and a variety of other parameters including signaling pathways.
Intracellular protein p16 overexpression	Commonly overexpressed	Commonly not overexpressed
Histopathologic features	Non-keratinizing, well-differentiated.	Keratinizing, poorly differentiated.
Clinical sites involved	Fenestrated surface epithelium overlying lymphoid tissue, i.e. palatine tonsils and lingual tonsils at the base of the tongue.	All mucosal epithelium in the oral cavity and pharynx

Table 1 Overview of head and neck cancer patients

departments worldwide. TORS – first with the transoral micro laser surgery, later with the robotic approach – has led to a significant shift in the treatment approach and now offers patients a minimal invasive surgical approach(33). HPV+ OPSCC has been a catalysator for the implementation of TORS in light of the favorable outcomes for HPV+ patients, and the introduction of TORS has been well-timed given the epidemiology of HPV+ OPSCC as we now for the low-stage patients may offer this treatment. The conventional treatment of

OPSCC in Denmark is cisplatin-based chemoradiotherapy (34). Although this treatment has shown high efficacy, the accomplishment requires high costs in quality of life for patients, including late radiotherapy damage(35).

The unanswered question regarding TORS and RT/C is which modality is superior regarding tumor control and toxicity. Studies have shown similar progression- and survival rates for the two treatment modalities, but it remains unknown how they differentiate regarding quality of life and functional outcome measures(33,36). Several ongoing studies are addressing this problem.

Incidence of HPV+ oropharyngeal cancer

Oropharyngeal cancer is one of the most rapidly rising cancers during the previous two decades (37,38) most obvious in Western countries but also observed in several Asian countries(38). In a global setting, the repartition of HPV+ in OPSCCs is estimated at approximately 33% although regions report HPV-fractions as low as 0% in e.g. Southern India. The highest HPV+ OPSCC prevalence is observed in the Northern European countries and North America, but Lebanon, China and South Korea are also high-prevalent areas (38). In Denmark, an increasing incidence in HPV+ OPSCC was observed in the year 2000 to 2017 rising from 0.9 to 3.2 age-adjusted incidence rates per 100,000 citizen equivalent to a three-fold increase (39).

In the US, alcohol and tobacco related HNSCC have decreased in incidence, and in the mid 2010's HPV+ oropharyngeal cancer is now the predominant cancer representing 40% of all head and neck cancers (40) and a cautious estimate is that the proportion is 50% today. In 2045, it is estimated that HPV+ OPSCC in middle-aged men will range in the top five of all cancers (40). Men are the greatest contributor to the rising incidence of HPV+ OPSCC but when analyzing data across age-groups, it is evident that the cancer is also rising in women being more pronounced in the younger population (39).

The first transcontinental study to consider the burden and incidence of HPV in HNSCC was performed by the Castellsagué group (The ICO study) in 2016 using centralized testing that standardized the histopathological evaluation (41). From 29 countries, 3,680 samples (1374 pharyngeal, 1264 oral cavities, and 1042 laryngeal tumors) were collected, and HPV-DNA detection was performed. Samples containing HPV-DNA were tested for

HPV E6*I mRNA, and using immunohistochemistry expressions of the intracellular protein p16 were performed.

The study provided several important results. First, the geographical difference of the impact of HPV on head and neck cancer was evident. A recent review updating the ICO results revealed that Central and Southern Europe had the lowest rates of HPV+ oropharyngeal cancers in contrast to Northern Europe, where the share of HPV+ OPSCC patients is as high as 65% (42). The reason for the large difference between regions is unknown and it seems less likely that sexual behavior, tobacco smoking, and alcohol consumption differ markedly between European countries.

Secondly, the ICO study was the first study to underline the importance of HPV evaluation of OPSCC diagnosis, which requires, besides the HPV-DNA detection, a minimum of one additional marker to conclude HPV-induced carcinogenesis. This biomarker could be mRNA as proposed by The ICO group, although, in a clinical setting, the most useful and easily performed analysis is the evaluation of the intracellular protein p16 and overexpression in IHC sections.

Genetics of HPV+ oropharyngeal cancer

HPV is a double-stranded DNA virus that may integrate into the human cell nucleus. The HPV subtypes may be classified into those responsible for benign lesions e.g. papillomas or warts or the mucosal types related to malignant lesions and hence classified as “high-risk”. The later are HPV16, -18, -31, -33, -34, -35, - 39, -45, -51, -52, -56, -58, -59, -66, -68 and -70 (39,43).

The HPV genome harbors three regions each with subregions with different functions: the early gene-coding region (E), late gene-coding region (L), and the long control region (LCR). The early genes e.g. E1, E2, E4–E7 all encode primarily viral replication of which three drive carcinogenesis: E5, E6, and E7. Following HPV infection of mucosal human cells the E1 and E2 advance viral replication at a relatively slow rate. When the basal cells differentiate into the suprabasal layer of the epithelia, the virus changes it replication rates and now replicates in a much higher rate.

The hypothesis regarding virus spreading centers around epithelia desquamation; e.g. virus is released and hence capable of infecting neighboring cells. HPV genome integration may appear in pre-malignant lesions although cells with genome integration are in high risk of malignant transformation (44). It seems paradoxically for the virus to integrate in the

human genome as this the “last stop” for the virus as this restricts itself in infecting other cells. Thus, the virus needs the human cellular tools to replicate as it is not able to do so on its own.

In the high-risk types, the decontrolled and increased expression of E6 and E7 are likely to produce genetic disorders in the infected cell, allowing the integration of viral episomes into the human DNA. This incorporation of viral DNA into the hosts DNA occurs frequently through E2 with the result of loss of E6/E7 regulation and will often lead to malignant transformation as the E6 and E7 proteins are capable of interrupting important regulatory pathways, e.g. pathways mediated by the retinoblastoma protein family and by the cellular p53 protein. The function of E6 is to recruit the ubiquitin-ligase E6AP, hence entailing the degradation of p53, and the function of E7 is bind to the retinoblastoma protein.

The definition of HPV+ OPSCC is an important issue that is not interpreted similarly across oncology centers. There is differences in the mutational profile of HPV+ and HPV- oropharyngeal SCCs(45). The most important difference is that HPV+ tumors generally do not harbor the TP53 mutation or loss of the chromosome arms 3p, 9p, have repeated deletions of TRAF3 but do have missense mutations in PIK3A(46). One of the most important cancer genes is located on the 9p arm being the tumor suppressor gene CDKN2A. This gene encodes the cell cycle-inhibiting protein (p16Ink4A, e.g., ‘p16’) and is commonly not lost in HPV+ tumors in contrast to HPV- squamous cell carcinomas. The p16-gene is a tumor suppressor gene that inhibits CDK4A. When the cell is infected with HPV, and the virus is active, the retinoblastoma protein (pRb) binds to the HPV oncoprotein E7, allowing the transcriptional activator E2F to be continually active and hereby eliminating the negative feedback of free pRb on p16Ink4A. In this state, the cell will continue to express the p16Ink4A protein, hence the term p16-overexpression. By IHC analysis, p16 was one of the pioneering cellular markers to determine prognosis between HPV+ and HPV- patients (47). A systematic review evaluated based on 39 studies and 3926 cases the correlation between HPV-positivity across different techniques and the definition of when p16 is overexpressed, e.g., positive (1). It was reported that p16 evaluation is better at predicting HPV-positivity when the positive cut-off is set at ≥70% of cytoplasmic and nuclear staining. This study reported highly different definitions of p16 overexpression ranging from +5% to +70% cytoplasmic and nuclear staining. The above concept was employed in the newly published UICC8/AJCC8 recommendations where the cut-off for p16 overexpression is diffuse tumor expression, but at least 75% staining with at least moderate (+2/3) staining intensity (48). The importance of

correct definition and classification of HPV is crucial for a number of reasons. Thus, several ongoing studies are including only 'true' HPV+ patients, e.g. requiring both HPV positivity based on PCR-analysis and p16-overexpression. The interpretation of p16 impacts the allocation of patients into low or high-risk disease progression groups and is imperative as clinical trials are investigating methods of treatment de-escalation in patients with HPV+ OPSCC based specifically on HPV status. Secondly, to compare data and publications across centers, homogenous and strict pathology guidelines are required. Finally, numerous novel methods for evaluating HPV status are investigated and in the near future liquid biopsies seem to be important in the categorization and follow-up regimes for especially HPV+ patients.

Impact of p16-overexpression in HNSCC

The relationship between HPV+ and other (non-HPV) HNSCC is discussed widely during the past 20 years, namely the association between oral cavity cancers and HPV. Here, the assumption is that the oral cavity is the first entrance for the virus exposing the oral cavity to viral infection. However, such an association between HPV and oral cancer has not been established. The discussion was fueled partly by the notion that p16 was included in the new UICC8/AJJC8 recommendations and partly because p16 overexpression might be caused by various intracellular misconfigurations other than HPV. The reason for p16 overexpression could – in part – be less important if p16-overexpression is associated to superior survival rates in all head neck squamous cell carcinomas. An US-based study from 2018 evaluated based on 387 non-oropharyngeal HNSCC cancers the impact of p16+ in relation to oropharyngeal cancers, and concluded that the p16-marker is equally important for both disease entities (non-oropharyngeal and oropharyngeal HNSCC) regarding overall survival (49). This study has several limitations. First, it is essential to centralize and standardize p16 testing; with de-centralized testing the definition and scoring of p16- positivity is highly likely to vary as reported in both Europe and the US (41,50). In the study by Bryant *et al* patients are included from 120 centers across the US. The authors report that only 29% of the non-oropharyngeal tumors that were classified as p16-positive were in fact "strong and diffuse" as is required when scoring oropharyngeal tumors. Secondly, p16-data was obtainable for only 8% of the tumors evaluated, providing concern for selection bias. Finally, it is concerning to generalize data from a cohort of veterans to the broader population due to differences such as comorbidities/physical disadvantages, current occupation, income,

education, and importantly tobacco and alcohol use. A study from 2020 with a centralized scoring board evaluated the difference in prognosis for patients with oral cavity squamous cell carcinomas (OSCC) stratified on p16+ (p16 overexpression) and p16- tumors (2). This study is in contrast to Bryant *et al* and showed that p16-overexpression status did not alter survival outcomes in patients with OSCCs (*Figure 3*). The patients in this study were treated based on the national guidelines and derived from all citizens living in Eastern Denmark. All patients in the study were offered the same follow-up scheme and hospital access was tax-financed. Although this study did not include an external patient cohort to validate the results, it is based on patients with standardized therapy regimes, precise anatomic site classification and relevant clinical variables in the statistical analysis (e.g. tumor stage, gender, tobacco smoking, age).

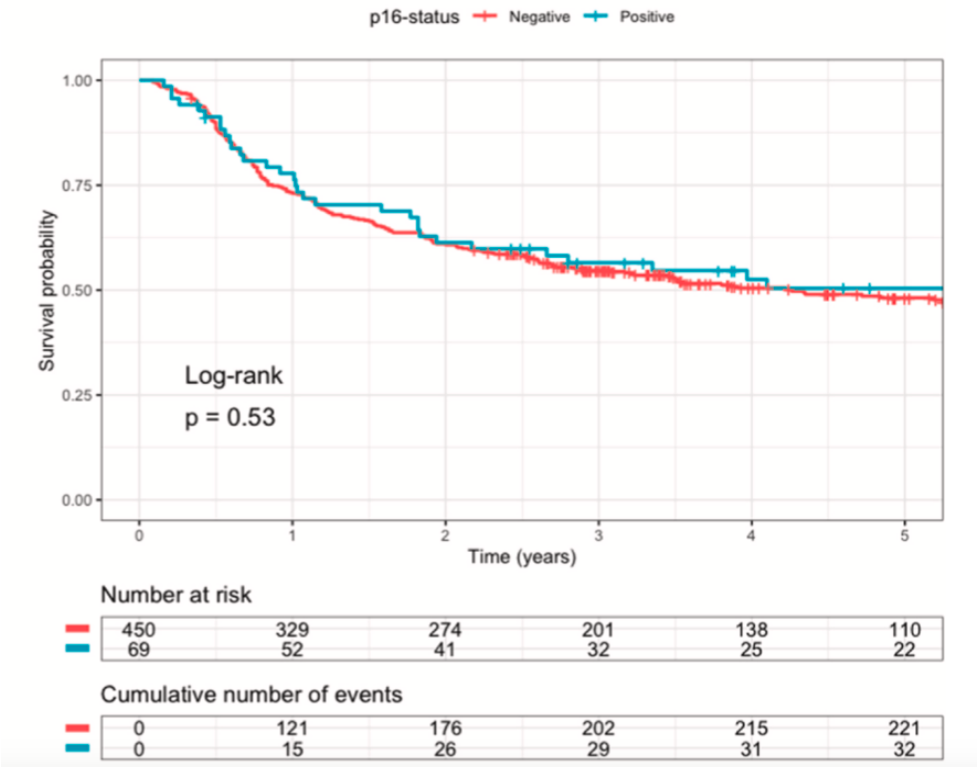

Figure 3. Kaplan-Meier plot depicting overall survival rates for OSCC patients treated with curative intent stratified on p16-overexpression status (2).

The survival graphs of patients with p16+ and p16- OSCCs are nearly identical. The poor survival chances of OSCC patients is noticeable with a 2-year survival rates of approximately 60%, and a 5-year survival rate of 50%. The relatively few p16+ cases in this analysis (n =

69, 13.3%) were derived from tumors from both the tongue and floor of the mouth and were frequently T1-T2 tumors. They were not HPV tested to explore the association between p16+ in OSCC and HPV infection (2).

For OSSC patients, the p16-biomarker should not be employed to classify patients into low or high risk groups contrary to OPSCC patients, and the above mentioned study is the largest to-date to confirm that the p16-marker is a poor biomarker when applied to patients with OSCC and unfit as a clinical variable when planning clinical study designs (2). The study underlined the justifiable distinguishment between OSCC and OPSCC patients – not just in anatomical location – but also in the light of tumor biology, e.g. p16 overexpression should be dealt with differently for OSSCs and OPSCCs. In the perspective of increasing demands and decreasing resources for head and neck pathologists, it should be discussed whether these findings should lead to fewer p16-stains for obvious OSCC resections, e.g. anterior tongue or floor of mouth tumors.

For OSCC patients, a strong and trustable biomarker as HPV for OPSCC or EBV for selected nasopharyngeal carcinomas, is still missing. Promising projects are employed here amongst the uPAR biomarker (51) which might also be an attractive target for molecular imaging in oropharyngeal cancer (52).

HPV+ oropharyngeal cancer and sexual transmitted diseases

Key findings in risk behavior and demographic differences between HPV+ and HPV- OPSCC patients are that HPV+ patients are typically younger, have higher socioeconomic status, are less likely to have a history of heavy consumption of alcohol and high tobacco smoking use, and have a history of more risky sexual behavior compared to HPV- patients. The increase in incidence of HPV+ OPSCC is thought to be mainly driven by sexual behavior e.g. sexually transmitted through trends in especially (oral) sexual behavior. Studies have strongly linked previous and current sexual behavior to the risk of acquiring HPV+ OPSCC.

In a cross-sectional US study of oral HPV infections, 5,579 healthy men and women (aged 14–69 years) provided an oral mouthwash sample. The prevalence of HPV infection showed a bimodal pattern with peaks between 30–34 year and 60–64 years age-groups. According to another US-based analysis, married or cohabiting men had a reduced risk of acquiring oral HPV infections (53). Thus, marital status might be a stronger predictor of oral HPV infection than the lifetime number of sexual partners and men who engage in sex with

men have a significantly higher risk of oral HPV infection. The largest reported case-controlled study of sexually transmitted diseases and head and neck cancer association included 39,405 HNC patients (63% men; all patients median 63.0 years at HNC diagnosis) and 393,238 controls from the general Danish population. Data were included from national databases (6). The controls were randomly picked from the background population matched on age and gender and did not have a history with head and neck cancer. A history of sexually transmitted diseases in HNC patients was 0.27% vs. 0.11% in age-gender matched controls. In an adjusted setting, a significantly higher risk of a previous sexual transmitted disease was identified in patients diagnosed with HNC compared to the matched population, exhibiting a hazard ratio (HR) of 2.5 (95% CI 2.0; 3.1) (*Figure 4*). A study-design as this harbor several bias and weaknesses such as selection and reporting bias, and examining such a large population, the results will easier lead to significant results; although the findings may be clinical relevant in future screening and preventive measures.

	Ref. population		Cancer patients		
Localization	Count	% with STD	Count	% with STD	*p* Value
All HNC	393,238	0.11	39,405	0.27	.00
Laryngeal cancer	93,051	0.07	9314	0.24	.00
Hypopharyngeal cancer	21,608	0.08	2176	0.32	.00
Nasopharyngeal cancer	11,289	0.07	1130	0.27	.04
Oropharyngeal cancer	71,626	0.12	7176	0.33	.00
Oral cancer	85,906	0.10	8626	0.31	.00
Sinonasal cancer	24,029	0.05	2406	0.25	.00
Salivary gland cancer	20,166	0.08	2019	0.05	.6
Thyroid cancer	59,157	0.21	5915	0.19	.7
Other	6406	0.00	643	0.78	.00
Prevalence of specific STD					
Gonorrhoea		0.07		0.13	.00
Syphilis		0.03		0.07	.00
HIV		0.02		0.06	.00

Figure 4 Table of head and neck cancer patients and the associated prevalence of STD in cancer patients and the reference population(6).

When considering sexual behavior, social economic status is an important factor as the term includes information such as occupation, marital status and education. Several studies have looked in to this. Dahlstrom *et al* (54) compared socioeconomic factors between

HPV+ and HPV- OPSCC patients: High income and educational level, reduced smoking history and a more frequent sexual risk behavior were more frequently associated to cases with HPV+ OPSCC cases compared to the HPV-.

Sexual behavior vary between men and women as well as within levels of income and educational level; women of lower socioeconomic status report and men of higher socioeconomic status report higher numbers of partners (55). Leigh *et al* published a US population-based survey of >2,000 adults and found that persons with college education or higher were more likely to report >5 sex partners in the previous 5 years compared to persons with lower education (55). The International Head and Neck Cancer Epidemiology Consortium published a multicenter, international case-control study, reported that the number of lifetime sexual partners and the probability of performing oral sex increased with educational status, and that more lifetime sexual partners and oral sex partners increased the odds of acquiring an oropharyngeal tumor but did not lead to any other head and neck tumor (56). This may be due to the correlation between the sexually transmitted HPV and the oropharyngeal cancers although it is debated.

For all mucosal diseases including oral and pharyngeal cancers, a significantly higher incidence of STDs was reported before HNC compared with cases diagnosed with the non-mucosal cancers of salivary gland and thyroid malignancies (*Figure 5*). Here, the salivary gland tumors and thyroid cancers practically function as “a negative control group”. No significant difference was found between men and women concerning the prevalence of a

	Association (HR) between HNC and STD before index date			
	Univariate analysis		Multivariate analysis[a]	
Localization	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
All HNC	2.5 (2.0; 3.1)	.00	2.5 (2.0; 3.1)	.00
Laryngeal cancer	3.6 (2.2; 5.9)	.00	3.2 (2.0; 5.3)	.00
Hypopharyngeal cancer	3.9 (1.6; 9.3)	.00	3.6 (1.5; 8.6)	.00
Nasopharyngeal cancer	3.8 (1.0; 14.2)	.05	3.7 (0.9; 14.2)	.06
Oropharyngeal cancer	2.8 (1.8; 4.3)	.00	2.5 (1.5; 3.9)	.00
Oral cancer	3.1 (2.0; 4.8)	.00	2.7 (1.8; 4.2)	.00
Sinonasal cancer	5.5 (2.0; 14.8)	.0	5.3 (2.0; 14.1)	.00
Salivary gland cancer	0.6 (0.1; 4.7)	.65	0.6 (0.1; 4.4)	.59
Thyroid cancer	0.9 (0.5; 1.6)	.68	0.8 (0.5; 1.6)	.58
Other	7.2 (2.3; 22.6)	.00	6.5 (2.1; 20.7)	.00
Specific STD				
Gonorrhoea	2.1 (1.5; 2.8)	.00	1.9 (1.4; 2.6)	.00
Syphilis	2.8 (1.8; 4.2)	.00	2.5 (1.7; 3.8)	.00
HIV	4.0 (2.5; 6.4)	.00	3.4 (2.1; 5.4)	.00

[a]Adjusted for gender, CCI at index date, and year of birth.

Figure 5 Table of association between STD and patients with a HNC; results of logistic regression analyses and the Cox regression analyses (6).

STD diagnosis before the HNC (p=0.2)(6). This contrasts with the oral infection studies as mentioned above where the prevalence of oncogenic HPV infection was significantly higher in men than women in an adjusted analysis.

The specific anatomical mucosal site of first HPV exposure may be important for the immunological and serologic response and it has been suggested that debut with first HPV exposure via oral sex provides a lower serological response compared to first-exposure after vaginal sex (57). The theory remains that oral HPV exposure without previous genital HPV contact could increase the odds for persistent, oral-mucosal HPV infection and lead to the development of HPV+ OPSCC later in life. A recent study of 163 HPV+ OPSCC cases and 345 matched controls examined differences in sexual behavior, relationship-history and changes, and corresponding serologic response (58). In conclusion, oral sex timing, number of oral sex intercourses, and intensity of partners were associated with the diagnosis of HPV+ OPSCC. There was a strong association between a history of sexually transmitted infection and development of HPV+ OPSCC compared with controls similar to the larger study above (6).

Recently a systematic review showed the effect of vaccination on the prevalence of oral HPV infection (59). Here, the relative prevention percentages is reported as high as 80% in patients immunized with HPV vaccines. Although oral infection cannot be correlated to oropharyngeal cancer development, these numbers are promising to prevent OPSCC carcinogenesis.

HPV+ oropharyngeal cancer and tobacco smoking

Whereas sexual behavior has *á priori* been considered a risk factor in the development of HPV+ OPSCC, the role of moderate tobacco use and alcohol is still debated (60). For HPV- OPSCC patients, we know that heavy alcohol and tobacco smoking are important risk factors, but the impact of heavy use of these substances in HPV+ OPSCC patients is uncertain.

Tobacco contains more than 4000 chemical components of which 70 are carcinogenic to humans. It is available in several forms including flammable cigarettes, pipes, and cigars, and in recent years a prompt increase in the use of e-cigarettes is observed. The e-cigarette is a battery-powered, electronic product able to heat a solution of nicotine, none-organic and flavoring agents to produce an aerosol containing several carcinogens. Numerous adverse

effects are observed with use of these including cellular and immunological toxicity(61,62). Still, the long-term cancer risks of e-cigarette practice remain unknown (63).

Globally the areca nut is the 4th most used addictive substance with more than 500 million users (62). Especially in India and Pakistan, the areca nut is likely the main contributor to the development of head and neck cancer especially oral and oropharyngeal cancers (64,65). The areca nut is the seed of the fruit of the Areca catechu palm and may be consumed in a number of ways with or without tobacco. It is labeled class I carcinogen by the International Agency for Research on Cancer (IARC) and the substance is shown to have several adverse effect including mucosal fibrosis and carcinogenicity (62).

The correlation between the risk of upper respiratory mucosal tumors and exposure to tobacco is evident although unanswered questions stand; the data on impact of tobacco exposure on survival in HPV+ patients quantified on pack-years in an adjusted setting is sparse, particularly in a study design where HPV- patients are included in comparison. A multi-national study investigated the impact of tobacco smoking on survival for patients treated for an OPSCC specifically with the focus on number of packyears (5). The study included patients from high and low HPV-areas; e.g. Eastern Denmark and Germany with HPV proportions diverging as much as 57% vs 20% for patients with HPV+ tumors for the Danish vs German cohort, respectively. There were several key points to extract, one of which is the quantification of smoking exposure in the HPV+ group compared to the HPV-. As visualized in *Figure 6*, there was a significant and high use of tobacco in the HPV+ group, and it is observed that overall survival probability was influenced by smoking status at diagnosis. One might speculate that high use of tobacco could be a determinant of the development of HPV+ OPSCC, and as visualized in the Kaplan-Meier plots, it is shown that the subgroup of high tobacco smoking users also perform significantly worse than those with no or little tobacco use (*Figure 7*).

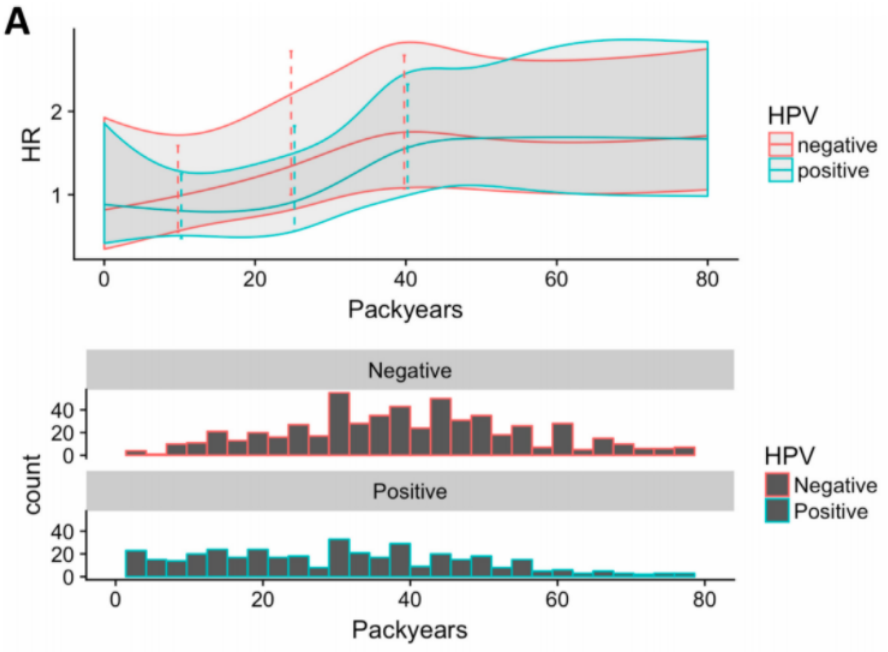

Figure 6 The impact of packyears is visualized with continuous smoking exposure for overall survival. In the top panel; the hazard-ratio for death is depicted in relation to packyears. In the bottom panel; absolute number of packyears are visualized for all OPSCC patients(5).

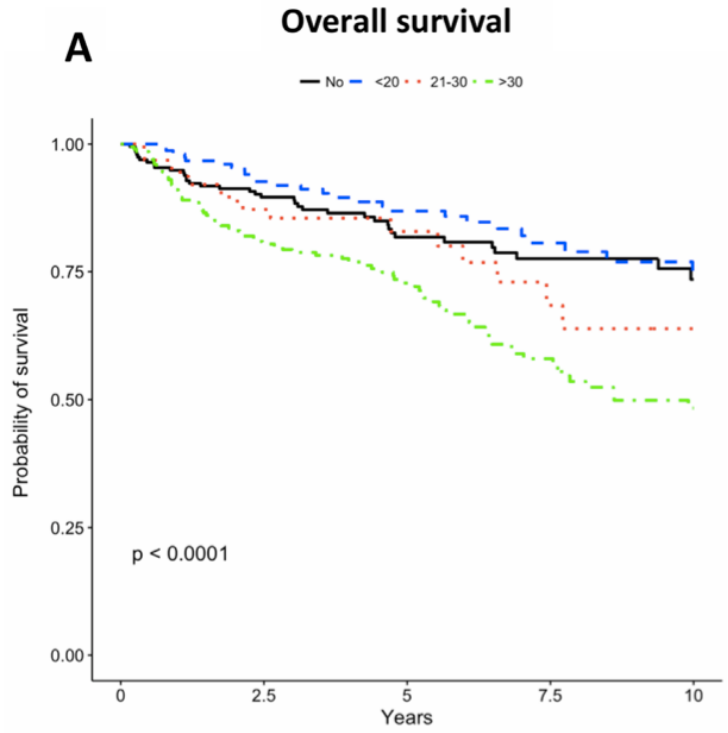

Figure 7 Kaplan-meier curves for overall survival for patients with a HPV+ oropharyngeal cancer stratified by amount of smoking in packyears (5)

In this series of 1316 patients, a high consumption of smoking tobacco is observed for the HPV+ group and the use is significantly higher comparing to other large cohorts in Europe and the US (66–69). This is either due to the Danish and German population, coherent tobacco use, misinformation from patients (e.g., registration bias), or that other similar studies underreport tobacco smoking use.

It is found that having more than 10 packyears for HPV+ OPSCC patients would negatively impact survival probability for patients treated for a HNSCC (70). However, in the Danish/German study including a similar US-based report (70), patients should have a smoking history of more than 20 packyears before it significantly impacts survival (5). For the HPV- group, the impact of smoking on survival is evident for patients with few packyears (5). Other studies report that a few cigarettes daily may be harmful supporting the benefit of quitting or reducing cigarette smoking (71). Especially for the HPV+ group, it remains unknown if a synergy exists between HPV-infection and tobacco in the malignant transformation.

Patients diagnosed with a HPV+ OPSCC are homogenous across Europe with similar demographics. It may be considered whether a proportion of HPV+ patients are underreporting tobacco smoking use and in fact have had a substantial tobacco exposure. Indeed, there is a group of HPV+ OPSCC patients who have never smoked being the "true" etiologic HPV+ patients and many of these report few sexual partners, are married, and report the same sexual partner the previous 30-40 years (56,72). These patients are unable to clear the HPV infection likely conceived decades before the diagnosis of the OPSCC. It has been hypothesized that the depth of the tonsillar crypts harbor the ideal environment for nesting and protecting the HPV from immunological clearing. What triggers the cancerogenic cellular transition is unknown, and we lack an explanation for the difference in age-distribution at diagnosis, e.g. the vast majority of patients are diagnosed in the late fifties whereas the HPV- patients are diagnosed in the early sixties; this is a small clinical but statistical difference.

When assessing the impact of smoking in OPSCC patients, it is important to stratify patients based on HPV-status(5,73). In *Figure 7* the impact of smoking is visualized for HPV+ patients, and here, the Kaplan-Meier curves are further sorted into never-smokers, 1-20, 21-30 and above 30 pack years to evaluate how number of cigarettes daily consumption alter survival outcomes. It is shown that the HPV+ group with +30 pack-years hold the same prognosis as the HPV- group of smokers with a history of less than 20 pack years (5). Likely, the HPV+ tumors with a high number of pack years is strongly associated with the mutational profile of the HPV- tumors with low to none pack years although this is controversial(70). Based on a study population of 37 non-smokers and 25 smokers in HPV+ OPSCC patients, smoking did not increase the mutation rate of genes that are frequently mutated in traditional smoking-related HNSCC(70).

For the HPV+ patients with high tobacco exposure, it may be hypothesized that the oncogenic transformation is caused by the tobacco exposure and a synergy between the tobacco exposure and the HPV-infection exist. For these patients the "tobacco mutational profile" may overrule the HPV+ profile providing similar tumor biology and hence a prognosis as HPV- patients. It may also be likely that the tobacco exposure causes a *modified* HPV+ mutational signature with tobacco mutations, i.e. a third mutational profile different from the "true" HPV+ or HPV- smoking profile. The reasons that these patients exhibit a poorer prognosis following treatment remain unknown; either due to the above *modified*

mutational profile, hence poorer response to treatment, or because of the co-morbidities related to smoking e.g. secondary primary cancers including lung and other head and neck cancers along with cardiovascular and lung diseases. The increased tobacco smoking exposure should also be part of the explanation why these patients acquire the OPSCC. The tobacco exposure might lower the robustness of the mucosal immunological response to HPV-infection allowing a persistent infection.

When addressing the impact of tobacco exposure on survival it is important to include information of tobacco smoking during and after treatment. A key paper on this topic is from the Gillison group. Here, the authors reported information on the impact of tobacco smoking during radiotherapy (RT) (74). In the multivariate overall survival analysis, the authors showed that the hazard ratio of death was 2.18 (95% CI: 1.48 to 3.19) for patients smoking during RT and that presumably continued to smoke in the follow-up period underlining the importance of smoking cessation during and following treatment. In p16-negative, non-oropharyngeal cancer SCCs, e.g. OSCC, a recent paper also addressed the importance of smoking cessation following treatment (75). This paper also underlines the importance of smoking cessation. Patients that continued to smoked faced a hazard ratio of 1.53 (1.25-1.87) for death compared to patients that quit smoking at the time of diagnosis.

Perspectives on the UICC 7 vs. UICC 8 classification and de-escalation initiatives

Based on the work from Princess Margaret Cancer Centre, Toronto, Canada, a new TNM classification scheme was published in 2016 (76,77). This classification introduced a few but clinically important changes to the diagnostic and prognostication schemes in head and neck cancer patients. The changes are that all squamous cell carcinomas should be tested for p16-overexpression, and nodal descriptors and the TNM scheme are redefined. Treatment recommendations remain unaltered, and staging should be viewed as a prognostic tool, not a treatment recommendation scheme. The new TNM-classification should not drive treatment decisions and should not be a license for treatment de-intensification. Nevertheless, in the era with a very large HPV-survivor group, it is imminent to discuss the quality of life after treatment and address de-escalation strategies as is the forefront of HPV-related research. The recent literature on published de-escalation studies focuses mainly on these strategies: A)

investigating the epidermal growth receptor (EDGR) directed antibody cetuximab vs. the platinum-containing agent cisplatin given with concurrent radiotherapy; B) reduction of the dose or the fields of radiotherapy as primary treatment; C) in the surgical setting, upfront treatment with TORS alone or TORS followed by reduced adjuvant radiation with or without chemotherapy; and D) induction chemotherapy to omit either radiotherapy or surgery. Regarding de-escalation of treatment it is evident that the HPV+ tumor is more radio- and chemotherapy sensitive and fewer secondary primary tumors are observed. Roughly 2% of the HPV+ group will experience a secondary primary tumor compared with the HPV- group with 10% (78). As the HPV+ group typically is younger with fewer comorbidities, this patient group is also destined to live with the side-effects of the treatments longer than the HPV- group. The arguments against de-escalating treatment are also persuasive because15-20% of the HPV+ group suffer cancer-specific mortality and a significant proportion of these are due to metastatic disease being the leading cause of death in patients with solid tumors.

To support the above research, several studies examined the impact of HPV in oropharyngeal cancers. Several papers have shown the importance of dual testing in OPSCC patients, meaning both HPV and p16, especially when considering the risk of distant metastasis (79,80). This information is crucial to accurately classify the population of patients and aid in the selection process for de-escalation or escalation of treatment. Discordant OPSCC cases – e.g. p16+/HPV- or p16-/HPV+ cases – have clinically significantly worse progression-free and overall rates survival rates compared with p16+/HPV+ cases. It is important for the clinician to acknowledge that if p16 immunostaining is used solitary, approximately 10% of p16+ patients might be incorrectly classified as HPV+, although actually not related to the HPV virus (HPV-/p16+). This can have significant implications to treatment selection, as conflicting cases have poorer outcomes than true HPV+/p16+ cases. Some of these patients may in fact benefit from additional or intensified treatment.

Failures in OPSCC patients: prognostic factors, timing, location, treatment options, and survival following failures

A large share of patients with HNSCC are treated upfront with surgery. Following surgical intervention several factors determine if postoperative treatment is indicated, which highly influence prognosis for the patient. Essential factors include extra-nodal extension (ENE) in the resected lymph nodes, positive resection margins in the primary tumor, lympho-

vascular invasion, perineural invasion, advance T-stage (>pT3) and N stage (N2 to N3), high grade/low differentiation. Other factors of importance also discussed in the postoperative setting are nodal yield, nodal ratio in neck dissection specimens, tumor location, comorbidities, ongoing tobacco smoking/alcohol consumption, and pattern of invasion. These factors are also important when addressing the risk of progression. Studies that report failures from both in HPV+ and HPV- OPSCC patients are sparsely reported in the literature, particularly when including time-to and detailed site of failure.

HNSCC is generally considered and treated as a loco-regional disease; hence, it is important to stratify recurrences into loco-regional and distant metastasis. In this manner, it is achievable to evaluate the impact of disease progression in patients with OPSCC. Although overall survival evaluated at the time of diagnosis remains important, addressing the risk, anatomical locations and the timing of recurrence in OPSCC patients are key factors not only for survival but also for follow-up regimes, quality of life in both short- and long-term survivors' time in hospital and in improving rehabilitation. When addressing none-selected cohorts, this data is limited; the largest study comes from a non-selected population with stratified HPV data (3). The authors evaluated how HPV+ vs. HPV- patients differed in time-to-progression stratified on loco-regional and distant failures. Both HPV+ and HPV- patients have a steep 'progression' curve within in the first year following treatment, but patients differ with two noticeable differences. The HPV+ patients experience fewer relapses in this period as most relapses occur within the first 6 months. Following this period the HPV+ patients have almost a linear development of patients with progression. On the contrary, the HPV- group of patients treated for an OPSCC, have a steeper curve the first year following treatment and the curve expands nearly a year longer compared to the HPV+ patients before flattening (*Figure 8; Figure 9*.).

For patients with OPSCC, data is sparse on the impact of survival for patients that encounter loco-regional vs. distant recurrence. In summary, patients with OPSCC with loco-regional recurrence (HPV+ and HPV- patients in a pooled analyzed), the median overall survival (OS) is approximately one year (3,81–83). The impact of experiencing loco-regional disease progression differs markedly once patients are stratified on HPV-status. The 5-year OS for the HPV+ and HPV- group is 25% and 5%, respectively (*Figure 8*) (3).

For the group of patients with distant progression survival rates diminish markedly. The median OS for patients with distant recurrence unstratified on HPV status is approximately six months. For the patient with a HPV+ tumor with distant progression, they usually hold a 5-year OS of 20% and for the HPV- patients no patients are alive after five years (*Figure 9*). Noteworthy, it is observed that significantly more HPV- patients experience recurrence compared to the HPV+, and distant metastatic progression occurred in both groups predominantly to the lung.

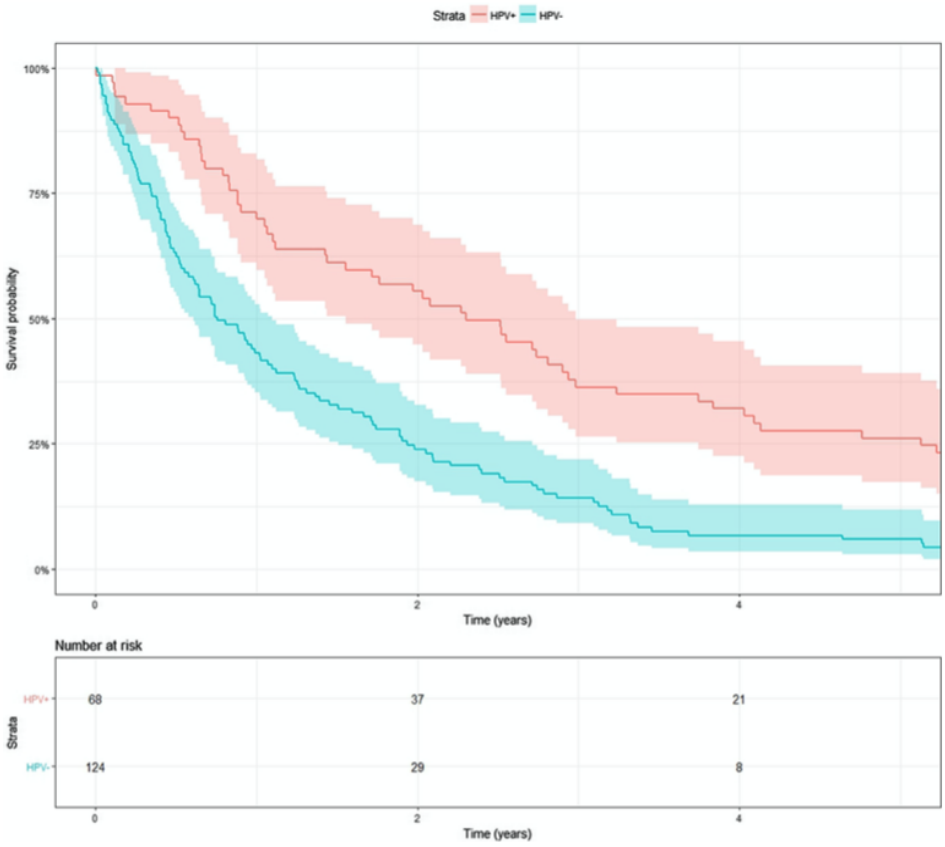

Figure 8. Kaplan-Meier plot depicting overall survival after loco-regional recurrence in years. The redline shows the HPV+ patients, and the blueline the HPV- patient (3).

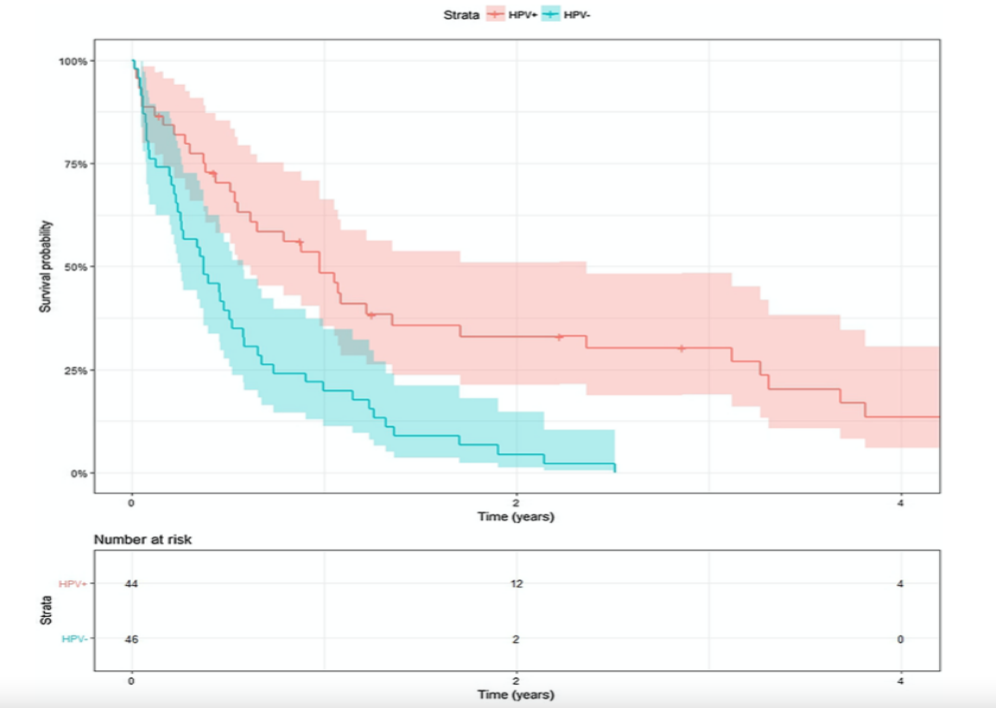

Figure 9. Kaplan-Meier plot depicting overall survival after distant recurrence in years. The redline shows the HPV+ patients, and the blue line the HPV- patients(3).

A systematic review showed distant progression to be a predictor for particularly bad outcomes due to poor treatment response and treatment choices (4). The most frequent site of distant progression irrespective of HPV-status is found to be the lung (*Figure 10*). Patients with HPV+ OPSCC were more disposed to dissemination involving multiple sites (risk ratio = 16.49). A small group of HPV+ OPSCC patients present at the time of diagnosis with rapidly growing and aggressive tumors and within weeks or months have multiple metastases, including metastasis to the lungs, bones, or the brain. In the review, the authors did not find a difference in time to distant progression when stratifying patients on HPV status, although a tendency was observed toward patients with HPV+ OPSCCs advancing progression later than patients with HPV- OPSCCs. In conclusion, the pattern of distant metastasis but not time to distant metastasis is different in patients with OPSCC when categorized on HPV status.

It is uncertain whether distant progression, is actually a new tumor (e.g. a secondary primary tumor) or, in fact, a metastasis from the primary OPSCC. This is a concern for both patient groups (HPV+ vs. HPV-) but a new lung primary cancer for the HPV- patients with a long, high tobacco consumption is more likely. However, heterogenous and unprecise reporting of distant progression make interpretation difficult, e.g. as many studies report sites as "non-regional" (4).

Organs with DP	Number of p16+ cases	Number of p16− cases	Relative risk	95% CI
Lungs	160	72	0.94	(0.82;1.08)
Liver	24	14	0.76	(0.41;1.41)
Bone	39	23	0.72	(0.45;1.13)
Nonregional lymph nodes	34	3	4.79	(1.51;15.21)
Skin	26	5	2.32	(0.92;5.84)
Brain	19	3	2.68	(0.82;8.83)
Mediastinum	6	3	0.89	(0.23;3.48)

Figure 10 Distribution of distant progression in p16+ and p16− OPSCC (4).

Comorbidity and survival outcomes in head and neck cancer patients with a focus on HPV+ OPSCC patients

Treatment of head and neck cancer is often associated with complex, prolonged and chronic functional sequelae and morbidities that severely impact quality of life and life expectancy (35). This is despite the fact that both surgical techniques and a general tendency concerning structural organ preservation strategies are employed along with the significant improvements in radiotherapy approaches. The importance of comorbidities not only serves as risk factors for death or severe disability but might also affect the ability to complete therapy and stick to follow-ups, and clinicians should be highly aware of the impact of comorbidities.

Overall survival for head and neck cancer patients are dramatically improved during the last 20-30 years (30), although the 5-year mortality remains very high at approximately 50% across the general population of patients treated for a squamous cell carcinoma. The risk

of mortality is mainly related to disease progression where patients face either limited, non-curative chemotherapy or debilitating surgical interventions. However, competing causes of death are acknowledged to significantly affect the overall survival for HNSCC patients, and especially during the last ten years a focus has been set on decreasing the impact of these in an effort to lower mortality. The comorbidities for HNSCC patients may not differ from the general population, but the comorbidities are likely to impact the patient more as upper respiratory, swallowing and digestive organs may be considerably functionally compromised due to previous treatment for the cancer.

To address the impact of comorbidities, a large Korean cohort study reported the impact of non-HNSCC deaths in advanced stage HNSCC (*Figure 11*). The Kaplan-Meier graph in the figure visually presents the incidence of non-cancerous deaths. These events were more likely in the elderly population and were more frequently associated with respiratory events. For all HNSCC cancer patients in advanced cancer stage, cardiac-pulmonary comorbidities were the leading competing mortality factor second to the primary cancer (84).

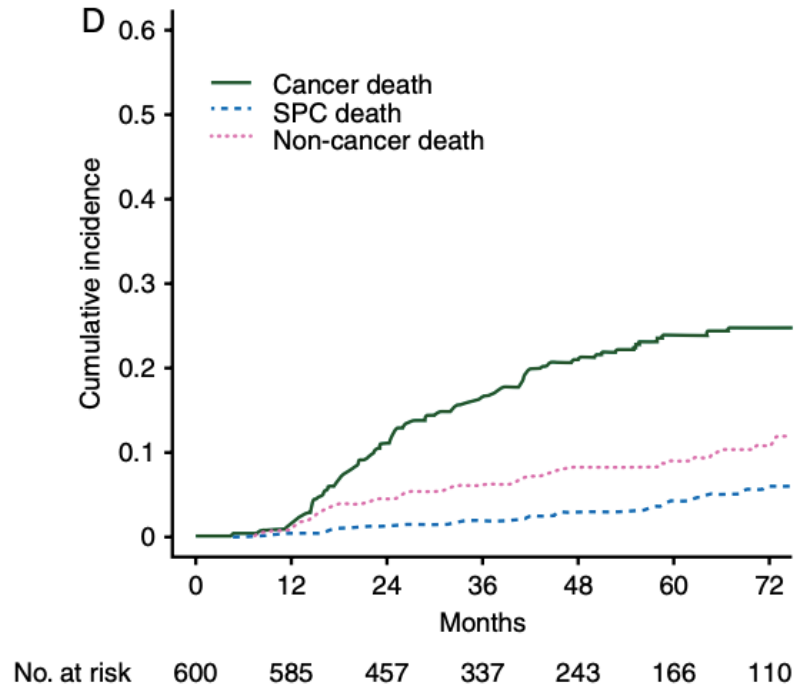

Figure 11 Cumulative incidence probabilities for causes of death in advanced stage HNSCC patients. SPC= second primary cancer (84)

The median age at the diagnosis of HNSCC is crudely 62 years both in Denmark (30) and globally (85), and knowingly the risk of acquiring an HNSCC increases with age. Elderly patients have increased in life expectancy, hence the impact of and the quantity of comorbidities in patients with HNSCC are also expected to increase.

Several attempts have been performed to gather the complexity of comorbidity into a single score to measure expected impact for the individual patient. These include among others the Charlson Comorbidity Index (CCI), American Society of Anesthesiologists Physical Status (ASA-PS), Elixhauser Comorbidity Index (ECI), Adult Comorbidity Evaluation-27 (ACE-27), National Cancer Institute Comorbidity Index (NCI-CI), and Washington University Head and Neck Comorbidity Index (WUHNCI) (86). Each of these have pros and cons although the general concept and purpose are to consolidate the impact of comorbidities into a numerical value which may be useful for the clinician or researcher.

The cumulative amount of most comorbidities will lower expected survival time and increase total morbidity, and are likely to affect both surgical outcomes and RT-outcomes. This is likely also to be the case for HNC patients, although data is sparse to back this up. Studies are either from smaller populations or from selected settings, e.g. surgical patients only (87–90). Patients with multiple comorbidities experience higher complication and mortality rates compared with patients with lower comorbidity burden (9). Patients with cumulative comorbidities are less likely to tolerate long-lasting procedures or deem fit for the extensive recovery and the prolonged rehabilitation following treatment(s). Additionally, health requirements might be applicable before initiating the demanding therapies. For OPSCC patients, few studies report demographical, descriptive data and associated comorbidities on a stratified population treated for a HPV+ and HPV- OPSCC despite the growing focus on defining and understanding the new entity of HPV+ OPSCC and considering the differences between the disease-entities in terms of impact of comorbidity.

Three key papers assessed the impact of comorbidities in HPV+ OPSCC patients (7,91,92). The largest registry report was from the Surveillance, Epidemiology, and End Results (SEER)–Medicare-linked databases. A limitation of this report is that the analysis is based on HPV-related (e.g. p16+) patients and not true HPV+ patients (HPV+/p16+) but strengthened by including 8,025 patients with HPV-unrelated (p16-) HNC and 2499 with

HPV-related (p16+) HNC (92). The study showed that patients with a high burden of comorbidities at the time of diagnosis had a significantly increased risk of early death compared with the patients without comorbidities. Comorbidities that occurred during the surveillance period also highly impacted survival and significantly increase mortality-hazards. Some of the comorbidities are related to treatment e.g. anemia, dysphagia, and weight loss (Table 2). No difference were found between patients with HPV-related and -unrelated HNC which is not seen in other reports.

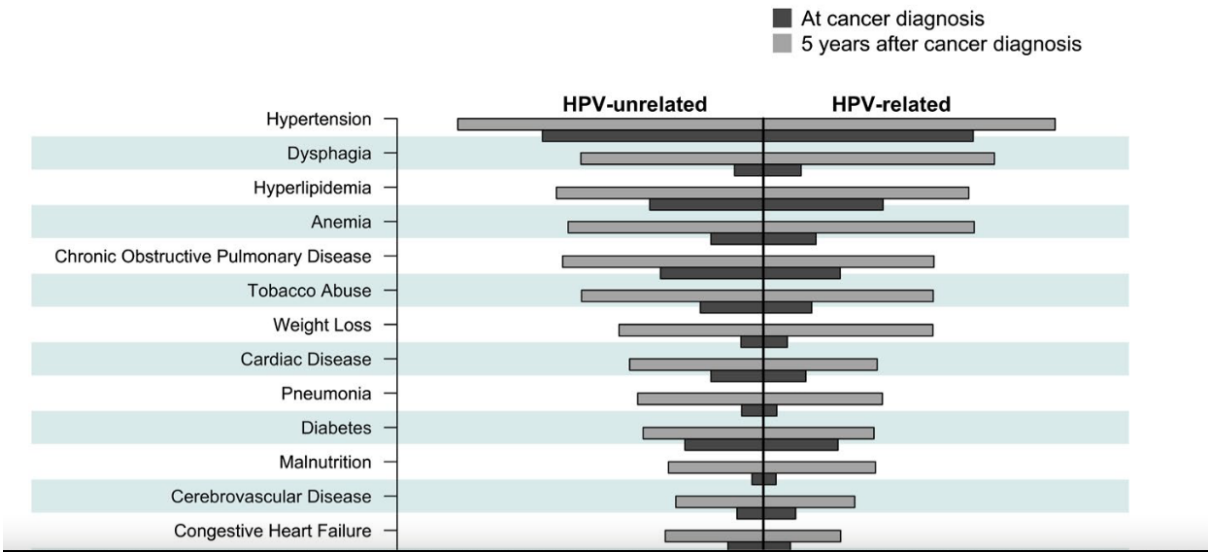

Table 2: Top 13 items of relevance in the prevalence of comorbid conditions in patients with HPV+ and HPV- head and neck cancer at diagnosis and cumulative probability at 5 years based on data from the SEER-database (92)

A similar report included true HPV+ patients, e.g. matched on HPV and p16 (7). Here, 1,499 OPSCC patients (55.0% HPV+, n = 824) and 14,990 age- and sex-matched controls from the general population were evaluated. Irrespective of HPV status, patients with an OPSCC had significantly more comorbidities than the general background population, but patients with HPV- tumors had more comorbidities than HPV+ patients at the time of diagnosis and accumulated more comorbidities following treatment. Also evident is the finding that significantly more HPV+ patients had no (zero) comorbidities at the time of diagnosis than those diagnosed with the HPV- patient group(7). Both groups had a significantly higher risk of (secondary) malignancy compared to the background, general population but the HPV- group showed significant higher risks of being diagnosed with alcohol and tobacco-related diseases such as liver cirrhosis, gastrointestinal ulcers, and

cerebrovascular disease. The population of HPV- HNSCC patients remain the subset of patients with the most influential and highest number of comorbidities. A means to highlight the impact of comorbidities on overall survival (OS) and recurrence-free survival (RFS) is to address the impact in a subset of known HPV- HNSCC patients, e.g. patients with oral cavity squamous cell carcinoma (OSCC). Here, it is fundamental to include the general population as controls to compare with the expected prevalence of comorbidities, and if so, which ones dominate in the population. In a tax-financed setting providing equal access to the health care system, one study was identified to report data on this topic. A study with only OSCC patients compared this population to a reference age and gender-matched population (8). This

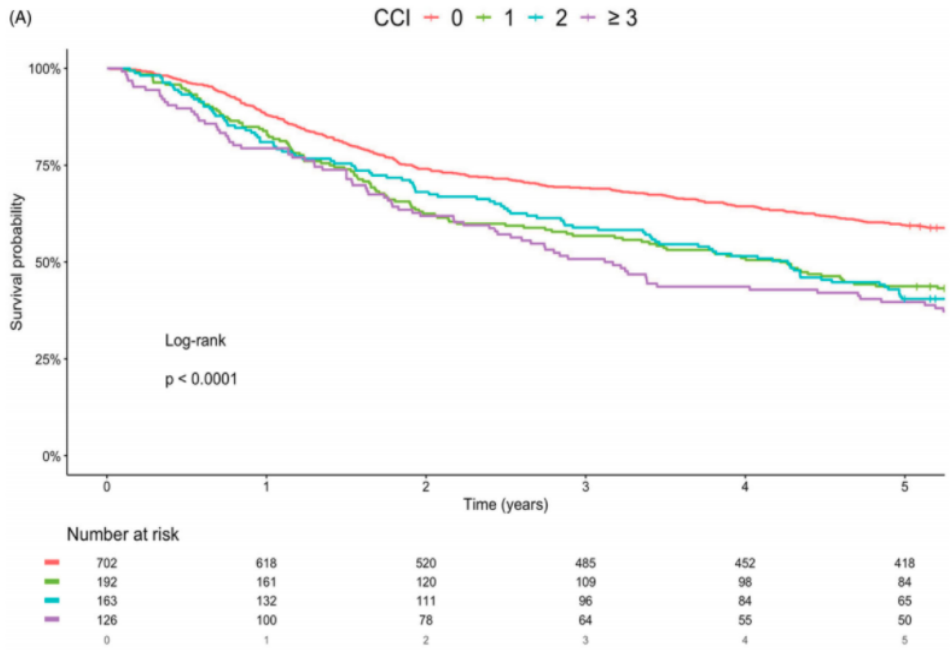

Figure 12 Kaplan-meier curve depicting survival probabilities stratified on CCI for OSCC patients (8)

study emphasized the impact of comorbidities in the subgroup of specifically smoking-alcohol-induced tumors, and underlined why clinicians should employ the concept of comorbidity-evaluations as part of the algorithm when choosing the correct treatment and follow-up scheme for non-HPV patients. OSCC patients had a significantly higher comorbidity burden at diagnosis and risk of developing more comorbidities after treatment than the reference population. An important finding of this study is how Charlson Comorbidity Index (CCI) impacts survival when stratified on CCI 0, 1, 2 and >3; e.g. survival outcomes decreased significantly with higher CCI score (8). This graphical

illustration emphasizes the significant differences between the subset of CCI-categories and the relative survival difference (*Figure 12*).

It is evident that comorbidity is a strong predictor of survival in HNSCC patients (7–9,93). A review from 2014, gathered available data published before the year 2012 on the survival impact of comorbidities in HNSCC patients (94) and included 10 studies with 22,932 cases in a meta-analysis. Although a variety of tools to assess and classify comorbidity were employed across the ten studies, it was concluded that comorbidities increased the mortality risk with a hazard ratio of 1.38 (95% CI: 1.32–1.43) when comparing higher comorbidity scores to lower. A systematic review of 116 studies published in 2021 addressed the impact of comorbidities in all head and neck cancer patients when treatment at some stage involved surgery(95). The authors concluded that the literature was highly inconsistent as to data-reporting and choice of comorbidity-tool.

The largest paper to assess the impact of comorbidity in HNC patients with patients treated with both surgery and radiotherapy is based on data from the Danish population (9). The paper is supported by two previous studies including partly the same patients but with a smaller time-period or restricted to the population only treated with radiotherapy (94,96). The above mentioned study (9) employed data from the Danish Cancer Registry and the DNPR, extracting all HNSCC patients diagnosed between 1980 and 2014 and 1:10 age-gender matched controls. Patients were evaluated based on the Charlson Comorbidity Index (CCI). Patients diagnosed with any head and neck SCC were compared to age- and gender-matched controls and the comorbidity burdens were estimated. The mean CCI at the time of diagnosis for patients and controls increased throughout the study period 1980–2014 (*Figure 13*). Several reasons might explain this, e.g., the overall improvement in medical services, prolonged lifespan and the improved data collection, registration, or both. For HNC patients, the mean CCI at diagnosis was 0.81, significantly higher than for controls (the general population) with a mean of 0.48 (not including the newly diagnosed cancer). At the time of diagnosis, 15,743 patients had a CCI of 0, while 4,393 patients (17.30%) a CCI of 1; 2,847 (11.20%) a CCI of 2; 1,152 (4.54%) a CCI of 3; and 1,253 (4.94%) a CCI of ≥4. Patients acquired significantly more comorbidities following treatment compared with controls and at the 5-year follow-up, the mean CCI among the group of 5-year survivors was 1.34 and for controls 0.86. Further, survivors had lower comorbid conditions at the time of diagnosis compared to diseased patients. The increase in CCI might solely be caused by the hospital

contact, where comorbidities were registered, and comorbidities were likely underreported in the control group without hospital contacts.

The pronounced survival impact of CCI is visualized in the Kaplan Meier curves (*Figure 14*) and the multivariate statistical analysis adjusting for gender, treatment and site of the HNC. The increase in CCI was significantly associated with a decrease in overall survival at the time of diagnosis and 5 years later.

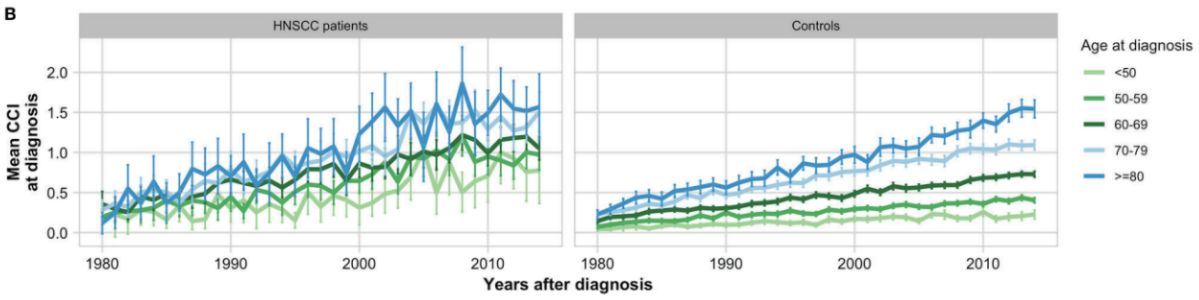

Figure 13. Historical change of Charlson Comorbidity Index (CCI) in HNSCC patients and controls (9)

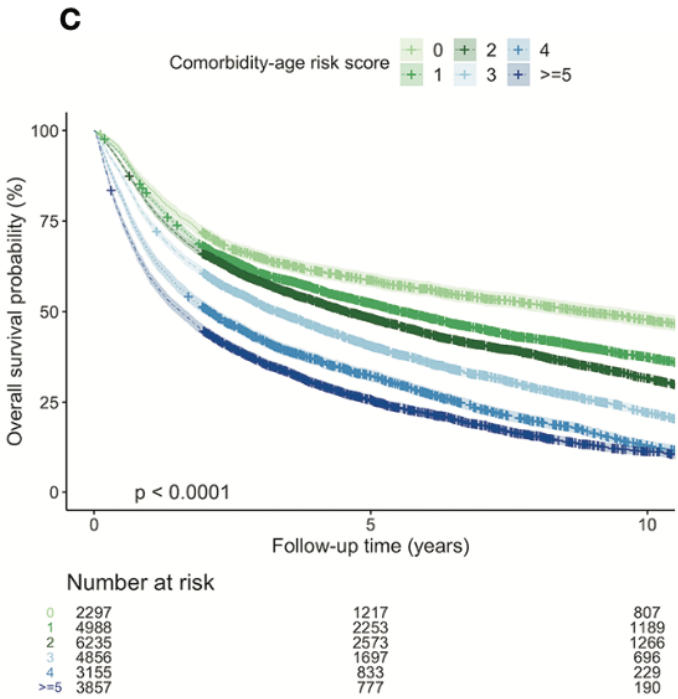

Figure 14. Kaplan Meier curves for HNSCC patients with head and neck squamous cell stratified by CCI score (9)

Multiple studies have demonstrated comorbidity burden as a strong prognostic factor for survival, and it could therefore be a confounder in clinical trials. In the past, older patients were often excluded from clinical trials thus comorbidity burden was considered to be an exclusion criteria. These studies lack the important relevance to the clinical setting and the "standard" HNSCC patient. Pre-treatment evaluation along with interventions known as pre-habilitation could preferably include the evaluation of patients' lung status (e.g. spirometry), physical status (e.g. "the chair-exercise"), social, nutritional, economic and mental status. The biological/functional age of HNSCC patients may be far more important than the chronological age as these patients compared with the age-matched background population have a higher comorbidity-burden.

General strengths and limitations of registry-based studies

Two papers comprised in this thesis are systematic reviews and seven papers are registry-based studies. The Danish national, public health registers provide invaluable, well-structured, non-commercial, and cost-effective data sources for research, and through especially the last 20 years of technological development, the opportunities with such strong data-wholeness and the extension of follow-up periods, are numerous. In Denmark, all individuals regardless of income and address, are registered and followed from birth to death, and for the majority of registries, the collection of data started in the 1970s.

In observational studies, investigators describe or define associations or correlations between predefined exposures and outcomes. Bias is unavoidable in most research projects as such but may be reduced if patients and controls are selected from the same source population or even better from the country's total population as this will diminish the risk of selection bias. The OPSCC and OSCC studies included in this thesis were derived from half of Denmark and are consequently 'selected' although all inhabitants from Eastern Denmark are incorporated in the study and ought to be assessed as population-based. We have used the possibilities within data combinations employing Rigshospitalet's Department of Oto-Rhino-Laryngology local databases (97) in combination with the national, readily available databases (98). This is only possible using the unique, individual Danish identifier, e.g. the CPR-number as the unique individual identifier. Data might be combined with many more variables employed in these studies, e.g., address, medication, education, financial status,

marriage, number of kids and occupation. The data available is also useful in clinical trials – although rarely used – to combine clinical findings with registry data.

Biases – e.g., the unidentified, systematic variance between the exposed cases and controls, resulting in a flawed estimation of the conclusion – is common in observational research and may occur in all steps of the process from the planning to the publication phase. Our reports are also subject to common biases associated with registry-based studies such as inconsistencies, historical medical practice changes, including the use of diagnosis codes (i.e., the change from the ICD-8 classification to the ICD-10). One should remember that associations, correlation, and predictions may be identified quite easily in large populations when performing observational research, although this is generally not related to causality. Observational studies should be used for generating hypotheses, but to assess or demonstrate causation, a randomized-controlled clinical trial is needed.

The concept of confounding should be fully considered in observational research. The association between exposure and outcome is inclined by the occurrence of variable(s) not included in the analysis. The most important variables missing for the large studies are tumor stage and smoking after treatment(9). Confounding is difficult to reduce, although it may be improved when complying with randomization or matching in study designs.

Future research perspectives in HPV-related HNSCC research

A significant amount of literature has backed up the notion of how to define the overexpression of the intracellular p16 marker in OPSCCs but little is known about the relationship between p16-overexpression and the immune system, specifically the non-cell cycle-related roles. Recent studies suppose that p16 may be a regulator of tissue immunological surveillance supporting the hypothesis that p16 is a regulator of tumor immunity(99). It has also been shown how that the local immune response in vulvar squamous cell carcinomas are depend on activation of p16 (100). Information on this topic for OPSSCs is sparse.

The future research in understanding tumor biology will most likely focus on how our immune system reacts to the presence of tumor cells with specific characteristics – being both as driver mutations and in the presence of tumor heterogeneity – and how we are able to stimulate the immune response. Research in tumor biology in the coming decades might answer why some HPV+ OPSSC are slow-growing, only metastasize to regional lymph

nodes in the neck – typical level 2-3 on the neck – and why some patients at the time of diagnosis have multiple distant metastasis; that is, typically lung metastasis. Patients with distant metastasis are, referring to the above studies, most prevalent in HPV- patients but do also occur in the "true" HPV+ patients; the patients that have never smoked and consume a moderate amount of alcohol. Knowledge is modest on these patients as they typically present with aggressive tumors and short survival lengths (101). A small fraction responds to chemo/immunotherapy or have a single lung metastasis that may be cured with lung surgery, but the vast majority are not alive 12 months following the diagnosis. We need to uncover which tumor-specific alterations are present in the primary tumor and in the lung metastasis to treat these patients with precision. The tool of circulating tumor DNA might contribute and prospective studies should collect blood from patients before, during and after treatment to assess developments in free HPV-DNA and free tumor DNA.

A number of new immune- and chemotherapies are waiting to be tested in clinical trials. Though new therapies are wanted for the group of patients with relapsed or metastatic disease, it is also important to test drugs already in-use for safe ways to decrease doses while maintaining effect. It could be meaningful to decrease side-effects to treatment in the last months of a patient's life, and it should be remembered that all drugs for metastatic disease will prolong life but rarely cure the patient. It is expected that a proportion of HPV+ tumors that do not respond to chemoradiation and present with relapsed disease share genomic aberrations with the HPV- tumors such as p53 mutations. However, this is poorly understood and should be better uncovered before initiating escalation treatment for this patient group.

Cancer immunotherapy is a major research field for patients with relapsed/metastatic disease and might prove to be safer and more effective than current therapy. Immune checkpoint inhibitors (ICIs) has led to a breakthrough in cancer treatment and has improved disease and survival outcomes for HNSCC patients. However, this treatment modality is very expensive with costs up to one million DKK/patient and also harbors risks of severe adverse side-effects while only prolonging patients life with months. A new generation of immunotherapies stimulating T-cells has emerged. T-cells may be stimulated to recognize mutated antigens using personalized vaccines targeting tumor specific mutations. This strategy may increase effectiveness of immunotherapies when sufficient mutations on the cancer surface is present. Antiviral vaccine design is under development with the purpose of targeting cancer associated viruses. This vaccine will target endogenous retroviruses (ERVs)

which are relics of past infections and once these viruses become reactivated the proteins encoded by these genes become potential targets for the immune system (102,103). Trials are awaiting to test drugs that target ERVs' antigens upregulated in solid tumors and the possible clinical effect.

Clinical trials are still initiated and published without stratifying or block-randomizing patients on tobacco smoking exposure. It may be proposed that future clinical studies that are treating 'true' HPV+ patients merely include never-smokers, alternatively allocate patients into never-smokers, 1-20, and patients with more than 20 pack years. In future trials, tumors as well as liquid biopsies should preferably allocate patients to "an ideal group" based on the combination of genetic alterations identified in the blood and in the primary tumor in an attempt to evaluate tumor aggressiveness. Evaluating the primary tumor for specific genetic mutations and including these in clinical trials is also a key research area with great potential as HNC patients often experience T-site failures. The combination of a mutational profile of the primary tumor, liquid biopsy along with smoking history and a comorbidity index might be the optimal tool to allocate patients in clinical trials and to appropriate follow-up. Addressing clinical trials, the categorization of clinical and pathological features is interesting in the era of escalating treatment for the group of HNC patients with a bad prognosis but without distant progression. A small group of patients that are never-smokers present with oral cavity or oropharynx aggressive HPV- tumors. These patients hold a very poor prognosis likely due to the hostile tumor biology although we know very little on the difference between the smoking and alcohol induced HPV- tumors contrary to the non-smoking patients with HPV- tumors.

Future preventive strategies must include keen awareness on vaccination as this strategy has the ability of altering the anticipated epidemic of HPV+ oropharyngeal cancer. Though the incidence among 30-50 year old persons might begin to decrease in the next 20 years the modeling provided by Zhang *et al* implies that HPV vaccination will have a minor impact on the overall oropharyngeal cancer incidence as it is likely to have effect following the year 2045 and forward (40). It is likely, given that the vaccination program as planned today keeps momentum, may provide a reduction in the incidence of oropharyngeal cancers.

Danish Summary

Denne afhandling præsenterer epidemiologiske, kliniske og biologiske aspekter vedrørende patienter diagnosticeret med humant papilloma virus-associeret (HPV) mundsvælgkræft. Antallet af patienter diagnosticeret med pladecellekarcinomer i hoved og hals området er stigende på trods af reduktionen af de vigtigste risikofaktorer navnligt tobak og alkohol. Forklaringen findes bl.a. i den stigende forekomst af patienter med højrisiko-HPV-positiv mundsvælgskræft. Patienter med sidstnævnte er yngre, udviser en længere overlevelse og får sjældnere sygdomstilbagefald end personer med ikke-HPV associeret mundsvælgskræft. Betydningen af det intracellulære protein p16 diskuteres i afhandlingen, og der gives indsigt i klassificeringen af p16 for HPV+ tumorer såvel som betydningen af p16 for ikke-HPV-tumorer. Tiden til og forekomsten af sygdomsprogression for patienter med mundsvælgskræft behandles i et lokalt (Østdansk) og internationalt perspektiv. Risikoadfærden hos patienter med hoved- og halskræft belyses herunder betydningen af tobaksrygning og associationen mellem hoved-hals kræft og tidligere diagnose med seksuelt overførte sygdomme. Endeligt anskueliggøres forekomst og betydning af komorbiditeter hos patienter med hoved- og halskræft.

English Summary

This thesis presents epidemiological, clinical, and biological aspects of patients with human papilloma virus (HPV) associated oropharyngeal squamous cell carcinomas in perspective to non-HPV patients. Head and neck squamous cell carcinomas are increasing despite the reduction in exposure to the major risk factors, tobacco and alcohol. The increase is partly explained due to the rising proportion of patients with high-risk HPV+ oropharynx squamous cell carcinomas (OPSCC). Patients with the latter are younger, exhibit a longer overall survival, and present fewer recurrences than individuals with HPV-negative OPSCC. The role of the intracellular protein p16 is discussed providing insights into the classification of p16 in tumor slides for HPV+ tumors as well as the impact of p16 in non-HPV tumors. The timing and occurrence of disease progression is dealt with in a local (Eastern Danish) perspective as well as in a broader outlook in a systematic review. The risk behavior of head and neck cancer patients are reviewed regarding the impact of tobacco smoking for OPSCC patients and the association to sexual transmitted diseases discussed. Finally, the incidence and impact of comorbidities in head and neck cancer patients are reviewed.

List of figures and tables

References

1. Grønhøj Larsen C, Gyldenløve M, Jensen DH, Therkildsen MH, Kiss K, Norrild B, et al. Correlation between human papillomavirus and p16 overexpression in oropharyngeal tumours: a systematic review. Br J Cancer. 2014 Feb 11;110(6):1587–94.
2. Schneider K, Jakobsen KK, Jensen JS, Wessel I, Christensen A, Specht L, et al. Impact of p16-overexpression on overall and progression-free survival outcomes in oral cavity squamous cell carcinomas: A semi-national, population-based study. Oral Oncol. 2020 Dec 1;111(105031).
3. Grønhøj C, Jakobsen KK, Jensen DH, Rasmussen J, Andersen E, Friborg J, et al. Pattern of and survival following loco-regional and distant recurrence in patients with HPV+ and HPV− oropharyngeal squamous cell carcinoma: A population-based study. Oral Oncol. 2018;83:127–33.
4. Tiedemann D, Jakobsen KK, von Buchwald C, Grønhøj C. Systematic review on location and timing of distant progression in human papillomavirus-positive and human papillomavirus-negative oropharyngeal squamous cell carcinomas. Head Neck. 2019 Mar 23;41(3):793–8.
5. Grønhøj C, Jensen JS, Wagner S, Dehlendorff C, Friborg J, Andersen E, et al. Impact on survival of tobacco smoking for cases with oropharyngeal squamous cell carcinoma and known human papillomavirus and p16-status: a multicenter retrospective study. Oncotarget [Internet]. 2019 Jul 23 [cited 2019 Aug 3];10(45):4655–63. Available from: http://www.oncotarget.com/fulltext/27079
6. Grønhøj C, Jakobsen KK, Wingstrand VL, Jensen D, Iachina M, Egeberg A, et al. Association between head and neck cancer and sexually transmitted diseases: a Danish nationwide, case-control study. Acta Otolaryngol. 2020 Jul 2;140(7):615–9.
7. Grønhøj C, Kronberg Jakobsen K, Kjær E, Friborg J, von Buchwald C, C. G, et al. Comorbidity in HPV+ and HPV− oropharyngeal cancer patients: A population-based, case-control study. Oral Oncol [Internet]. 2019 Sep 1 [cited 2019 Oct 26];96:1–6. Available from: https://pubmed.ncbi.nlm.nih.gov/31422200/
8. Ghanizada M, Jakobsen KK, Jensen JS, Wessel I, Filtenborg Tvedskov J, Grønhøj C, et al. The impact of comorbidities on survival in oral cancer patients: a population-based, case-control study. Acta Oncol (Madr). 2021;60(2):173–9.
9. Ruud Kjær EK, Jensen JS, Jakobsen KK, Lelkaitis G, Wessel I, von Buchwald C, et al.

The Impact of Comorbidity on Survival in Patients With Head and Neck Squamous Cell Carcinoma: A Nationwide Case-Control Study Spanning 35 Years. Front Oncol. 2021 Feb 17;10.

10. Weinbauer MG. Ecology of prokaryotic viruses. FEMS Microbiol Rev [Internet]. 2004 May [cited 2022 May 29];28(2):127–81. Available from: https://pubmed.ncbi.nlm.nih.gov/15109783/
11. Reche I, D'Orta G, Mladenov N, Winget DM, Suttle CA. Deposition rates of viruses and bacteria above the atmospheric boundary layer. ISME J [Internet]. 2018 Apr 1 [cited 2022 May 29];12(4):1154. Available from: /pmc/articles/PMC5864199/
12. Kedrov A V., Durymanov M, Anokhin K V. The Arc gene: Retroviral heritage in cognitive functions. Neurosci Biobehav Rev [Internet]. 2019 Apr 1 [cited 2022 May 29];99:275–81. Available from: https://pubmed.ncbi.nlm.nih.gov/30772431/
13. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Heal [Internet]. 2020 Feb 1 [cited 2022 May 29];8(2):e180–90. Available from: https://pubmed.ncbi.nlm.nih.gov/31862245/
14. Payne J. On the contagiousness of common warts. Br J Dermatol. 1891;3:185–188.
15. Parsons RJ, Kidd JG. Oral Papillomatosis of rabbits: a virus disease. J Exp Med [Internet]. 1943 Mar 1 [cited 2021 Dec 1];77(3):233–50. Available from: https://pubmed.ncbi.nlm.nih.gov/19871279/
16. Purola E, Savia E. Cytology of gynecologic condyloma acuminatum. Acta Cytol [Internet]. 1977 [cited 2022 Nov 25];21(1):26–31. Available from: https://pubmed.ncbi.nlm.nih.gov/264754/
17. Meisels A, Fortin R, Roy M. Condylomatous lesions of the cervix. II. Cytologic, colposcopic and histopathologic study. Acta Cytol. 1977;21(3):379–90.
18. Zur Hausen H, Gissmann L, Steiner W, Dippold W, Dreger I. Human papilloma viruses and cancer. Bibl Haematol [Internet]. 1975 [cited 2021 Dec 1];No.43(43):569–71. Available from: https://pubmed.ncbi.nlm.nih.gov/183728/
19. Hausen H Zur, Schulte-Holthausen H, Wolf H, Dörries K, Egger H. Attempts to detect virus-specific DNA in human tumors. II. Nucleic acid hybridizations with complementary RNA of human herpes group viruses. Int J cancer [Internet]. 1974 [cited 2021 Dec 1];13(5):657–64. Available from: https://pubmed.ncbi.nlm.nih.gov/4367341/
20. Durst M, Gissmann L, Ikenberg H, Zur Hausen H. A papillomavirus DNA from a

cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci U S A [Internet]. 1983 [cited 2021 Dec 1];80(12):3812–5. Available from: https://pubmed.ncbi.nlm.nih.gov/6304740/

21. Quick CA, Faras A, Krzysek R. The etiology of laryngeal papillomatosis. Laryngoscope [Internet]. 1978 Nov 1 [cited 2021 Dec 1];88(11):1789–95. Available from: https://onlinelibrary.wiley.com/doi/full/10.1288/00005537-197811000-00009
22. Syrjänen K, Syrjanen S, Pyrhdnen S. Human Papilloma Virus (HPV) Antigens in Lesions of Laryngeal Squamous Cell Carcinomas. ORL [Internet]. 1982 [cited 2021 Dec 1];44(6):323–34. Available from: https://www.karger.com/Article/FullText/275612
23. Syrjänen K. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. Int J Oral Surg. 1983 Dec 1;12(6):418–24.
24. Kirnbauer R, Taub J, Greenstone H, Roden R, Dürst M, Gissmann L, et al. Efficient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles. J Virol [Internet]. 1993 Dec [cited 2021 Dec 1];67(12):6929–36. Available from: https://pubmed.ncbi.nlm.nih.gov/8230414/
25. You EL, Henry M, Zeitouni AG. Human papillomavirus – associated oropharyngeal cancer : review of current evidence and management. 2019;26(2):119–23.
26. Brandsma JL, Abramson AL. Association of papillomavirus with cancers of the head and neck. Arch Otolaryngol Head Neck Surg [Internet]. 1989 [cited 2021 Dec 1];115(5):621–5. Available from: https://pubmed.ncbi.nlm.nih.gov/2539843/
27. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst [Internet]. 2000 May 3 [cited 2015 Oct 20];92(9):709–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10793107
28. Mellin H, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E. Human papillomavirus (HPV) DNA in tonsillar cancer: clinical correlates, risk of relapse, and survival. Int J Cancer. 2000;89:300–4.
29. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Sep;
30. Kathrine Kronberg Jakobsen, Grønhøj C, Jensen DH, Karnov KKS, Agander TK, Specht L, et al. Increasing Incidence and Survival of Head and Neck Cancers in

Denmark: A nation-wide study from 1980-2014. 2017;

31. Grønhøj Larsen C, Channir HI, Kiss K, Charabi B, Lajer C, von Buchwald C. Udredning af knude på halsen hos voksne. Ugeskr Laeger. 2015 Sep 21;177(39).
32. Channir HI, Rubek N, Nielsen HU, Kiss K, Charabi BW, Lajer CB, et al. Transoral robotic surgery for the management of head and neck squamous cell carcinoma of unknown primary. Acta Otolaryngol. 2015 Oct;135(10):1051–7.
33. Rubek N, Channir HI, Charabi BW, Lajer CB, Kiss K, Nielsen HU, et al. Primary transoral robotic surgery with concurrent neck dissection for early stage oropharyngeal squamous cell carcinoma implemented at a Danish head and neck cancer center: a phase II trial on feasibility and tumour margin status. Eur Arch Otorhinolaryngol. 2017 May;274(5):2229–37.
34. DAHANCA. Larynx og Pharynx retningslinjer [Internet]. 2011. p. 1–69. Available from: www.dahanca.dk
35. Høxbroe Michaelsen S, Grønhøj C, Høxbroe Michaelsen J, Friborg J, von Buchwald C. Quality of life in survivors of oropharyngeal cancer: A systematic review and meta-analysis of 1366 patients. Vol. 78, European Journal of Cancer. 2017. p. 91–102.
36. Isenberg A Lou, Channir HI, von Buchwald C, Rubek N, Friborg J, Kiss K, et al. Transoral robotic surgery: a 4-year learning experience in a single Danish Cancer Centre. Acta Otolaryngol [Internet]. 2020 Feb 1 [cited 2021 Dec 1];140(2):157–62. Available from: https://pubmed.ncbi.nlm.nih.gov/31849248/
37. Gooi Z, Chan JYK, Fakhry C. The epidemiology of the human papillomavirus related to oropharyngeal head and neck cancer. Laryngoscope. 2016 Feb;126(4):894–900.
38. Carlander A, Jakobsen K, Bendtsen S, Garset-Zamani M, Lynggaard C, Schmidt Jensen J, et al. A Contemporary Systematic Review on Repartition of HPV-Positivity in Oropharyngeal Cancer Worldwide. Viruses [Internet]. 2021 Jul 1 [cited 2021 Oct 9];13(7). Available from: https://pubmed.ncbi.nlm.nih.gov/34372532/
39. Zamani M, Grønhøj C, Jensen DH, Carlander AF, Agander T, Kiss K, et al. The current epidemic of HPV-associated oropharyngeal cancer: An 18-year Danish population-based study with 2,169 patients. Eur J Cancer [Internet]. 2020 Jul 1 [cited 2020 Jul 7];134:52–9. Available from: https://pubmed.ncbi.nlm.nih.gov/32460181/
40. Zhang Y, Fakhry C, D'Souza G. Projected Association of Human Papillomavirus Vaccination With Oropharynx Cancer Incidence in the US, 2020-2045. JAMA Oncol [Internet]. 2021 [cited 2022 May 29];7(10). Available from: https://pubmed.ncbi.nlm.nih.gov/34473210/

41. Castellsagué X, Alemany L, Quer M, Halec G, Quirós B, Tous S, et al. HPV Involvement in Head and Neck Cancers: Comprehensive Assessment of Biomarkers in 3680 Patients. J Natl Cancer Inst [Internet]. 2016 Jun [cited 2016 Jul 27];108(6):djv403. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26823521
42. Stjernstrøm KD, Jensen JS, Jakobsen KK, Grønhøj C, von Buchwald C. Current status of human papillomavirus positivity in oropharyngeal squamous cell carcinoma in Europe: a systematic review. Acta Otolaryngol [Internet]. 2019 Sep 27 [cited 2019 Oct 26];December:1–5. Available from: https://www.tandfonline.com/doi/full/10.1080/00016489.2019.1669820
43. Carlander A-LF, Grønhøj Larsen C, Jensen DH, Garnæs E, Kiss K, Andersen L, et al. Continuing rise in oropharyngeal cancer in a high HPV prevalence area: A Danish population-based study from 2011 to 2014. Eur J Cancer. 2017;70:75–82.
44. Yeo-Teh NSL, Ito Y, Jha S. High-Risk Human Papillomaviral Oncogenes E6 and E7 Target Key Cellular Pathways to Achieve Oncogenesis. Int J Mol Sci [Internet]. 2018 Jun 8 [cited 2022 Oct 14];19(6). Available from: /pmc/articles/PMC6032416/
45. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018 May;18(5):269–82.
46. Grønhøj C, Jensen DH, Agander T, Kiss K, Høgdall E, Specht L, et al. Deep sequencing of human papillomavirus positive loco-regionally advanced oropharyngeal squamous cell carcinomas reveals novel mutational signature. BMC Cancer. 2018;18(1).
47. Klussmann JP, Gultekin E, Weissenborn SJ, Wieland U, Dries V, Dienes HP, et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. Am J Pathol [Internet]. 2003 Mar [cited 2016 Jan 4];162(3):747–53. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1868106&tool=pmcentrez&rendertype=abstract
48. Lydiatt WM, Patel SG, O'Sullivan B, Brandwein MS, Ridge JA, Migliacci JC, et al. Head and Neck cancers-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. CA Cancer J Clin [Internet]. 2017 Mar [cited 2021 Nov 29];67(2):122–37. Available from: https://pubmed.ncbi.nlm.nih.gov/28128848/
49. Bryant AK, Sojourner EJ, Vitzthum LK, Zakeri K, Shen H, Nguyen C, et al. Prognostic Role of p16 in Nonoropharyngeal Head and Neck Cancer. JNCI J Natl

Cancer Inst [Internet]. 2018 Dec 1 [cited 2022 Sep 13];110(12):1393–9. Available from: https://academic.oup.com/jnci/article/110/12/1393/5033454

50. Grønhøj Larsen C, Gyldenløve M, Kiss K, von Buchwald C. Who evaluates p16 immunohistochemistry? Apmis. 2015 Oct;123(10):912–3.
51. Christensen A, Kiss K, Lelkaitis G, Juhl K, Persson M, Charabi BW, et al. Urokinase-type plasminogen activator receptor (uPAR), tissue factor (TF) and epidermal growth factor receptor (EGFR): tumor expression patterns and prognostic value in oral cancer. BMC Cancer [Internet]. 2017 Aug 25 [cited 2022 Jun 1];17(1). Available from: https://pubmed.ncbi.nlm.nih.gov/28841839/
52. Christensen A, Grønhøj C, Jensen JS, Lelkaitis G, Kiss K, Juhl K, et al. Expression patterns of uPAR, TF and EGFR and their potential as targets for molecular imaging in oropharyngeal squamous cell carcinoma. Oncol Rep [Internet]. 2022 Aug 1 [cited 2022 Sep 13];48(2). Available from: https://pubmed.ncbi.nlm.nih.gov/35775375/
53. Kreimer AR, Pierce Campbell CM, Lin H-Y, Fulp W, Papenfuss MR, Abrahamsen M, et al. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. Lancet (London, England). 2013 Sep;382(9895):877–87.
54. Dahlstrom KR, Bell D, Hanby D, Li G, Wang LE, Wei Q, et al. Socioeconomic characteristics of patients with oropharyngeal carcinoma according to tumor HPV status, patient smoking status, and sexual behavior. Oral Oncol [Internet]. 2015 Sep 1 [cited 2022 Sep 12];51(9):832. Available from: /pmc/articles/PMC4532617/
55. Leigh BC, Temple MT, Trocki KF. The sexual behavior of US adults: results from a national survey. Am J Public Health [Internet]. 1993 [cited 2022 Sep 12];83(10):1400. Available from: /pmc/articles/PMC1694838/?report=abstract
56. Heck JE, Berthiller J, Vaccarella S, Winn DM, Smith EM, Shan'gina O, et al. Sexual behaviours and the risk of head and neck cancers: a pooled analysis in the International Head and Neck Cancer Epidemiology (INHANCE) consortium. Int J Epidemiol [Internet]. 2010 Dec 18 [cited 2022 Sep 12];39(1):166. Available from: /pmc/articles/PMC2817092/
57. Pittrof R, Sully E, Bass DC, Kelsey SF, Ness RB, Haggerty CL. Stimulating an immune response? Oral sex is associated with less endometritis. Int J STD AIDS [Internet]. 2012 [cited 2022 Nov 7];23(11):775. Available from: /pmc/articles/PMC3639487/
58. Drake VE, Fakhry C, Windon MJ, Stewart CM, Akst L, Hillel A, et al. Timing, number, and type sexual partners associsated with risk of oropharyngeal cancer.

Cancer [Internet]. 2021 Apr 4 [cited 2022 Sep 12];127(7):1029. Available from: /pmc/articles/PMC8035131/

59. Nielsen K, Jakobsen K, Schmidt Jensen J, Grønhøj C, Von Buchwald C. The Effect of Prophylactic HPV Vaccines on Oral and Oropharyngeal HPV Infection-A Systematic Review. Viruses [Internet]. 2021 Jul 1 [cited 2021 Oct 9];13(7). Available from: https://pubmed.ncbi.nlm.nih.gov/34372545/
60. El-Mofty SK. Human papillomavirus (HPV) related carcinomas of the upper aerodigestive tract. Head Neck Pathol. 2007 Dec;1(2):181–5.
61. Nethan ST, Gupta S, Warnakulasuriya S. Risk Factors for Oral Squamous Cell Carcinoma in the Indian Population. Microbes Oral Squamous Cell Carcinoma. 2022;9–40.
62. Microbes and Oral Squamous Cell Carcinoma. Microbes and Oral Squamous Cell Carcinoma. Springer Nature Singapore; 2022.
63. Stefaniak AB, LeBouf RF, Ranpara AC, Leonard SS. Toxicology of flavoring- and cannabis-containing e-liquids used in electronic delivery systems. Pharmacol Ther [Internet]. 2021 Aug 1 [cited 2022 Nov 8];224:107838. Available from: /pmc/articles/PMC8251682/
64. Guha N, Warnakulasuriya S, Vlaanderen J, Straif K. Betel quid chewing and the risk of oral and oropharyngeal cancers: a meta-analysis with implications for cancer control. Int J cancer [Internet]. 2014 Sep 15 [cited 2022 Nov 8];135(6):1433–43. Available from: https://pubmed.ncbi.nlm.nih.gov/24302487/
65. Merchant A, Husain SSM, Hosain M, Fikree FF, Pitiphat W, Siddiqui AR, et al. Paan without tobacco: An independent risk factor for oral cancer. South Asia Int J Cancer [Internet]. 2000 [cited 2022 Nov 8];86:128–31. Available from: https://onlinelibrary.wiley.com/terms-and-conditions
66. Gillison ML, Zhang Q, Jordan R, Xiao W, Westra WH, Trotti A, et al. Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. J Clin Oncol. 2012 Jun;30(17):2102–11.
67. Granata R, Miceli R, Orlandi E, Perrone F, Cortelazzi B, Franceschini M, et al. Tumor stage, human papillomavirus and smoking status affect the survival of patients with oropharyngeal cancer: an Italian validation study. Ann Oncol. 2012 Jul;23(7):1832–7.
68. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: An emerging epidemic of human papillomavirus-associated cancers? Vol. 110, Cancer. 2007. p. 1429–35.

69. Maxwell JH, Kumar B, Feng FY, Worden FP, Lee JS, Eisbruch A, et al. Tobacco use in human papillomavirus-positive advanced oropharynx cancer patients related to increased risk of distant metastases and tumor recurrence. Clin Cancer Res [Internet]. 2010;16(4):1226–35. Available from: http://clincancerres.aacrjournals.org/content/16/4/1226.long
70. Wyss A, Hashibe M, Chuang SC, Lee YCA, Zhang ZF, Yu GP, et al. Cigarette, cigar, and pipe smoking and the risk of head and neck cancers: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Am J Epidemiol [Internet]. 2013 Sep 1 [cited 2022 Nov 8];178(5):679–90. Available from: https://pubmed.ncbi.nlm.nih.gov/23817919/
71. Winn DM, Lee YC, Hashibe M, Boffetta P, Agudo A, Ahrens W, et al. The INHANCE consortium: toward a better understanding of the causes and mechanisms of head and neck cancer. Oral Dis [Internet]. 2015 Sep 1 [cited 2022 Sep 12];21(6):685–93. Available from: https://pubmed.ncbi.nlm.nih.gov/25809224/
72. Rettig E, Kiess AP, Fakhry C. The role of sexual behavior in head and neck cancer: implications for prevention and therapy. Expert Rev Anticancer Ther. 2015 Jan;15(1):35–49.
73. Chaturvedi AK, D'Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. Oral Oncol. 2016;60:61–7.
74. Gillison ML, Zhang Q, Jordan R, Xiao W, Westra WH, Trotti A, et al. Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. J Clin Oncol. 2012;30(17):2102–11.
75. Andersen AO, Jensen JS, Jakobsen KK, Stampe H, Nielsen KJ, Wessel I, et al. The impact of tobacco smoking on survival of patients with oral squamous cell carcinoma: a population-based retrospective study. Acta Oncol [Internet]. 2022 [cited 2022 Sep 17];61(4):449–58. Available from: https://pubmed.ncbi.nlm.nih.gov/35114883/
76. O'Sullivan B, Huang SH, Su J, Garden AS, Sturgis EM, Dahlstrom K, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. Lancet Oncol [Internet]. 2016 Feb 26 [cited 2016 Mar 5]; Available from: http://www.thelancet.com/article/S1470204515005604/fulltext
77. Huang SH, O'Sullivan B. Overview of the 8th Edition TNM Classification for Head

and Neck Cancer. Curr Treat Options Oncol [Internet]. 2017 Jul 1 [cited 2022 Oct 24];18(7):40–40. Available from: https://europepmc.org/article/med/28555375

78. Xu CC, Biron VL, Puttagunta L, Seikaly H. HPV Status and second primary tumours in Oropharyngeal Squamous Cell Carcinoma. J Otolaryngol - Head Neck Surg [Internet]. 2013 [cited 2022 Jun 1];42(1):36. Available from: /pmc/articles/PMC3668914/
79. Rasmussen JH, Grønhøj C, Håkansson K, Friborg J, Andersen E, Lelkaitis G, et al. Risk profiling based on p16 and HPV DNA more accurately predicts location of disease relapse in patients with oropharyngeal squamous cell carcinoma. Ann Oncol [Internet]. 2019 Jan 18 [cited 2019 Jan 31]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/30657857
80. Burd EM. Human Papillomavirus Laboratory Testing: the Changing Paradigm. Clin Microbiol Rev [Internet]. 2016 Apr [cited 2016 Feb 29];29(2):291–319. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26912568
81. Fakhry C, Zhang Q, Nguyen-Tan PF, Rosenthal D, El-Naggar A, Garden AS, et al. Human papillomavirus and overall survival after progression of oropharyngeal squamous cell carcinoma. J Clin Oncol [Internet]. 2014;32(30):3365–73. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4195851&tool=pmcentrez&rendertype=abstract
82. Hamill CS, Muller RG, Clancy K, Vu B, Gui S, Thuener JE, et al. Treatment failure patterns are similar between p16- and p16+ oropharyngeal squamous cell carcinomas. Laryngoscope Investig Otolaryngol [Internet]. 2022 Aug 1 [cited 2022 Sep 13];7(4):988–93. Available from: https://pubmed.ncbi.nlm.nih.gov/36000056/
83. Lee NCJ, Kelly JR, Park HS, An Y, Judson BL, Burtness BA, et al. Patterns of failure in high-metastatic node number human papillomavirus-positive oropharyngeal carcinoma. Oral Oncol [Internet]. 2018 Oct 1 [cited 2022 Sep 19];85:35–9. Available from: https://pubmed.ncbi.nlm.nih.gov/30220317/
84. Kwon M, Roh JL, Song J, Lee SW, Kim SB, Choi SH, et al. Noncancer health events as a leading cause of competing mortality in advanced head and neck cancer. Ann Oncol. 2014 Jun 1;25(6):1208–14.
85. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Paula Curado M, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. J Clin Oncol. 2013;31(36):4550–9.

86. Sarfati D. Review of methods used to measure comorbidity in cancer populations: no gold standard exists. J Clin Epidemiol [Internet]. 2012 Sep [cited 2022 Nov 17];65(9):924–33. Available from: https://pubmed.ncbi.nlm.nih.gov/22739245/
87. Homma A, Sakashita T, Oridate N, Suzuki F, Suzuki S, Hatakeyama H, et al. Importance of comorbidity in hypopharyngeal cancer. Head Neck. 2010 Feb;32(2):148–53.
88. Piccirillo JF. Importance of comorbidity in head and neck cancer. Laryngoscope. 2000;110(4):593–602.
89. Mowery A, Light T, Clayburgh D. Long-term Trends in Head and Neck Surgery Outcomes. Otolaryngol Head Neck Surg [Internet]. 2018 Dec 1 [cited 2022 Sep 19];159(6):1012–9. Available from: https://pubmed.ncbi.nlm.nih.gov/29986636/
90. Lin HW, Bhattacharyya N. Contemporary assessment of medical morbidity and mortality in head and neck surgery. Otolaryngol - Head Neck Surg. 2012 Mar;146(3):385–9.
91. Hess CB, Rash DL, Daly ME, Farwell DG, Bishop J, Vaughan AT, et al. Competing causes of death and medical comorbidities among patients with human papillomavirus-positive vs human papillomavirus-negative oropharyngeal carcinoma and impact on adherence to radiotherapy. JAMA Otolaryngol Head Neck Surg. 2014;140(4):312–6.
92. Eytan DF, Blackford AL, Eisele DW, Fakhry C. Prevalence of comorbidities and effect on survival in survivors of human papillomavirus–related and human papillomavirus–unrelated head and neck cancer in the United States. Cancer. 2019 Jan 15;125(2):249–60.
93. Boje CR, Dalton SO, Gronborg TK, Primdahl H, Kristensen CA, Andersen E, et al. The impact of comorbidity on outcome in 12 623 Danish Head and Neck Cancer Patients: A population based study from the DAHANCA database. Acta Oncol (Madr) [Internet]. 2013 Feb [cited 2022 Sep 19];52(2):285–93. Available from: https://www.tandfonline.com/doi/abs/10.3109/0284186X.2012.742964
94. Bøje CR, Dalton SO, Primdahl H, Kristensen CA, Andersen E, Johansen J, et al. Evaluation of comorbidity in 9388 head and neck cancer patients: a national cohort study from the DAHANCA database. Radiother Oncol [Internet]. 2014 [cited 2022 Sep 19];110(1):91–7. Available from: https://pubmed.ncbi.nlm.nih.gov/24412015/
95. Pai K, Baaklini C, Cabrera CI, Tamaki A, Fowler N, Maronian N. The Utility of Comorbidity Indices in Assessing Head and Neck Surgery Outcomes: A Systematic Review. Laryngoscope [Internet]. 2022 Jul 1 [cited 2022 Sep 19];132(7):1388–402.

Available from: https://onlinelibrary-wiley-com.ep.fjernadgang.kb.dk/doi/full/10.1002/lary.29905

96. Bøje CR, Dalton SO, Grønborg TK, Primdahl H, Kristensen CA, Andersen E, et al. The impact of comorbidity on outcome in 12 623 Danish Head and Neck Cancer Patients: A population based study from the DAHANCA database. Acta Oncol (Madr). 2013 Feb;52(2):285–93.
97. Schmidt Jensen J, Jakobsen KK, Mirian C, Christensen JT, Schneider K, Nahavandipour A, et al. The Copenhagen Oral Cavity Squamous Cell Carcinoma database: protocol and report on establishing a comprehensive oral cavity cancer database . Clin Epidemiol. 2019;
98. Lynge E, Sandegaard JL, Rebolj M. The Danish national patient register. Scand J Public Health. 2011;39(7):30–3.
99. Leon KE, Tangudu NK, Aird KM, Buj R. Loss of p16: A Bouncer of the Immunological Surveillance? Life 2021, Vol 11, Page 309 [Internet]. 2021 Apr 2 [cited 2022 Oct 24];11(4):309. Available from: https://www.mdpi.com/2075-1729/11/4/309/htm
100. Sznurkowski JJ, Żawrocki A, Biernat W, Sznurkowski JJ, Żawrocki A, Biernat W. Local immune response depends on p16 INK4a status of primary tumor in vulvar squamous cell carcinoma. Oncotarget [Internet]. 2017 May 3 [cited 2022 Oct 24];8(28):46204–10. Available from: https://www.oncotarget.com/article/17581/text/
101. Takatsu F, Suzawa K, Okazaki M, Shien K, Yamamoto H, Watanabe M, et al. Clinical features of patients with second primary lung cancer following head and neck cancer. Ann Thorac Surg [Internet]. 2022 May [cited 2022 Jun 1]; Available from: https://pubmed.ncbi.nlm.nih.gov/35595090/
102. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer. 2010 Dec;10(12):878–89.
103. Kassiotis G. Endogenous Retroviruses and the Development of Cancer. J Immunol [Internet]. 2014 Feb 15 [cited 2022 Oct 25];192(4):1343–9. Available from: https://www.jimmunol.org/content/192/4/1343

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