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Prognostic indicators of survival for patients with oral cavity squamous cell carcinoma in Norway

Outcomes in a retrospective, multicenter cohort, with special focus on oral tongue squamous cell carcinoma, 2005-2009

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Abbreviations

AJCC	American Joint Committee on Cancer
AKF	The Unit of Applied Clinical Research
CRF	Case report form
CRN	The Cancer Registry of Norway
DAHANCA	The Danish Head and Neck Cancer Group
DOI	Depth of tumor invasion
DSS	Disease-specific survival
ECOG	Eastern Cooperative Oncology Group
EHR	Electronic health record
ESAS-r	Edmonton Symptom Assessment System-revised version
FNAC	Fine needle aspiration cytology
H&E	Hematoxylin and eosin
HN	Head and neck
HPV	Human papillomavirus
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th Revision
IHC	Immunohistochemistry
IRB	Institutional Review Board
ISH	*In situ* hybridization
MDT	Multidisiplinary team
NCCN	National Comprehensive Cancer Network
NOROC	NORwegian Oral Cancer study
OCC	Oral cavity cancer
OCSCC	Oral cavity squamous cell carcinoma
OS	Overall survival
OTSCC	Oral tongue squamous cell carcinoma
REK	The Regional Committee for Medical Health Research Ethics
REMARK	REporting recommendations for tumor MARKer prognostic studies
RT	Radiation therapy
SCC	Squamous cell carcinoma
SEER	The Surveillance, Epidemiology and End Results Program
SNOMED	Systematized NOmenclature of MEDicine
SPSS	Statistical Package for the Social Sciences
TB	Tumor budding
TMA	Tissue microarray
TNM	Tumor-Node-Metastasis
cTNM	clinical presentation TNM
pTNM	pathological presentation TNM
UICC	Union for International Cancer Control
UNN	University Hospital of North Norway
WHO	World Health Organization
WPOI	Worst pattern of invasion

List of papers

This thesis is based on the following papers. They are referred to as **Papers I-IV** in the manuscript.

Paper I

Characteristics and prognosis of primary treatment-naïve oral cavity squamous cell carcinoma in Norway, a descriptive retrospective study. Bjerkli IH, Jetlund O, Karevold G, Karlsdóttir Á, Jaatun E, Uhlin-Hansen L, Rikardsen OG, Hadler-Olsen E, Steigen SE. PLoS One. 2020 Jan 16;15(1): e0227738. doi: 10.1371/journal.pone.0227738. eCollection 2020.

Paper II

High-risk human papilloma virus was not detected in a Norwegian cohort of oral squamous cell carcinoma of the mobile tongue. Søland TM, Bjerkli IH, Georgsen JB, Schreurs O, Jebsen P, Laurvik H, Sapkota D. Manuscript submitted for publication.

Paper III

Tumor budding score predicts lymph node status in oral tongue squamous cell carcinoma and should be included in the pathology report. Bjerkli IH, Laurvik H, Nginamau ES, Søland TM, Costea D, Hov H, Uhlin-Hansen L, Hadler-Olsen E, Steigen SE. Manuscript submittet for publication.

Paper IV

A combined histo-score based on tumor differentiation and lymphocytic infiltrate is a robust prognostic marker for mobile tongue cancer. Bjerkli IH, Hadler-Olsen E, Nginamau ES, Laurvik H, Søland TM, Costea D, Uhlin-Hansen L, Steigen SE. Manuscript submitted for publication.

Abstract

Background: Oral cavity cancer (OCC) is the most frequent of all head and neck (HN) cancers, and has distinct survival outcomes compared to other sites. The oral tongue is the most common site of cancer in the oral cavity. About 90% of these are squamous cell carcinomas (SCC), and they can be very aggressive with a high mortality rate. The treatment of these cancers is based on the tumor (T), lymph node (N), and metastasis (M) classification, although tumors with the same TNM classification may act differently in aggressiveness. Treatment of oral cavity (OC)SCC is preferentially surgery when the tumor is regarded as resectable, with additional neck-dissection for many of them. For some patients, postsurgical radiotherapy (RT) is added, and chemotherapy can be used in a palliative setting. Today there are no established histopathological or molecular markers in use to differentiate between those who will benefit from additional neck-dissection, or RT, when no lymph node metastases primarily are suspected. Such additional markers would be useful for supplementing the commonly used TNM classification in treatment decisions. **Objective:** The goal of this PhD-project was to collect clinical and histopathological information for a national cohort of OCSCC in the period 2005-2009, the Norwegian oral cancer (NOROC) study, to assess survival and prognostic factors. We explored whether high-risk Human Papilloma Virus (HPV) was present in oral tongue (OT)SCC, and assessed the prognostic value of different tumor growth patterns of these cancers. **Methods:** Clinical information was retrieved from the patients' electronic hospital files. Histologic sections were reexamined. Data from the national Cause of Death Registry was used to calculate overall and disease-specific survival. Statistical evaluation was performed to determine correlations, and find independent and significant predictors of survival. **Results:** We identified 643 patients with OCC, 535 of these were primary treatment-naïve OCSCC. Age at time of diagnosis, and low-stage disease correlated with higher survival outcome. We did not detect high-risk Human Papilloma Virus in the OTSCC. Tumor depth of invasion shifted many of the tumors to a higher T-status. Tumor differentiation, tumor budding, and lymphocytic infiltrate were the most important histopathologic prognosticators. **Conclusions:** Histopathological variables such as tumor budding, tumor differentiation, and lymphocytic infiltration, can add significant prognostic information to aid clinicians in treatment decisions and follow up, especially for low-stage tumors.

Sammendrag

Bakgrunn: Munnhulekreft er den hyppigst forekommende kreftformen i hode- og halsområdet. Munnhulekreft kan vokse aggressivt, og har lav overlevelsesprosent, nesten halvparten av pasientene dør i løpet av de første fem år etter behandling. Behandlingen avgjøres basert på pasientens generelle helsetilstand, men mest av alt med bakgrunn i klassifiseringen av kreftsykdommen, en såkalt TNM-klassifisering. T står for svulststørrelse, N for spredning til lokale lymfeknuter og M for fjernspredning. Sykdom med samme klassifisering kan ha forskjellig forløp. Munnhulekreft behandles først og fremst ved kirurgi, i tillegg kan det gis strålebehandling og i sjeldnere tilfeller kjemoterapi. Vi har så langt ingen veletablerte histopatologiske eller molekylære markører til bruk for å differensiere mellom behandlingsmodaliteter for munnhulekreft. **Målsetting:** Vårt mål var å etablere en nasjonal kohort av munnhulekreft. Vi reklassifiserte patologiske vevsprøver fra pasienter med kreft i den mobile tunge. Vi ønsket å undersøke om høyrisiko humant papillomavirus (HPV) er tilstede i tungekreft. Vi ønsket også å undersøke om ulike aspekter ved vekstmønster i disse kreftsvulstene er av prognostisk verdi. **Metode:** Dette doktorgradsprosjektet er basert på en retrospektiv innsamling av datamateriale fra pasienter som fikk påvist og behandlet munnhulekreft i Norge i årene 2005-2009, og reklassifisering av tilgjengelige histopatologiske prøver. Studien fikk navnet NOROC, Norwegian Oral Cancer study. Vi innhentet data fra Dødsårsaksregisteret for å kunne beregne overlevelse. Forskjellige statistiske metoder ble brukt for å undersøke sammenheng mellom variabler og overlevelsesanalyser. **Resultat:** Vi har beskrevet en stor kohorte med munnhulekreft fra Norge, og fant 643 tilfeller der munnhulekreft var satt med ICD-10-diagnose. 535 av disse var førstegangs tilfeller av kreft i munnhulen. Median alder for diagnosen var 67 år, fem års total overlevelse var 47%, fem års sykdoms-spesifikk overlevelse var 52%. Vi fant at yngre alder, små svulster med lav sykdomsutbredelse (N0) korrelerte med høyere overlevelse. Vi fant ikke høyrisiko HPV i vårt materiale av tungekreft. For vekstmønster fant vi at dybdevekst forskjøv mange av tumorene til høyere klassifisering. Tumor budding, differensieringsgrad sammen med lymfocytt infiltrat, de siste to i en kombinert histo-skår, kan være med som supplement til TNM-klassifiseringen. **Konklusjon:** Vi har demonstrert at histopatologiske variabler som tumor budding, differensieringsgrad og lymfocytt infiltrat, kan tilføre viktig informasjon. Dette kan brukes til å predikere for aggressivitet i tumor og bidra sammen med TNM-klassifiseringen i omfanget av behandling og videre oppfølging.

1 Introduction

Oral cavity cancer (OCC) is one of the most common subsites of head and neck (HN) cancers. In all treatment of cancer, the patients should be able to know what disease they are facing, by receiving current and correct information. Clinicians and patients need to decide upon treatment based on the most updated knowledge and most likely outcome, and the patient has to provide consent for treatment from this perspective. Consequently, there is a need for continuous research in epidemiology, diagnosis, management protocols, and outcomes for all forms of cancer. In this way, we are able to make decisions according to the principles of evidence-based medicine.

1.1 Epidemiology

HN cancers constitute 2-5% of all cancers in the world (1). Lip and OCC, when joined together, are the most common subtypes of HN cancers, and these comprised 354,864 new cases worldwide in 2018, 2% of all cancers. Oropharyngeal (oral pharynx) cancer comprised 92,887 new cases the same year, 0.5% of all cancers (1). These subsites should not be merged as they are different entities.

In 2012, the global incidence of oral cancer was estimated at 275,000 per year, and the incidence is steadily rising worldwide, according to global cancer statistics in 2018. Lip and OCC rank as the 18th most common of all cancers with 354,864 new cases, and rank as the 16th most frequent of deadly cancers in the world, with 177,384 deaths in 2018 (1). In general, men are at higher risk for having OCC than women (1). Median age for OCC is in the mid-sixties, but for oropharyngeal cancer, especially the human papillomavirus (HPV)-positive oropharyngeal cancer, the median age is ten years younger (2-4). Some studies report patients with oral tongue cancer to be younger than patients with other cancers of the oral cavity (5, 6).

The numbers of HN cancers have been rising in Norway, as well as globally (1, 7, 8). In the latest annual surveys from the Cancer Registry of Norway (CRN), OCC has been recorded without merging that with cancer of the lip or base of tongue (oropharyngeal part). The

cancers are classified to their anatomical location according to the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) diagnosis (9). Previously the national cancer registry tended to merge oral cavity and oropharyngeal sites; the number of new cases by primary sites and sex, from the CRN, for 2008 and 2018, is shown in Figure 1 (7, 8).

Table 4 Number of new cases by primary site and sex - 2008

ICD-10	Site	Males	Females	Total
C00-96	All sites	14000	12121	26121
C00-14	Mouth, pharynx	262	189	451
C00	Lip	54	50	104
C01-02	Tongue	62	32	94
C03-06	Mouth, other	43	44	87
C07-08	Salivary glands	15	23	38
C09-14	Pharynx	88	40	128

https://www.kreftregisteret.no/globalassets/publikasjoner-og-rapporter/cin2008part1_web.pdf

Table 5.1: Number and age-standardised rates of new cases by primary site and sex, 2018

		Cases			Age-standardised rates			
					Norwegian std.		World std.	
ICD-10	Site	Males	Females	Total	Males	Females	Males	Females
C00–96	All sites	18 321	15 869	34 190	700.0	548.8	359.9	311.1
C00–14	Mouth, pharynx	427	228	655	16.1	7.8	9.3	4.3
C00	Lip	49	30	79	2.0	1.0	0.9	0.4
C02–06	Oral cavity	136	108	244	5.1	3.7	3.0	2.0
C07–08	Salivary glands	46	27	73	1.7	0.9	1.0	0.5
C09–10, C01, C14	Oropharynx	172	54	226	6.3	1.9	3.9	1.2

https://www.kreftregisteret.no/globalassets/cancer-in-norway/2018/cin2018.pdf

Figure 1 Estimates of head and neck cancers in Norway in 2008 and 2018. From "Cancer in Norway", 2008 and 2018 (with permission, and available from https://www.kreftregisteret.no/globalassets/) (7, 8).

In Norway, the total number of patients with new cases of OCC (ICD-10; C02-C06) was 181 in 2008 (although this number includes C01, an oropharyngeal site), and 244 in 2018 (7, 8). The oral tongue is the most common site for OCC, comprising up to 50% of the cases (2, 10, 11).

Cancers occurring in different anatomical sites often have distinct etiologies and different treatment options, resulting in different survival outcomes. HN cancers are often presented and discussed as one type of cancer although the term includes different entities of cancer (12). It is important to describe precisely the sub-cohort of HN cancer patients when

estimating the most probable outcome. Oral cavity cancer and oropharyngeal cancer are two distinct cancer sites with different etiology, treatment, and survival outcome (13). In earlier investigations, data from oral tongue cancer (oral cavity site) and base of tongue cancers (oropharyngeal site) have often been combined and called tongue cancers or oral cancers, without being distinguished, and these studies are hampered by bias with respect to both risk factors and survival outcomes (14-16). In other reports, OCC incidence is presented together with cancer in the lip, and global cancer statistics combine these two locations in their presentations (1). Lip cancers tend to act like non-melanoma skin cancers and are for the most part less aggressive than cancers of the oral cavity (1, 17, 18). As a consequence, it is important to describe cohorts with validated oral cavity sites that are not merged with other HN sites in order to have reliable information.

In most countries, studies on patients with OCC report a five-year survival rate around 50% (4, 12, 18). In 2019, the Surveillance, Epidemiology, and End Results (SEER) program database in the U.S., published a five-year relative survival rate of 53% for the floor of mouth, and 66% for tongue, for the years 2009-2015 (19). It is difficult to compare different reports, as many merge anatomical sites, or do not specify precisely the anatomical sites they are describing, and there is no consistency in reporting survival as relative survival, overall survival (OS), or disease-specific survival (DSS). In some cases, differences in reporting cancer cohorts occur regardless of treatment options, and some studies report survival of the patients treated in curative intent, and exclude those with metastasized cancer, and this will also preclude any proper comparison of cohorts (2, 20). The anatomical regions are nowadays better clarified by the World Health Organization (WHO), and one should describe cohorts according to this resource (21).

Squamous cell carcinomas (SCC) are the most common malignancy in the oral cavity, representing 90% of the cases. Less common are verrucous carcinomas, adenocarcinomas, melanomas, lymphomas, and sarcomas (21).

In this thesis, we present the Norwegian Oral Cancer (NOROC) study, in which we focus on SCC of the oral cavity, with both clinical data and histopathological data, documenting diagnosis, treatment choices, and follow-up. All this in order to present a homogeneous cohort concerning the ICD-10 diagnosis, one type of cancer cells, and survival outcome.

1.2 Anatomy and classification of tumors

The oral cavity consists of the oral tongue, floor of the mouth, hard palate, buccal mucosa including the retromolar areas, and upper and lower alveolus and gingiva (gum), as shown in Figure 2 a-b. With respect to discussing locations and management of OCC, the designation "oral tongue" is applied to the mobile/anterior two-thirds of the tongue. The remaining posterior one-third is the base of the tongue and considered as part of the oropharynx (22).

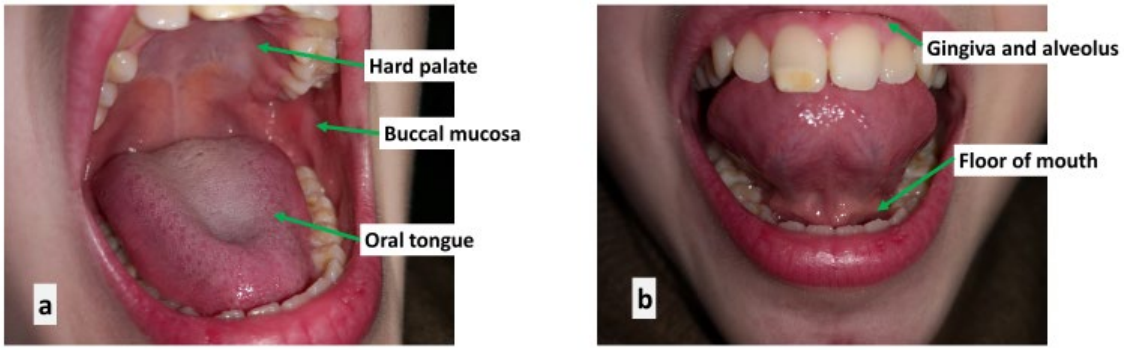

Figure 2 Oral cavity anatomy: a) oral tongue, buccal mucosa, and hard palate; b) floor of mouth, and gingiva and alveolar mucosa. Photographs: IH Bjerkli.

Tumors and cancers are classified by anatomical location according to ICD-10 with an alphanumeric code, possibly with an eventual fourth character. ICD-10 diagnoses applied to cancer in oral cavity are C02-C06 (9), but do not include C05.1 (soft palate) and C05.2 (uvula), as these are regarded oropharyngeal sites. C02.4 (tongue-tonsils) should also be exclusively used for oropharyngeal cancers (22). The ICD-10 diagnoses for OCC are shown in Table 1.

Table 1. ICD-10 diagnoses and anatomical sites of oral cavity cancer.

ICD-10 diagnosis	Anatomical site of oral cavity cancer
C02	Oral part of tongue
C03	Upper and lower gum
C04	Floor of mouth
C05	Hard palate
C06	Buccal mucosa

The anatomical extent of disease is classified in the TNM system. The TNM system describes the extent of the primary tumor (T); for head and neck cancers, the extent (absence or presence) of cervical (regional) lymph node metastasis (N); and the absence or presence of distant metastases (M). The TNM classification and staging of disease (I-IV) are described according to TNM Union for International Cancer Control (IUCC) and American Joint Committee on Cancer (AJCC) (17, 21-23). The simplicity of the TNM system promotes clinical utility, but the prognostic outcome can be difficult to determine in patients with oral cavity squamous cell carcinoma (OCSCC) (24).

The TNM system has been revised on an irregular basis, but in 2017 a new classification was introduced (22, 24). In the new TNM 8th edition, for OCC, tumor dimension now including depth of invasion (DOI), is a standard assessment in the T classification. Cervical lymph nodes surgically removed will be described with or without extranodal extension (22). For the oropharyngeal cancers, HPV-status was included. The categories in the former TNM in use for the years 2005-2009, and those in the new 8th edition of TNM classification for OCC, are shown in Table 2.

Table 2. TNM classification and stage for oral cavity cancer and the changes in the classification between 5th and 8th editions. Adapted from «TNM Atlas, 5th Edition» (23) and «TNM Classification of Malignant Tumours, 8th Edition» (22).

	TNM 5th edition	TNM 8th edition (if changed)
Primary tumor (T) classification, size and extent		
TX	Primary tumor cannot be assessed	
T0	No evidence of primary tumor	
T1	Size ≤2 cm in greatest dimension	and 5mm or less in depth of invasion (DOI)
T2	Size > 2cm<4 cm in greatest dimension	Size≤2cm and DOI 5-10mm, or size >2 cm<4cm and DOI≤10mm
T3	Size >4 cm in greatest dimension	or DOI >10 mm
T4a/b	Tumor invades adjacent tissue	
Cervical node (N) metastasis classification		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node assessment	
N1	Metastsis in a single ipsilateral node, ≤3 cm in greatest dimension	and without extranodal extension
N2	a) Metastasis in a single ipsilateral>3 cm< 6cm	a) and no extranodal extension
	b) Ipsilateral, multiple ≤6 cm	b) and no extranodal extension
	c) Bilateral, contralateral≤6cm	c) and no extranodal extension
N3	>6 cm	a) and no extranodal extension
		b) Any N with extranodal extension
Distant metastasis (M) classification		
MX	Distant metastasis cannot be assessed	considered to be inappropriate to use
M0	No distant metastasis	
M1	Distant metastasis	
Stage of disease on basis of TNM classification		
Stage I	T1 N0 M0	
Stage II	T2 N0 M0	
Stage III	T1-T2 and N1 M0 T3 and N0-N1 M0	
Stage IVA	T1-T3 N2 M0 T4a N0-2 M0	
Stage IVB	Any T N3 M0 T4b any N M0	
Stage IVC	Any T any N M1	

The use of the TNM classification system guides in the choice of treatment and provides a rough prediction of probable outcome. Moreover, the TNM system of classification facilitates treatment evaluation and the exchange of information between clinicians, supports cancer control activities, and contributes to standardize cancer research. For these reasons, it is important that the classification system remains stable, in order to evaluate factors contributing to long-term survival outcomes in a population (22).

1.3 Etiology of oral cavity cancer

Globally, there are large geographical variations in incidence of HN and cancer of the oral cavity (1). Cultural habits, such as betel nut quid chewing and eating areca nuts, increase the OCC incidence in South and South East Asia (4, 25, 26). Cigarette (tobacco) smoking and excessive alcohol consumption are major risk factors (27-29) . We also know there is a synergistic effect of smoking and alcohol consumption (28). Poor dental health status has also been considered to be a risk factor, although reports are inconsistent (30, 31). On the other hand, other factors like consuming vegetables and fruits might forestall development of cancer (29, 32).

Viruses (oncoviruses) play a role in some HN cancers; especially documented is the Epstein-Barr virus in nasopharyngeal cancer, and HPV in laryngeal and oropharyngeal cancers (17). In the last decade, high-risk HPV has been established as playing an important role in oropharyngeal cancers, and this has therefore been implemented in the new TNM classification (22). Some studies have reported a small proportion of OCC presenting as HPV-positive (33, 34). HPV detection and confirmation can be done by several methods (35, 36). We lack larger studies on high-risk HPV in OCC where the anatomical locations for the tumors are precisely described.

The risk of recurrence or second primary tumors is present for the patients (37, 38). Genetic factors influence the initiation and progression of cancers, but the molecular mechanisms remain uncertain; several biological concepts have been described by Hanahan and colleagues in «The Hallmarks of Cancer» in 2011 (39).

1.4 Diagnosing oral cavity cancer

1.4.1 Clinical and radiological considerations

Early detection is important to improve the likelihood of good survival outcomes (2, 12, 40). The most common clinical manifestations of OCC are pain or numbness, wounds not healing, dental-symptoms, and cervical lymph node presentation (palpable lumps or mass) (21).

Patients can present with their signs and symptoms to the general practitioner, an ear-nose-throat /HN-specialist, or a dentist or dental hygienist. In Norway, patients with suspected HN cancer are referred to one of the four university clinics in the country, either Oslo University Hospital–Rikshospitalet in Oslo, Haukeland University Hospital in Bergen, St. Olavs University Hospital in Trondheim, or University Hospital of North Norway (UNN), Tromsø. The patients are clinically examined, documented by radiological imaging such as ultrasound, computed tomography (CT) scan, orthopantomogram (OPG), and sometimes magnetic resonance imaging (MRI) and positron emission tomography (PET)-CT (41, 42). CT is the most-used imaging modality for OCSCC in the Nordic countries (43). The clinical and radiological appearance forms the basis for the clinical (c)TNM classification of cancer disease.

1.4.2 Histopathology

Nearly 90% of the cancers of the oral cavity are SCC, originating from within the epithelial (mucosal) lining (21). The SCC in the HN region is heterogenous in histopathologic presentation, clinical appearance, and response to treatment. Samples, such as biopsies, and fine needle aspiration cytology (FNAC), are examined histologically for signs of pathology by a clinical pathologist. The histopathological verification forms the pathological (p)TNM classification (22). Histopathological confirmation of the clinico-radiological diagnosis is the foundation for tumor classification and further treatment decisions.

OCSCC are further classified according to morphological evaluation, although grading alone does not correlate well with prognosis (21). WHO distinguishes three morphological grades

divided into well, moderately, and poorly differentiated (grade I-III). Within a tumor, the degree of differentiation varies as well (21).

Within the same TNM classification, individual differences in clinical outcome is present, especially for the lower stages. Low-stages (early-stages) tumors are stage I and stage II (T1N0M0 and T2N0M0, in TNM 8th edition, with tumor size <4cm and/or DOI ≤ 10 mm, with no lymph node or distant metastasis) of OCSCC (44).

Tumor budding (TB) has been proposed as a possible additional variable for colorectal cancer (22, 45), and some have argued that this characteristic may also be of value for appraising OCSCC evaluation (46, 47). In fact, before DOI was introduced into the new TNM classification, some studies also suggested applying a combined TB and DOI score (BD score) as a prognostic indicator (48, 49).

Guidelines for recognizing patterns of the invasive front of the tumor and histologic risk models have been proposed by Anneroth and coworkers in 1987, Bryne and coworkers in 1998, and by Brandwein-Gensler and coworkers in 2005 (50-52). These latter classifications have been tried in later studies, but with only moderate success, as differentiating high-risk tumors and low-risk tumors is very challenging (44, 53-55). Proposed histopathological risk-parameters of tumors are: WHO differentiation grade, worst pattern of invasion (WPOI), degree of keratinization, nuclear polymorphism, perineural invasion, lymphocyte infiltration, and vascular infiltration (17, 21, 50-52). Some of the different histopathological growth pattern assessments presented in literature over recent years are shown in Table 3.

Table 3. Histopathological risk-parameters often presented in literature.

Histopathological variables	**References**
Tumor depth of invasion (DOI)	(22)
Tumor budding (TB)	(45, 46)
Tumor budding and depth of invasion score	(48)
WHO differentiation, whole tumor	(17, 21)
WHO differentiation, worst pattern	(52)
Degree of keratinization, whole tumor	(50)
Degree of keratinization, tumor front	(51)
Nuclear polymorphism, whole tumor	(50)
Nuclear polymorphism, tumor front	(51)
Perineural infiltration	(52)
Lymphocytic infiltrate	(52)
Worst pattern of invasion (WPOI)	(52)

Several studies have tried to adapt to these oral cancer risk-parameters in histopathology. However, few studies have been large enough, or based strictly on one anatomical site, to allow conclusive results. Different pathologists notice different patterns (inter-rater) and they will not always agree with themselves (intra-rater). The reproducibility between pathologists has potential for improvement with respect to inter-rater and intra-rater agreement (53, 56).

1.5 Treatment options and treatment complications

Multidisciplinary team (MDT) meetings are central in all cancer treatment and care (57, 58). The treatment options of today require accurate tumor classification and staging as well as additional molecular details. As a consequence, an increasing number of procedures for diagnosing and staging of cancer in patients is needed to determine any treatment decision (59). Today, treatment requires involvement of experts within different medical disciplines, and specialists from these disciplines meet in MDT.

For many cancers, a range of innovative surgical and medical treatments have been developed over recent decades. Examples include minimal invasive surgery techniques, stereotactic radiation therapy (RT), numerous chemotherapeutics, and involvement of targeting therapies and immune-therapies, all broadening the choice of treatment for most cancer types (60).

For OCC, the most common choice of treatment is still surgery of the primary site when the tumor is regarded resectable, with or without surgical neck dissection (61). Additional postoperative RT may be given (62). In some of the cases, RT is given before surgery to decrease the size and extent of the tumor (59, 63-65). A more advanced stage of disease (≥stage III) will, for some patients, lead to additional medical treatment with chemotherapy or combined radiochemotherapy. Chemotherapy alone is for the most part used for metastasizing disease and in a palliative setting (66, 67). The treatment in many cases leads to some sequela, depending on the size of the tumor and the extent to which the patient has had neck dissection surgery, reconstructive surgery, and postoperative RT.

As many as one-fifth of patients with low-stage tumors might experience neck node metastasis and recurrence within 1-3 years (68, 69). Two options are discussed for treatment of the neck in clinically node-negative (N0) OCSCC. One is elective neck dissection, and the

other is wait-and-see. In some cases for low-stage diseases, clinicians might suggest watchful waiting rather than neck dissection surgery (70-72). A patient with a small tumor and a N0-status in the neck region does not necessarily need neck dissection or postoperative RT, but early-stage node-negative OCSCC, that later develops regional metastasis, has poor prognosis (70, 71).

Complications of OCC treatment are associated with physical and psychological debility. Some patients feel socially inhibited due to a changed facial appearance and disfiguring scars, or because of persistent speech, swallowing, or tracheostomy difficulties. Xerostomia is common and can cause poorer dental health. Alterations in taste, malnutrition, and trismus are other complications. Moreover, a late complication of RT is induced osteoradionecrosis of the mandible, and some report an increased risk of ischemic stroke many years after RT (73). The patients may perceive themselves as altered, and many are not able to work again, part-time or full-time. Any impact on quality of life will be a cost for the individual as well as for society (74-76).

There is no established tumor growth pattern or biomarker profile to identify patients with occult neck metastasis that would allow for more individualized treatment, especially for the patients with low-stage disease (77). If such a diagnostic tool were available, we could predict aggressiveness of the tumor and limit treatment-related adverse effects. Due to the severe side-effects of the treatment, it is important to reduce overtreatment of OCC patients. In the absence of a national guideline for treatment of HN cancers in Norway, the Danish Head and Neck Cancer Group (DAHANCA) and the U.S. National Comprehensive Cancer Network (NCCN) guidelines have been used (59, 63).

Many previous studies that describe treatment and survival outcome of patients with OCC have not been consistent in reporting the anatomy and ICD-10 diagnosis or the actual treatment given. Some studies only included a small number of patients, and in some studies different anatomical sites have been merged (12, 78). In several studies, SCC of the oropharynx may be presented as part of the oral cavity and vice versa (13, 16). This predicament makes discussions of etiology and survival outcome inconclusive. To better understand OCC and answer our own and our patients' questions, it was considered important to describe a cohort of solely primary OCC, that is, squamous cell carcinomas, since they make up 90% of oral cancer patients. We wanted to provide a more precise description of epidemiology, etiology, treatment, and survival outcome in a national cohort that is

comparable to other studies. Only in this manner can we describe and argue for the choice of treatment and give prognostic outcomes to both our patients and our colleagues. A transparent cohort would be valuable for future studies to adhere to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines (79-81).

2 Aims of the thesis

2.1 Purpose of the thesis in general

The general aim was to establish a large cohort of oral cavity squamous cell carcinomas with a broad overview concerning clinical and histopathological characteristics. All this in order to find characteristics with prognostic value. The specific aims of my PhD-work were:

1. To establish a national, multicenter cohort of patients strictly limited to OCSCC, excluding other HN cancer sites;
2. To describe this cohort with respect to clinical and histopathological characteristics, and to calculate survival data;
3. To determine if high-risk HPV is present in oral tongue (OT) SCC;
4. To assess prognostic value of various morphological tumor traits that have previously been suggested as prognostic markers for OCSCC.

Our contributions fulfilling the stated aim of this thesis are presented in four separate articles, each with different research aims.

2.2 Aims of the included papers

- In **Paper I** we wanted to characterize a retrospective, multicenter cohort of solely OCSCC from all four health regions in Norway. We aimed to find clinical characteristics that significantly influenced survival, which we could compare with other cohorts.
- In **Paper II** we aimed to determine if high-risk HPV was found in a cohort with OTSCC.
- In **Paper III** we wanted to explore how depth of tumor invasion and tumor budding in OTSCC can impact survival outcomes.
- In **Paper IV** we aimed to describe how different high-risk histopathological patterns, even when made less complex with fewer options, can predict survival outcomes in OTSCC.

3 Materials and methods

This section provides an overview of the patient material retrieved retrospectively as the basis for the NOROC study, a collaborative multi-center study in Norway, outlining methods used. In Norway, treatment of HN cancer is centralized to the four university hospitals. The Institutional Review Board (IRB) of the Northern Norwegian Regional Committee for Medical Research Ethics (REK Nord) approved the NOROC study in 2013, with an expansion in 2015, and an extension in 2019. The protocol numbers are REK Nord 2013/1786 and 2015/1381 (Appendices I, II, III and IV). Ethical and methodological considerations are summarized in chapter 5.

3.1 The web-based case report form

The main build-up of the case report form (CRF), was developed in cooperation with the clinicians and pathologists of the NOROC group. Data collection was enabled by a web-based data collection system developed and administered by The Unit of Applied Clinical Research (AKF), Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway (82). The web-based CRF was administered on different servers, all situated in Norway. To be able to record, the person had to log in with a personal ID and a two-step message-access control code (double-authentication). Each of the four university hospitals had one clinician and one pathologist with access to record data into the CRF, a total of eight persons. The vast majority of the recording of clinical data was made by the PhD-candidate (IHB) as a clinician, supplemented by the other clinicians at the other hospitals. Four different pathologists were responsible for the histopathological re-assessment and recording. A list of questionnaire-specific questions and answers is included in Appendix V.

3.2 Data collecting process

3.2.1 Extracting clinical data from the patient cohort

Electronic health records (EHR) have been introduced in hospitals in many countries and, for Norwegian hospitals, from the beginning of 2000. Digitalized journals were implemented in Norway from 2002, but the latter were only fully available from 2005. After the introduction of EHR, we could search for the diagnoses electronically, and we did not have to find and store large collections of health records on paper (83). The time span 2005–2009 was chosen for data collection because we wanted to have a minimum of 500 patients and to have a minimum of a five-year follow-up in 2015; hence the last year for diagnosis had to be 2009. The collection of clinical data was achieved between August 2015 and February 2017.

The inclusion criteria were that patients had to be diagnosed with OCSCC (ICD-10 C02-C06) in the time span 1 January 2005–31 December 2009, documented in the EHRs. Exclusion criteria were: any reservation about research recorded in the EHR, the cancer not being in the oral cavity but in a neighboring site (like C05.1, C05.2, or in the tongue-tonsil C02.4), cancers other than SCC, recurrent cancer or second primary from a cancer before 2005, or any record of previous cancer treatment.

The ICD-10 diagnoses were matched with the pathologists' archive for patient tumors with coding T51 (mouth) and T53 (tongue). These are codes in the pathologists' archive called the Systematized Nomenclature of Medicine (SNOMED) (84). Some patients did not have the correct diagnosis in the EHR, and some were registered twice with different codes within the oral cavity or twice in different hospitals. We found 643 patients with OCC (ICD-10; C02-C06). The NOROC study focused on SCC, so therefore we excluded other histopathological diagnoses such as verrucous carcinoma, malignant melanoma, adenoid cystic carcinoma, lymphomas, and sarcomas. When all diagnoses, clinical data, and available histopathological samples were assessed, we ended up with 535 unique primary treatment-naïve OCSCC, as described in **Paper I**. When we extracted the strictly oral tongue cancers from the original 643, we found a total of 273 patients that had OTSCC; 34 of these were recurrences or second primaries. Those of the 273 patients with OTSCC available for tissue microarray (TMA) technique (n=146), formed the cohort for **Paper II**. When focusing on the primary treatment-naïve OTSCC, we ended up with 239 patients. Those of the 239 patients who were primary

treatment-naive and treated in curative intent eligible for histopathological reassessment (n=150), formed the cohort for **Paper III** and **Paper IV**, as illustrated in the flow-chart, Figure 3.

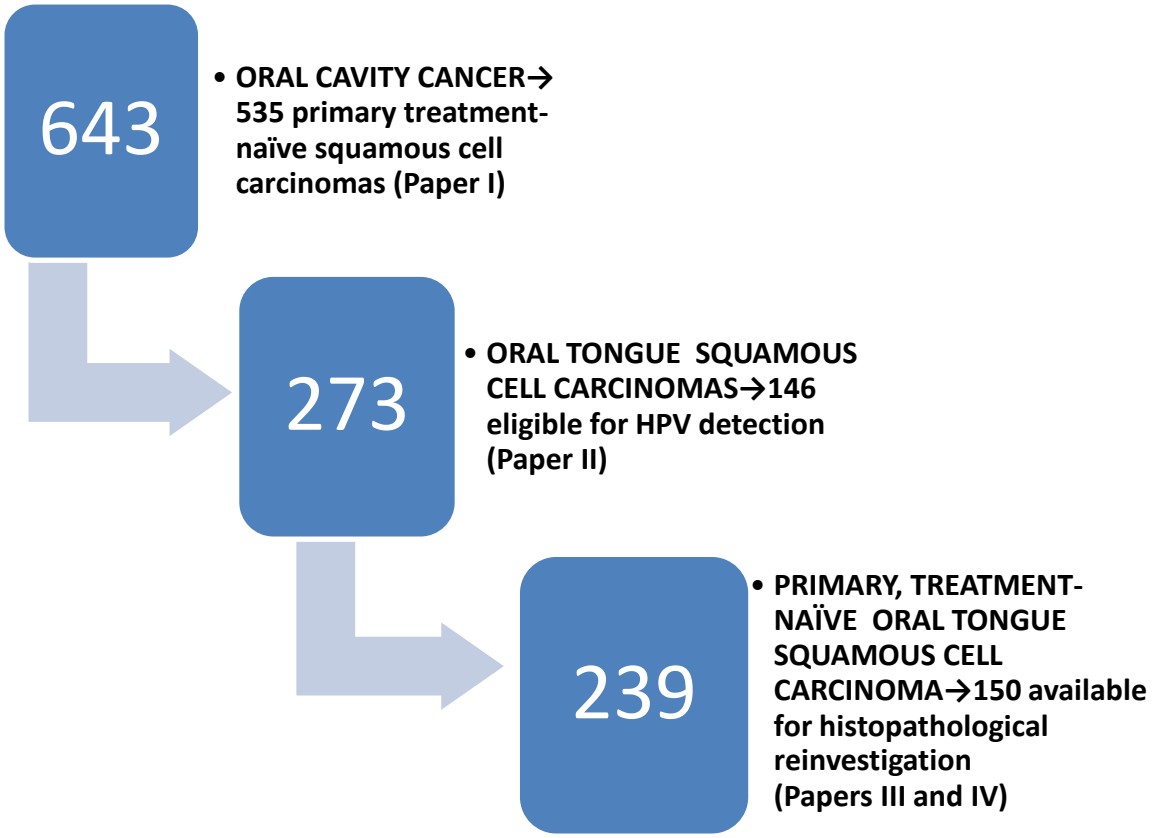

Figure 3 Flow-chart outlining the number of patients and available histopathological samples in the NOROC cohort presented in Papers I-IV.

Rikshospitalet in Oslo is the national referral hospital. When a patient was located in EHRs at both the Rikshospitalet and another university hospital (registered twice), the case was excluded from one of the hospital's CRF in order to be registered as a single patient at a single hospital. Most cases were recorded in the CRF belonging to the hospital that completed the follow-up. The proportion of patients in each of the four different hospitals is shown in Figure 4.

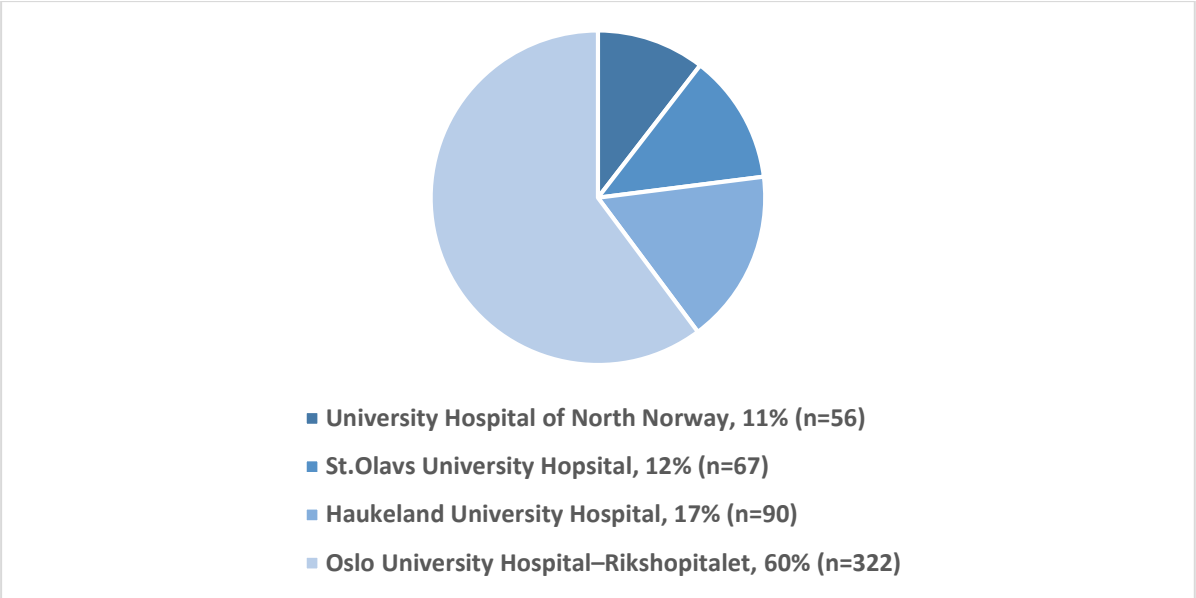

Figure 4 Percentage and number of patients diagnosed with primary OCSCC at the four university hospitals in Norway, 2005-2009.

We retrieved clinical data such as gender, age, county, treating hospital, cigarette smoking, alcohol consumption, medication, comorbidity, ICD-10 diagnosis, treatment of surgery, radiation therapy and chemotherapy, follow-up, residual disease, recurrence of disease, second primary, and survival at fold-in date. See Appendix V for details.

3.2.2 HPV assessment

HPV analysis can be done by several methods: immunohistochemistry (IHC) for tumor suppressor protein (p)16, and HPV *in situ* hybridization (ISH) for both DNA and mRNA (35). To be able to explore high-risk HPV in OTSCC, we used TMAs (85, 86). This is a method to analyze huge amounts of molecular analyses of, in this case, DNA, RNA, and p16, in so-called micro-matrices shown in Figure 5.

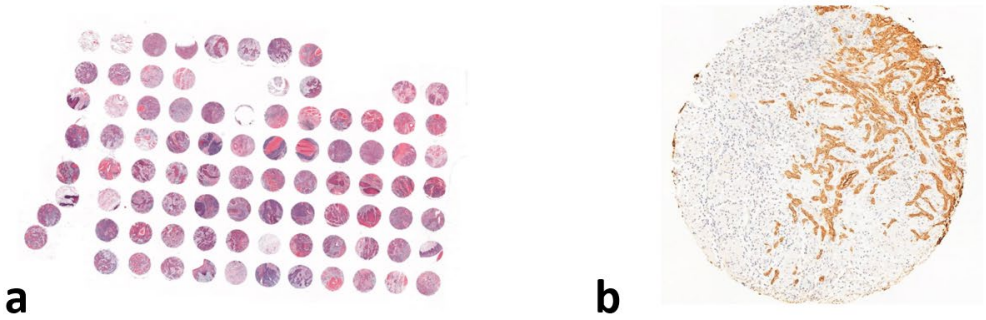

Figure 5 Tissue microarrays of oral tongue squamous cell carcinoma: a) overview H&E-stained TMAs; b) example of one pan-keratin stained TMA block (EA1EA3). Photographs: T Søland.

TMAs were designed from formalin-fixed, paraffin-embedded tissue blocks from OTSCC samples. We used a fully automated TMA machine (Ventana). The p16 IHC, the HPV DNA ISH, and the HPV RNA ISH, were all performed on TMAs. *In situ* hybridization is used to map and order (in this case) DNA and RNA to localize a specific DNA or RNA in a section of tissue. For details regarding methodology, consult **Paper II**.

3.2.3 Extracting histopathological data

The histopathological re-evaluation was fully discussed among the NOROC collaborating pathologists before beginning the task and documenting into the CRF. The pathologists also had a more detailed written worklist corresponding to the pathology part in the CRF, shown in Appendix VI. The collaborating pathologists had workshops prior to histopathological assessment. The histological re-evaluation for the OTSCC was done on available Hematoxylin and Eosin (H&E)-stained tissue sections and the pathologists were blinded for clinical outcome.

3.2.4 Depth of tumor invasion and tumor budding

Depth of tumor invasion or just depth of invasion is the depth the tumor invades into the tissue. The DOI was implemented for the T-status for OCC in the 8th edition of TNM in 2017 (22). This was also described by the International Consortium for Outcome Research in 2014 (24), and is also explained in Appendix VI. The pathologists re-evaluated the OTSCC and included the new DOI in the available H&E-slide sections.

Tumor budding is suggested as a new risk parameter for OTSCC (46). TB is a microscopic finding defined as the presence of isolated single tumor cells or small clusters of tumor cells, of four or fewer tumor cells at the invasive tumor front (47, 87), as shown in Figure 6. More details are explained in **Paper III**.

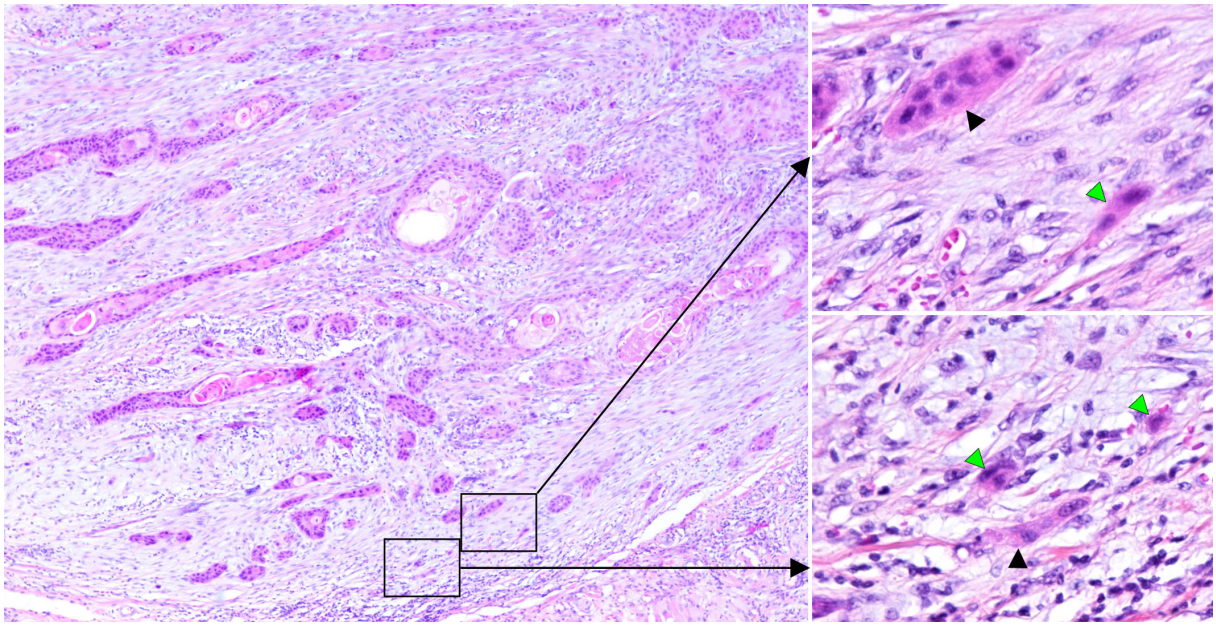

Figure 6 H&E-stained section of OTSCC with tumor budding; green arrowheads indicating buds<4, and black arrowheads indicating the tumor islands with>4 buds. Photographs: O Schreurs/T Søland.

3.2.5 Histopathological assessment of risk-patterns

Oral cavity SCC is for the most part described with the WHO differentiation of the whole tumor in the reports from the pathologists to the clinicians (21). Bryne and coworkers in 1998 and Brandwein-Gensler and coworkers in 2005, introduced assessment of histopathological risk-patterns in oral cancer (51, 52, 88). The additional histopathological information includes the WHO differentiation for worst pattern, nuclear polymorphism in the whole tumor, nuclear polymorphism at tumor front, keratinization of the whole tumor, keratinization in tumor front, lymphocyte infiltrate, perineural infiltration, and WPOI. A recent work by Steigen and coworkers (2020) provides an overview of the variables (56), which is shown in **Paper IV.** Details about grading are shown in Appendix IV. In **Paper IV** we also describe a less complex scoring model with fewer options, as previously shown to increase inter-rater and intra-rater agreement (56). All the histopathological variables assessed and presented in this thesis are listed below in Table 4.

Table 4. The various variables in oral tongue squamous cell carcinomas considered in this thesis.

Variable	Score	Paper
High-risk HPV	Present or not	II
Depth of tumor invasion (DOI)	<5mm, 5-10mm, and >10mm	III
Tumor budding (TB)	Low-high (2-tier) or low-intermediate-high (3-tier)	III
Tumor budding and depth of invasion score	Low-high (2-tier) or low, intermediate, and high (3-tier)	III
WHO differentiation, whole tumor	Well-moderately-poor	IV
WHO differentiation, worst pattern	Well-moderately-poor	IV
Degree of keratinization, whole tumor	Highly-moderately-minimal-no keratinization	IV
Degree of keratinization, tumor front	Highly-moderately-minimal-no keratinization	IV
Nuclear polymorphism, whole tumor	Little-moderately-abundant-extreme	IV
Nuclear polymorphism, tumor front	Little-moderately-abundant-extreme	IV
Perineural infiltration	No nerves-at invasive front-in tumor center	IV
Lymphocytic infiltrate	Marked-moderate-little/none	IV
Worst pattern of invasion (WPOI)	Pushing-Infiltrating-small groups-marked-tumor satellites (Type 1-5)	IV

3.3 Data from the Cancer Registry of Norway

This study also uses data from the CRN. To be able to verify whether we had found the majority of patients diagnosed and treated for OCC, we contacted the CRN in October 2015 to obtain the total number of patients diagnosed with OCC (ICD-10, C02-C06) recorded in their registry between 2005–2009. The data presented in this thesis are those selected by the author from that official public document of the CRN. Data was provided according to current regulations; Legal Authority Cancer Registry § 3-4(1).

3.4 Data from Norwegian Cause of Death Registry

Cause of death was acquired from Norwegian Cause of Death Registry. In this manner we could calculate overall survival and disease-specific survival (89).

3.5 Statistical analyses

In this thesis, the following statistical methods have been performed by using the IBM Statistical Package for the Social Sciences (SPSS) version 25-26 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive data with range, mean, median, and frequencies, were used to characterize the cohort in the four included papers. Chi-square was used to describe association among variables (90). Spearman bivariate correlation (2-tailed) including bootstrapping was used to identify correlation between variables (91). To avoid risk of bias from sparse-data, the variables with few data were excluded from these calculations (92). The Log-Rank (Mantel Cox) univariate survival analysis, giving Kaplan-Meier survival curves, was used to carry out a survival plot where all patients were censored at death or at follow-up after five years (93). When statistical variables were significant in univariate analysis, they were entered onto Cox regression multivariate analyses to assess their independent value as a prognostic factor of survival in the presence of other variables (90). For survival evaluation in multivariate analyses, Cox regression allowed us to describe significance, hazard ratio (HR),

and 95% confidence interval (CI) after bootstrapping. Collinearity was evaluated with linear regression (90). All results were considered significant if $p \leq 0.05$.

4 Summary of results

This is a summary of the papers in the present thesis. **Paper I** is based on the clinical characteristics of the oral cavity cancer patients in the NOROC study. **Paper II** is based on tissue microarrays assessment of high-risk HPV in oral tongue cancer. In **Paper III** and **Paper IV**, we focus on the different histopathological patterns in oral tongue squamous cell carcinomas and their association with survival outcomes.

4.1 Paper I

Characteristics and prognosis of primary treatment-naïve oral cavity squamous cell carcinoma in Norway, a descriptive retrospective study. Bjerkli IH, Jetlund O, Karevold G, Karlsdóttir Á, Jaatun E, Uhlin-Hansen L, Rikardsen OG, Hadler-Olsen E, Steigen SE. PLoS One. 2020 Jan 16;15(1): e0227738. doi: 10.1371/journal.pone.0227738. eCollection 2020.

In **Paper I**, we outline a large and retrospective study of primary OCSCC from all four university hospitals in Norway, the NOROC study. We describe the incidence of the cancer at the different anatomical sites of the oral cavity, together with descriptive data such as gender, age, smoking, alcohol consumption, treatment of choice, follow-up, and five-year survival outcomes. The patients in the NOROC study form the basis of the other papers in this thesis. All patients were diagnosed in the years 2005–2009, and validated against comparable cohorts.

- We identified 535 patients with primary treatment-naïve squamous cell carcinomas from the years 2005–2009.
- The male: female ratio was 1.2. Median age at diagnosis was 67 years, the range from 24 to 101 years. Men were eight years younger at diagnosis compared to women, median of 64 years and 72 years, respectively.
- Forty-five percent of the cases were oral tongue cancer.
- Age at time of diagnosis, tumor-status and node-status significantly influenced survival, but there was no gender difference in survival.
- The five-year disease-specific survival for the whole cohort was 52%. Patients treated with curative intent had a 62% five-year DSS.

4.2 Paper II

High-risk human papilloma virus was not detected in a Norwegian cohort of oral squamous cell carcinoma of the mobile tongue. Søland TM, Bjerkli IH, Georgsen JB, Schreurs O, Jebsen P, Laurvik H, Sapkota D. Manuscript submitted for publication.

In **Paper II**, we wanted to describe to what extent high-risk HPV was present in OTSCC. Tissue microarrays of tumor tissue from 146 Norwegian OTSCC patients were assessed for presence of high-risk HPV. We compared different and independent approaches for HPV detection. We used DNA and RNA in situ hybridization assays and immunohistochemistry to evaluate the expression of HPV surrogate marker p16. We hypothesized that high-risk HPV infection is uncommon in OTSCC.

- We did not identify any transcriptional active HPV in these cases.
- None of the tumors were positive for either high-risk HPV DNA or for *E6/E7* mRNA by *in situ* hybridization.
- Only two cases showed strong and uniform p16 in more than 70% of the cells (both cytoplasmic and nuclei staining).
- The main conclusion was that high-risk HPV is an unlikely causative factor in OTSCC.

4.3 Paper III

Tumor budding score predicts lymph node status in oral tongue squamous cell carcinoma and should be included in the pathology report. Bjerkli IH, Laurvik H, Nginamau ES, Søland TM, Costea D, Hov H, Uhlin-Hansen L, Hadler-Olsen E, Steigen SE. Manuscript submitted for publication.

In **Paper III**, 150 cases of H&E-stained slide sections from OTSCC treated in curative intent were described. Survival outcome was compared with clinical and histopathological variables to evaluate their prognostic significance. We elucidated the prognostic value of the TNM 8th edition pT classification, including DOI, as well as lymph node status, TB, and a combined TB and DOI score, and whether these could supplement treatment decision.

- Tumors shifted to a higher T-status when classified according to the TNM 8th edition pT compared to the older pT. This indicates that DOI significantly complements T classification for prognostication.
- The age at diagnosis, TNM 8th edition pT classification, N-status, TB, DOI in a 3-tier category, and the combined TB and DOI scores, all significantly influenced prognosis in univariate analyses.
- A high TB score was associated with lymph node metastasis. For tumors with low TB score 22.5% had lymph node metastases, whereas for tumors with high TB score, 42.8% had lymph node metastases.
- The 8th edition T-status and TB in a 3-tier category were independent variables of five-year DSS in multivariate analyses.
- Tumor budding was associated with lymph node metastases, and can be used as a supplement to TNM classification in treatment decision for low-stage tumors.

4.4 Paper IV

A combined histo-score based on tumor differentiation and lymphocytic infiltrate is a robust prognostic marker for mobile tongue cancer. Bjerkli IH, Hadler-Olsen E, Nginamau ES, Laurvik H, Søland TM, Costea D, Uhlin-Hansen L, Steigen SE. Manuscript submitted for publication.

In **Paper IV**, we determined whether tumor histopathological differentiation and other histopathologic high-risk assessments, when made less complex through fewer options, can help in predicting survival outcome for oral tongue cancer and thereby supplement treatment decisions.

- One-hundred and fifty H&E-stained sections of OTSCC treated in curative intent were available for re-investigation; 77 of these were low-stage tumors (T1-2, N0M0).
- Five-year disease-specific survival for the whole cohort was 65%, and for the low-stage tumors, five-year DSS was 83%.
- For the whole cohort, lymph node status and risk-patterns including differentiation of the whole tumor, perineural infiltration, and lymphocytic infiltrate, were found to be survival predictors.

- The WHO whole tumor differentiation correlated with survival outcome following the traditional grading, but also when made less complex, both for the whole cohort and for low-stage diseases.
- Lymphocytic infiltration can be scored with fewer options, to increase reproducibility (inter-rater and intra-rater agreeability), without losing prognostic value.
- A histo-score combining WHO differentiation and lymphocytic infiltration identified a group of low-stage tumors with a five-year DSS worse than the high-stage tumors, and this group of patients should be given special attention concerning treatment planning and follow-up.

5 Discussion

The general objective of this thesis has been to gain knowledge about cancer of the oral cavity from a cohort of strict OCSCC where the clinical and histopathological registration has been done in a structured manner. We present a large and well-controlled group of cases. As there may be differences in risk factors and clinical course of OCSCC from different oral sub-sites, we performed separate and more extensive analyses on the OTSCC, which comprised almost half of the cohort. Our retrospective study has not interfered with any choice of treatment or survival outcomes, insofar as the patients included had had their treatment 10-15 years ago.

This section will first address the ethical considerations of this research, emphasizing issues regarding the patient cohort. Then methodological and statistical considerations specific to this thesis will be addressed. The findings from the four papers will then be comprehensively discussed and related to other findings from the literature.

5.1 Ethical considerations

The Declaration of Helsinki was developed by the World Medical Association (WMA) in 1964, and has been updated several times (94). The declaration is a statement of ethical principles for medical research when it involves human subjects, and also covers human material and research data. The principle of informed consent is essential.

Originally this research was planned as a multicenter-study with two Norwegian university hospitals and one Finnish university hospital, but inasmuch as we found it difficult to collect clinical data in an unrelated foreign language, we shifted to a multicenter-study comprising the four university hospitals in Norway, naming it the NOROC study.

For the NOROC study, a passive consent procedure was utilized. The NOROC study and approach for consent was approved by the IRB of the REK Nord (Appendix I and Appendix II). Approval from one of the four regional IRBs gives validated approval for the other national health regions. Patients alive at the retrospective inclusion date received a written informal consent letter informing them about the study (Appendix III). The patients were given the opportunity to decline participation (opt-out). Deceased patients and those who did

not opt-out were included in the analyses, except for three patients with written statements in the EHR about not participating in research; these were excluded at the outset.

This patient group is a vulnerable patient group having high mortality risk, with location of cancer in a part of the body that is important to vital functions and social life. Confidentiality of patient information is mandatory. Individual data such as information about burden of disease may have implications, for example, for the right to insurance; thus confidentiality is essential (95).

The study was planned retrospectively and approved in 2013/2014, five to ten years after the patients had had their cancer diagnosed and treated. The patients still alive were informed that they could withdraw from the NOROC study without concern. The letter was sent to patients when inclusion started between August 2015 and February 2017. We found the latest addresses given in the EHR; addresses are updated once or twice a year. No letters were returned from either patients or postal services. Three patients contacted the principal investigator by telephone to confirm they were agreeing to participate. One of them said, “do whatever you want, because this is very important research for our group of patients”, and another said, “you can do what you want at any time, and there is no need to inform me more about further research on my case”. My own experience with patients suggests that the majority of patients are very positive to research. None of the living patients contacted us to opt-out. As this was a retrospective study, many years after primary treatment, this study did not interfere with their treatment or outcome in any way. All data are kept anonymous, and the study register will be terminated when the time-period for the IRB approval closes, the study was given an extension in 2019 (Appendix IV).

If we had been obliged to have the patients to opt-in to the project, we could have been facing many patients not responding for several reasons, given their advanced age and perhaps not able to focus on an illness they survived many years ago. We would then only have had the clinical data and histopathological data of those not alive, and the analyses of five-year OS and DSS would not have been valid for all the patients with the cancer. With the consent approach we were given, we could gain insight into the patients who have survived, with both clinically and histopathological data, not only from those deceased. With our procedure, clinical data of all patients were available regardless of outcome or available tumor samples.

5.2 Methodological considerations

The study was conducted in collaboration with the four university hospitals in Norway, and with some of their most experienced clinicians and pathologists diagnosing and treating oral cavity cancer patients in Norway. All clinical and histopathological information was collected into a web-based CRF.

5.2.1 Web-based case report form

We considered other web-based collection systems, but for the security of data, we chose a national administration in Trondheim; AKF, with the servers situated in Norway and with a two-step authentication for the recorders (82).

The study register is a method of structuring the recording of patient data. The CRF was prepared to organize the clinical and histopathological data used for the NOROC study and the papers in this thesis. A basic CRF was prepared from knowledge at that time (2014), with a prospective model as guide, as there were few retrospective models to be found (27, 96). Previous studies from our group in Tromsø had collected clinical data such as gender, age, smoking, drinking, TNM stage, treatment, and survival outcome (34). We now wanted to collect more detailed data if these were available, but still anonymized, especially about treatment, follow-up, and histopathological variables in a broader manner.

We discussed and then designed a CRF with five main sections in addition to the first page with anonymized identification. Those five main sections were: basic patient data, ICD-10 diagnosis, histopathological information, treatment, and follow up (Appendix V). To some extent the sections were detailed, but given that members in the research group had different medical backgrounds, we focused on several objectives. We wanted to explore whether it was possible to record these data retrospectively. The recording was documented in the de-identified CRF. The information from the CRF was then uploaded as a SPSS file for statistical analyses. No birthdate or identification number is available in the CRF or in the SPSS file; a key list was held by one of the co-investigators.

The CRF was found to be functional for annotation and sufficiently nuanced by the clinicians and pathologists recording into it. We did not do a pilot, but from AKFs all-around experience, they had created a test session page that one should try out before adding patients in to the main CRF. The researcher who carried out the majority of data-collection into the CRF, as well as the other clinicians, had extensive experience in the diagnosing, surgical treatment, and follow-up of this patient group. Histopathological assessment was done by experienced clinical pathologists at each hospital.

When several professionals are collaborating, a CRF will naturally have to include questions that not all are equally familiar with. It is important to have standardized methods for collecting data, and to have systematic ways to evaluate the results as well. Among clinicians and pathologists, some of the questions and answers could be misunderstood from the original context, and one could also miss out on some questions due to fatigue during the collection process, but we did not find this a significant problem.

It was not possible to find and record all details into the CRF from the EHR. The EHR records are tools for diagnosis and treatment, and not designed for research (83, 97, 98). To use EHR directly as a study-register for research is difficult because of the variable quality of the details, since the documentation accumulates as a running record (83, 97, 98). That is why a CRF can be constructed in a manner that requires us to carefully read through the EHR and assess data in a systematic manner. Presently, compared to 10-15 years ago, assessments in the EHRs are more standardized; for example, patient symptom-burden is recorded with Edmonton Symptom Assessment System-revised version (ESAS-r) and patient performance status as Eastern Cooperative Oncology Group (ECOG) (99-101). As NOROC was a retrospective study, many of the details we wanted to assess were not fully available in the EHR, underlining how difficult it can be to find the information needed for a retrospective study. A prospective study could ask specific questions and use standardized questionnaires, and patients would answer directly; however, patients may not answer honestly, or not respond at all, particularly concerning smoking and drinking habits (102).

The data recording was done by reading the EHR and re-classifying the available histopathological samples, not by searching codes for diagnosis, histopathology, or procedures. Our CRF decreased the number of misclassifications, and enabled us to describe which treatment had been given and not just what was planned for. It is important to have a CRF that is built to assess crucial clinical data.

5.2.2 The tissue microarrays and H&E-slide section assessment

Histopathological evaluation of tumors into many variables is time-consuming, and in our work, we limited the workload to this point by including squamous cell carcinomas of the oral tongue. We had hoped to be able to evaluate histopathological samples from all registered OTSCC patients, but some lacked material in the archives. This resulted in a lower number of samples available for TMA and the other histopathological re-evaluations. In addition, some of the samples were excluded because of reduced quality of the collected sample-slides, or fragmented or shallow resections, or biopsies inappropriate for further assessments.

TMAs were used to explore high-risk HPV in the OTSCC. TMA has the advantage of examining many samples at the same time (less time-consuming), and for a lower cost. A TMA disadvantage is that only a small part of the tumor is examined, and that might not be representative of the whole tumor. The question is also whether one should choose material from the middle of the tumor or at the tumor front. The number of harvested cores needs to ensure representability for the whole tumor. Two or three cores have been said to be representative, and TMA is well accepted as a method (85, 86).

With full section H&E-stained slides, the pathologists normally look at one or a few slides that are harvested to be most representative for the tumor, and both the central and the peripheral parts can be evaluated, so that arguments about TMA being limited to small samples are less of a concern.

We wanted to explore the histopathological DOI and TB on the available H&E-stained tissue sections available on the OTSCC in our cohort (22, 24, 45). The pathologists also re-evaluated the parameters suggested in the risk-model: WHO differentiation grade, WPOI, degree of keratinization, nuclear polymorphism, perineural invasion, lymphocytic infiltration, and vascular infiltration (50-53, 88). This has been verified in other studies, but differentiating between high-risk and low-risk tumors is challenging, with poor reproducibility among pathologists (44, 53). The pathologist evaluates one slide at a time, and this is obviously time-consuming. A less complex categorization might be less time-consuming, and also seems to be of greater value with respect to better inter-rater and intra-rater agreeability (56).

Many pathologists welcome analyzing software that could diminish subjective results in the future. Digital pathology in the future, represented by new bio-imaging analysis platforms such as QuPath, might prove to analyze histopathological slides with a high grade of reproducibility. Such a system is time-consuming for importing and standardizing the data-input, but the system will then score the tumors in the same manner (103).

5.3 Statistical considerations

With 535 primary treatment-naïve patients in our cohort, the study was sufficient according to a-priori power calculation. But because there are so many different variants of tumor stage, etiological factors, and treatment, sub-grouping is sometimes important to control for known risk-factors, and such sub-groups could become small and thus be potential sources of bias. Statistical tests will always be debated and can be misinterpreted (104). We decreased the risk of sparse-data bias by excluding variables with fewer than five cases (92). The Chi-square test was used to test relationships between variables (90). The Spearman bivariate correlation (2-tailed), including bootstrapping at 95% CI was used to identify correlation between parameters. By bootstrapping we also decreased the bias of small sample size. When the cohort contains few individuals of a variable, and correlation is calculated, bootstrapping decreases the bias of small samples by calculating imagined samples from the cohort several times (105-107). In 2-tailed correlation (bivariate), cases with missing values are excluded pairwise. We used Spearman correlation because it does not require normal distributed data, and we can use Spearman correlation to analyze nonlinear relationship; thus we do not have to worry about extreme outliers in the cohort (91).

Log-Rank univariate survival analyses were used to give Kaplan-Meier curves for estimates of survival. It is easy to calculate, and gives a non-parametric estimate of the survival function. One can only calculate one variable, commonly used to compare two (or a few more) study populations within the same variable. It is easily applicable to small, medium, and large samples. When a patient dies within (in our calculations) five years, it is censored at the time of death. One limitation is that one should have >50% uncensored observations. The program gives the median survival time, and does not control for covariates; for that we then have to do multivariate analyses (93).

Multivariate analyses are means to control for confounding factors in univariate analyses. Only variables significant on univariate analyses were included into the multivariate tests. We tested independent variables for collinearity with linear regression when we suspected they could be correlated, and thus excluded them from the multivariate calculation (90). Cox regression analyses for survival allowed us to describe significance, HR, and 95% CI after bootstrapping for multivariate variables (90).

5.4 Discussions of main findings

The thesis is based on a newly established, large Norwegian cohort of patients with OCSCC. The NOROC cohort represents the majority of patients treated for OCSCC in all four health regions in the country during 2005-2009. To our knowledge, there are no previous studies aiming to include all patients treated with OCSCC in Norway, with a five-year follow-up. In **Paper I,** we describe the clinicopathological variables of the cohort and survival outcome. We explored the available OTSCC for high-risk HPV in **Paper II**, and re-investigated them histopathologically in **Paper III** and **Paper IV**.

The characterization of this cohort is important for clinicians in Norway. It is crucial to know the characteristics of our patients to be able to compare them with other cohorts. It is also of great impact for further studies on prognostic biomarkers to be able to report in a rigorous fashion, with sufficient information according to the REMARK guidelines (81).

5.4.1 Epidemiology

According to the CRN, the number of patients classified according to ICD-10 with label C02-C06 in the years of 2005–2009 was 788, or 158 patients annually. We found 643 unique patients, and of these 535 were primary treatment-naïve oral cavity cancers (annually, 107 patients). In 2018 (the last reports from CRN), the average annual number of new cases in oral cavity cancer (C02-C06) was 220 (male 123, female 97), so the annual incidence is increasing (7).

Four hundred unique patients were identified by searching for the relevant ICD-10 codes in the EHRs at Rikshospitalet. Patients treated at Haukeland, St. Olavs, and UNN were first identified by searching the hospitals' pathology archives for cancers with topographic SNOMED coding T51 and T53, which were subsequently matched with the relevant ICD-10 codes recorded in the EHR. Three hundred and eighty unique patients were found in the latter three hospitals, but screening of the files showed that 115 patients (30.3%) had been incorrectly coded and did not have oral cancer. The majority (91 patients) of these had oropharyngeal cancer, accounting for 24% of the 380 identified patients.

The total number of patients we identified with primary OCSCC was 535. If we calculate that 25-30 % of the patients recorded in the CRN during these years could be misclassified, the numbers of patients would be reduced from 788 to about 575, and we found 535 primary treatment-naïve patients. Some patients may have had histopathological diagnoses other than squamous cell carcinoma, and some patients with small tumors might have been treated at local hospitals. Also, patients with cancer at an advanced stage treated without curative intent might not have been transferred to the university hospital but treated palliatively at a local hospital. Given this background, we think we have documented a representative group of patients.

The 535 primary OCSCC found in this cohort included 322 patients (60.2%) from Rikshospitalet, 90 patients (16.8%) from Haukeland, 67 patients (12.5%) from St. Olavs, and 56 patients (10.5%) from UNN, respectively, as previously shown in Figure 4. This correlated well with the division between Health Regions of Norway: Rikshospitalet in Oslo should have had 58%, but actually had 60% of the patients. This correlates with the demographic pattern in Norway (2007 and today), and considering that Rikshospitalet in Oslo is a tertiary referral hospital (108).

For cancers of the oral cavity, the male: female ratio has been reported to be 1.4–1.8 (7, 20, 109), but the ratio tends towards no gender difference with a recent report from Finland having the male: female ratio of 0.9 (2). We found a ratio of 1.2. in **Paper I**. The numbers we had from the CRN were 433 men and 355 women, also giving a male: female ratio of 1.2.

In our cohort we found a median age 67 years. The ages reported from CRN corresponded to that median age (7). The median age from a Danish cohort reported retrospectively in 2017 was 63 years (20). From a Finnish study the median age of OTSCC was 66 years (2). In a

recent Spanish and Swedish report for HN cancers, the mean age at diagnosis was 64 and 67 years, respectively (40, 110). Our Norwegian cohort is therefore in line with other cohorts in Europe. We found the younger age-groups to present better five-year DSS, than older age groups (>70 years of age) (p=0.001). We did not have many young patients; only 13 patients (2.4%) were under 40 years. Other reports have found a larger proportion of younger patients with oral cancer —in Finland as high as 8-10%; a U.S. study reporting median age for OCSCC of 35 years (2, 111); and worldwide, increasing trends of younger patients with oral tongue cancers have been reported (6). This suggests that there might be different etiology in different countries as found in a U.S. report (109).

The median survival follow-up time after primary treatment (major surgery or definitive radiation therapy with or without chemotherapy) in our cohort was 48 months; 53% of the patients deceased within 5 years. Median follow-up at 48 months confirms the high rate of death during the first five years. Cause-specific survival/DSS is defined as the percentage of patients in a study or treatment group who have not died from a specific disease in a defined period of time (89). We used the phrase DSS in our studies and not disease-free survival (DFS). For both DSS and DFS, the period of time usually begins at the time of diagnosis or at the start of treatment and ends at the time of death. DFS was not considered, as this is defined as the length of time after a new, primary treatment of cancer and the period the patient survives without signs or symptoms of that particular cancer (or disease). Measuring DFS is largely used to evaluate how a new treatment works in a clinical trial and should be restricted to that use (89). We found the OS to be 47% and the DSS to be 52% in **Paper I**. Relative survival for HPV-unrelated oral tongue cancer was reported to be 57% in Germany, and in the same report the U.S. had 64% relative survival (112). For OCC (not including tongue and lingual tonsils), the Northern Europe five-year age-standardized relative survival was estimated to be 50% (12). We believe the five-year OS and DSS rates we found are reliable.

5.4.2 Anatomy and classification of tumors

When reading the EHR, we could verify what diagnoses the patients had, comparing the given diagnosis to the surgical documentation, and in some we corrected the diagnosis in the CRF accordingly. This led to exclusion of some patients, mostly oropharyngeal cancer documented

as tongue cancer, or very few maxillary cancers with breakthrough to hard palate documented improperly as hard palate cancer (a neighboring anatomical site).

In the NOROC study we included patients diagnosed with OCSCC between 1 January 2005–31 December 2009. Therefore, patients had originally been classified according to an older TNM edition (23). We based our anatomic classification in the CRF on the latest TNM edition, in which the description of anatomical location is improved (22). The relevant ICD-10 codes were C02-C06, referring to cancers of the oral cavity. We excluded lip cancers, and ICD-10 codes C05.1 and C05.2, which are regarded as oropharyngeal sites. C02.4 for tongue-tonsils was seldom used, but it also reflects an oropharyngeal anatomical site, and should be exclusively used for oropharyngeal cancers. In this way we present a homogenous cohort regarding anatomical sites. The numbers we obtained from the CRN indicated C02 to be 40.6%, C03–25.6%, C04–19%, C05–2.7%, and C06 to be 12.1%. In the NOROC cohort we found quite similar numbers: C02–44.9%, C03–20%, C04–19%, C05–1.3%, and C06 to be 14.8%. Hence, our cohort appears consistent, corroborating a previous cohort in Northern Norway (34), and oral cavity cancers in the U.S. (113). Today the anatomical regions are better clarified by the WHO and in TNM 8th edition, and we recommend describing cohorts according to these resources (21, 22). It is important to verify the diagnoses by reading the clinical presentation recorded in the EHR, and not only base the search for patients for inclusion in a cohort according to recorded ICD-10 diagnosis or a SNOMED-code. In this way, we have decreased the number of misclassifications.

Histopathological cancer types other than SCC were excluded. In **Papers I**, **III**, and **IV** we excluded patients with second primaries or previous cancer treatment to focus on the primary treatment-naïve SCC, and to decrease the bias of previous treatment interfering with development of new cancer.

The patient's TNM score reported to the CRN was found in the EHR, and the staging was done in the CRF according to which TNM was recorded in the EHR. Some lacked T, N, or M, and were registered in the EHR with TX, NX, and/or MX. These were few, only 22 of 535 patients ended up with unknown staging. The same trend of some unknown stages is described in Cancer in Norway 2018 (7).

In our cohort for **Paper I,** T1 constituted 20.6%, T2–32.2%, and T4 as much as 33.3%. N0-status made up 61.3% of the patients. A total of 42.1% of the tumors were stage I and stage II,

which is in line with data from a Swedish HN cohort where stage I and stage II tumors accounted for 43.1% (40). In a German/U.S. report of HPV-unrelated oral tongue cancer, 44% of the Germany patients versus 62% for the U.S. patients were stage I and II tumors (112). This might reflect an earlier awareness of oral cancer in the U.S., but our cohort seems in line with other European cohorts.

In **Paper III** and **Paper IV,** the re-evaluation of T-status including DOI increased the T-status of many of the tumors. Those who remained T1, improved survival outcome compared to the former T1, likewise for T2. We confirmed an upregulation of T with the implementation of DOI from previous TNM editions, as others have described (24, 114).

The N-status correlated with survival outcome. N0 disease is associated with better survival outcome than N+. In **Paper I**, we showed that patients with N0 disease had a five-year OS of 59% and five-year DSS of 65%; these were the five-year survival outcomes for all documented patients regardless of the T-status or treatment. In **Paper III** and **Paper IV**, patients with N0 disease had a five-year DSS of 82% and N+ five-year DSS of 38% ($p<0.001$). These findings of better survival with N0-status, is in line with other work on this matter (115-117).

Tumor-status together with node-status (stage of disease) influenced survival. There was no gender difference in tumor or node-status. Patients with low-stage disease (stage I and stage II; T1-T2, N0M0) at time of diagnosis had better survival outcome, as shown in **Papers I**, **III**, and **IV**. In **Paper I**, we found five-year DSS for T1–73%, T2–62%, N0–65%, Stage I–80.2%, and Stage II–67.7% (all $p\leq0.001$). In **Paper III** and **Paper IV**, patients treated in curative intent were investigated. Here we found five-year DSS to be 88% for pT1, 64% for pT2 and pT3 ($p=0.006$), and five-year DSS for pN0 to be 82% ($p<0.001$). In **Paper IV**, low-stage disease had a five-year DSS of 83% and high-stage disease a five-year DSS of 45% respectively ($p<0.001$). This is in line with other studies showing low-stage tumors to have better survival outcome than higher stages (2, 118).

5.4.3 Etiology

When presenting etiological factors for OCC, the following are often discussed: smoking, alcohol, the synergy of these last two, level of education, socioeconomic status, and high-risk HPV. A disadvantage of collecting data retrospectively is that clinicians tend to document such data differently. In part of the basic patient-data, we discovered that it was difficult to specify etiological factors such as level of education, family history of cancer, smoking, and drinking habits, as these are not consistently documented in the patient journal.

In as many as 35% of the EHRs, the documentation of smoking and drinking was lacking; our results in **Paper I** have to be considered with this in mind. In another retrospective study from our group, and also in other retrospective and prospective studies, smoking and alcohol data can be missing for up to 30% of the participants (34, 102, 111). We cannot specify smoking and drinking habits in detail, nor smoking in pack-years, as this was not a standard of documenting amount of smoking during 2005-2009.

For dental status we found 20% to have good dental status, 50% in need of treatment, 21% edentulous, and 9% without unknown dental status, in **Paper I**. Older people were more often edentulous. Patients with oral tongue cancer had better dental status than patients with other oral cavity cancer sites. For survival, five-year DSS was 69% for those with good dental status, whereas those in need of dental treatment had 55% five-year DSS, whereas those edentulous had five-year DSS of 29% ($p<0.001$). Good oral hygiene may reduce the risk of HN cancer (13, 31, 119), and oral hygiene may as well reflect socioeconomic status.

We wanted to explore and document the presence of high-risk HPV in our cohort of OTSCC in **Paper II**. One of the research-questions based on the OTSCCs assumed that high-risk HPV has no role in OTSCCs. There were several reasons to explore whether these SCC contained high- risk HPV. First of all, to explore the presence or absence of high-risk HPV in oral tongue cancer. Secondly, to know if HPV could impact further sample staining, infiltration, or other growth patterns. And finally, this was also to indicate whether or not we had homogeneous material, not biasing future research.

There are many available screening test for detecting high-risk HPV (36). We used the most recommended detection and confirmation analyses for high-risk HPV (35). p16 is a surrogate-marker for HPV. p16 protein can be upregulated by HPV, but it can also be present because

of other sources by contamination (120). With HPV DNA ISH, we cannot be sure if this is in the patient samples, or if it might be present by contamination, and we cannot determine whether this is virus replication. Also, with HPV RNA ISH (mRNA), we are exploring whether we have an active transcription process; there are few other studies so far that have done this in their samples (35).

In **Paper II**, we present both second primaries and primary oral tongue squamous cell carcinomas, to find whether there was any difference in high-risk HPV presentation between primary cancer and recurrent or second primary disease. There was none. We found no positive samples for high-risk HPV, and so have concluded that our material is not biased by high-risk HPV, and high-risk HPV will therefore not bias further staining and assessment of infiltration or other growth patterns in future work on the NOROC cohort. Our results are supporting a study from 2017 from Jansen and coworkers, that found no HPV-related oral tongue cancers in Germany or in the U.S. (112). For biomarkers, many clinicians ask for HPV-status, not only for oropharyngeal cancers, but also for oral tongue cancers; to our knowledge this is not necessary as a standard.

Within the five-year follow-up time, 18% of the patients had recurrence within three years. Second primaries, a new OCC more than three years after first presence of OCC, was the case for 10.5% of the patients. There are various definitions of second primaries, but second neoplasms after an HN index tumor is decreasing ultimate survival (121).

We wanted to explore whether we could document the patients' body mass index (BMI), but in many patient charts we might have been able to document either weight or height, but not both. This can also be lacking in prospective studies (102). Another drawback in the CRF was the lack of documentation of physical performance status (often reported according to Karnofsky performance status scale, ECOG-scale, or WHO scale) of the patients (122). The patient's physical performance could absolutely influence treatment decision and survival outcome. Patients with low performance status are more likely to have low survival outcome (40). By reading the EHR, we recognized that it was not a tradition in 2005–2009 to specify the patient's physical performance status; it was neither documented nor vaguely described, and it would have been difficult to register in this retrospective study. In a new prospective study-CRF, we would implement this.

5.4.4 Histopathological growth pattern findings

As part of the NOROC project, dedicated and experienced pathologists have now reassessed available histopathological oral tongue samples; other sites will be pursued later. The reassessment has reclassified one of the oral tongue cancer patients from SCC to verrucous carcinoma, thereby excluding this patient from statistical analyses. By reading the EHR, we found that the patient had had the appropriate treatment given the histopathological result, but due to this, this case was first included into the CRF on the basis of the ICD-10 diagnosis in **Paper I**, written before the histopathological re-evaluation. However, by histopathological re-evaluation, we then excluded this case from the other statistical analyses.

As part of this project we also wanted to explore whether histopathological patterns/morphological characteristics in OTSCC could be used to predict tumor aggressiveness. Hypothetically such characteristics may allow more personalized treatment, especially for low-stage tumors. In **Paper III** and **Paper IV**, histopathological growth pattern and survival outcome were explored.

Tumor thickness and DOI are used by many pathologists without further description (123). Tumor thickness can be reported as actual tumor thickness (TA) or reconstructed tumor thickness (TR), ad modum Woolgar from 1995 (Appendix V). The NOROC pathologists used TR to describe DOI in **Paper III**.

When integrating DOI into the T classification in the re-evaluation from an older TNM version to our newer TNM classification, we found that DOI provided complementary prognostic value for the classification. Tumors generally shifted to a higher T-status as described in **Paper III**. This is in line with other reports documenting that DOI generally upregulates the T-size (10, 24, 124). As a consequence of this, the five-year OS and DSS for those staying low-stage tumors improves (10, 124).

Tumor budding has been recommended to be an additional prognostic factor to the TNM classification in grading of colorectal cancer, and also to supplement the T classification of OTSCC (45, 46). A recent work by Shimizu and colleagues found TB to be a prognostic marker for low-stage OCSCC, together with mode of invasion (125). We found TB to be significant in univariate analyses. High budding-score was associated with a higher degree of lymph node metastases. In this way, TB predicts aggressiveness of the tumor, and can be of

important relevance, together with the TNM classification, as a supplement in treatment decision. A high TB score has been shown to correlate with lymph node (N+) involvement and recurrence of disease in other studies (126, 127). Our findings could also support a recommendation that low-stage tumors with high TB score could have neck dissection.

Other research questions were to investigate whether histopathological risk-patterns of the tumors can predict the aggressiveness of OTSCC (50-52), and we addressed this in our **Paper IV**. For the whole cohort, lymph node status and risk-patterns such as differentiation of the whole tumor, perineural infiltration, and lymphocytic infiltrate, were found to associate with survival. A recent work from Sinha and coworkers in 2018 also found WPOI, lymphocytic host response, and perineural invasion, to be histologic factors that could indicate a more aggressive treatment for some low-stage OCSCC (128). Wagner and colleagues found that Bryne's tumor histologic differentiation grading system was a useful method to predict survival outcome, where the well differentiated (grade I) had the highest survival outcome (129). Lymphocytic infiltrate, also called lymphocytic host response in the risk model, is histopathologically quantified as the density of lymphocytes at the tumor front (53, 130). High density of lymphocytic infiltration, has for a long time been considered as a positive prognostic sign (52, 53, 131). Also, we found that the WHO differentiation grading of whole tumor together with lymphocytic infiltration in a combined histo-score, was significant predictor for survival. In this work, we found that this combined histo-score of differentiation and lymphocytic infiltration as a combined variable can be used as a supplement into the TNM classification when assessing tumor aggressiveness. We could use this combined score especially when deciding whether a low-stage tumor should have additional neck dissection and/or postoperative RT. We recommend further clinical trials and biomarker research to explore this.

5.4.5 Treatment

Since 1 May 2015, we have had fast-track on diagnosing and startup of treatment of HN cancer patients in Norway. The goal is that a minimum of 80% of the patients should be diagnosed and commence treatment within 28 days (132). In the NOROC study we could not always determine the point when the patient was referred to the hospital until given treatment, because the referral letter was not always documented and filed in the EHR. We tried to

calculate time from first mention of referral or annotation in the EHR until start of treatment, and 52% of the patients had started treatment within 4 weeks, additionally 32% had started treatment within 8 weeks, 3% started the treatment 9 weeks or more after referral, and for 13 % we could not specify when they had been referred. Today, all corresponding letters from general practitioners, dentists, or other specialists are documented in the EHR the same day as they are received electronically; this was not the case in 2005–2009.

Most of the patients' data had been documented in MDT meetings (58). Today this is routine. It was a positive finding that this was also the standard in 2005–2009 as shown in **Paper I**.

Treatment procedure was not registered based on procedure codes in our CRF, but by reading through the EHRs as to what had actually been the treatment. For treatment assessment, planned treatment and actual treatment were not always aligned. Patients could deteriorate during treatment. Postoperative RT ended up as definitive RT without post RT surgery. It was necessary to read the surgical report to assess what surgical treatment had been given, not just trusting the codes and headlines, especially when defining what type of dissection of the neck the patients had. We tried to assess RT given, but to some extent it was difficult to find this, because at some hospitals the RT given is recorded in another file system, or the patients were given the RT at an oncological hospital, not the same one where they had the diagnosis or surgery treatment. We also discovered that split-course RT was missed when uploaded as a SPSS file for statistical analyses, because the answer option in the CRF was set as a single number without allowance for using combinations.

There were no great differences in treatment between the four hospitals. At one hospital, there was a slight tendency to give more preoperative RT to the patients, but the difference was not statistically significant. The cohort is uniform when we consider survival outcome from different hospitals. For the patients given preoperative RT, we only had the diagnostic biopsy. We did not have or include eventual resections if they had preoperative RT, to avoid influencing the histopathological assessment with bias from the actual given RT.

Chemotherapy was given in few cases, and in combination with RT with or without surgery, altogether only 44 patients (8%). This is quite in line with recent Swedish study that reported 12% given chemoradiotherapy (but for all HN cancer types) (40).

In our cohort in **Paper I,** we documented that 11% of the patients were given palliative treatment; this is in line with that recent Swedish study, though it includes all sites of HN

cancers, reporting 9% for palliative treating (40). This is also in line with the knowledge that most patients with this type of cancer usually present with local or regional cancer, seldom with metastasized cancer (112).

Concerning the follow-up time, we wanted to assess whether patients had many complications after treatment, but these were often not specified in the EHR. To some extent clinicians had asked the patients about complications and documented them, but this was not the standard.

It is important to diagnose and treat OCC at an early stage, and screening of premalignant lesions might prevent development of oral cancer. Public awareness is important, and each year there is one month focusing on oral cancer in the UK (Mouth Cancer Action Month, November 2020) (133). In the U.S., the 8-Step Oral Cancer Screening has been introduced to reduce people's risk of oral cancer, and April is Oral Cancer Awareness Month (134). In Norway, 16 September 2020 is «Munn-og halskreftdagen 2020» (postponed to November because of the coronavirus-lockdown situation), and this day will be organized for the fifth time (135).

6 Conclusion

With this thesis and cohort of patients, we have contributed to increased knowledge about one type of cancer for different specialty fields of medicine, especially the clinical and histopathological fields. We have described a large, Norwegian cohort of OCSCC where the inclusion criteria have been strict enough to exclude tumors at the base of the tongue, an oropharyngeal localization, and other neighboring sites. We have clarified the role of HPV in OTSCC, and have presented histopathological growth patterns that might supplement the TNM classification in choice of treatment for OTSCC, especially for the low-stage tumors.

In **Paper I**, we presented a national cohort of primary treatment-naïve OCSCC comparable to other cohorts. This can be used as a standard cohort for future research on biomarkers/proteomics following the REMARK guidelines. We found that younger age-groups (< 70 years of age) and low-stage tumors had better five-year survival. We stated in **Paper II** that we had a homogenous cohort with no detection of high-risk HPV in OTSCC. The implementation of DOI to the T-status according to the newest TNM classification shifted many of the tumors to a higher T-status as shown in **Paper III**. N0-status gave higher survival outcome compared to N+ status, and tumor budding can be used as a supplement to guide in treatment planning for low-stage tumors. Some of the high-risk histopathologic patterns as described in **Paper IV**, also when made less complex with fewer options, can indicate degree of aggressiveness and therefore guide in treatment planning. In our work a combined histo-score (WHO differentiation grade of whole tumor combined with lymphocytic infiltrate) identified a subgroup of patients with lower five-year DSS than high-stage disease, although they were diagnosed with low-stage disease. This histo-score could be a promising variable to be added to the TNM classification for treatment planning. From our research, especially for low-stage OTSCC, we may recommend TB, tumor differentiation and lymphocytic infiltrate (in a combined histo-score), as additional factors supplementing the TNM classification in treatment decisions and follow-up. In less aggressive tumors and a negative neck (N0), watchful waiting could still be the recommendation.

7 Future perspectives

There is no common consensus in Norway about how we collect clinical and histopathological data, except for the short reports we send to the CRN. Here we have established a study-registry with a web-CRF, with a fairly homogenous cohort of OCSCC patients. The work in conducting the CRF was necessary to develop a platform for data collection. Information could not have been taken directly from the diagnosis codes or the treatment codes; we had to validate both diagnosis and treatment. A national guideline for diagnosing, treatment, and follow-up of HN cancers in Norway was published recently in May 2020 (136). We could recommend that all patients be treated according to mutual treatment decisions, in national or Nordic clinical trials. We must seek the advantages of a common way of documenting the clinical and histopathological variables and to constitute data through time, in order to establish evidence-based knowledge. Part of the CRF has already been implemented in another project on dental health care and HN cancer.

For the future, we would present more clinical perspectives concerning choice of treatment for the patients. The calculations in the statistical file (SPSS) are necessarily time-consuming, and many details need to be rearranged within the statistical file to get variables to use in calculations for future research. In our CRF and associated SPSS documents, there is further material for continuing research from a clinical point of view. We will also proceed with further biomarker-studies, both looking into overall growth pattern and on protein and transcriptional level, with bioinformatics, proteomics, and nanotechnology of the tumors, to inspect for the presence or absence of certain markers in the tumors of those who survive or not (137-140). Our work has had as a future goal that henceforth studies on biomarkers will be reported according to the REMARK-guidelines, and that we can hope to find prognostic markers for the clinical management of patients allowing for personalized treatment (79-81).

This collaboration worked well; it is important to have a principal investigator who has a broad knowledge about different aspects of epidemiology and the disease, both clinical and histopathologic. To gain further insight into clinical appearance, treatment, and tumor samples, we could recommend collaboration in a prospective study with an updated CRF, one where the new general data protection regulation would be implemented (141). In the pipeline of the present NOROC collaboration, other papers on details in clinical appearance, evaluation of biomarkers, and proteomics are in progress. All of this will allow us to explore more the variance within different patients, treatment, and survival outcomes.

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Paper I

Characteristics and prognosis of primary treatment-naïve oral cavity squamous cell carcinoma in Norway, a descriptive retrospective study.

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RESEARCH ARTICLE

Characteristics and prognosis of primary treatment-naïve oral cavity squamous cell carcinoma in Norway, a descriptive retrospective study

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Abstract

Objectives

Incidence of oral cavity squamous cell carcinomas is rising worldwide, and population characterization is important to follow for future trends. The aim of this retrospective study was to present a large cohort of primary oral cavity squamous cell carcinoma from all four health regions of Norway, with descriptive clinicopathological characteristics and five-year survival outcomes.

Materials and methods

Patients diagnosed with primary treatment-naïve oral cavity squamous cell carcinomas at all four university hospitals in Norway between 2005–2009 were retrospectively included in this study. Clinicopathological data from the electronic health records were compared to survival data.

Results

A total of 535 patients with primary treatment-naïve oral cavity squamous cell carcinomas were identified. The median survival follow-up time was 48 months (range 0–125 months) after treatment. The median five-year overall survival was found to be 47%. Median five-year disease-specific survival was 52%, ranging from 80% for stage I to 33% for stage IV patients. For patients given treatment with curative intent, the overall survival was found to be 56% and disease-specific survival 62%. Median age at diagnosis was 67 years (range

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24–101 years), 64 years for men and 72 years for women. The male: female ratio was 1.2. No gender difference was found in neither tumor status (p = 0.180) nor node status (p = 0.266), but both factors influenced significantly on survival (p<0.001 for both).

Conclusions

We present a large cohort of primary treatment-naïve oral cavity squamous cell carcinomas in Norway. Five-year disease-specific survival was 52%, and patients eligible for curative treatment had a five-year disease-specific survival up to 62%.

Introduction

Oral cavity cancer (OCC) is the most common subtype of head and neck (HN) cancer [1], and includes cancers in the mobile tongue (anterior 2/3 of the tongue), floor of mouth, buccal and labial mucosa, upper and lower gingiva and alveolar mucosa, retromolar trigone, and hard palate [2–4]. The mobile tongue is the most common site for OCC, accounting for up to 50% of the cases [5–7].

In 2012 the global incidence of OCC was estimated to 275 000 [1], and is steadily rising worldwide. According to global cancer statistics in 2018, the estimated incidence of OCC together with lip location was found to be around 355 000 [8]. However, there is geographical variation; as many as 25% of all cancers in high-risk countries in South-East Asia are oral carcinoma [8, 9]. In Europe, the incidence is higher in Southern and Central/Eastern parts compared to Northern and Western parts [9–11]. For cancer of the tongue, there is a trend of increasing incidence in the Nordic countries as well as in the United States [5, 12–14]. The now recognized importance of HPV infection in developing oropharyngeal cancer has stressed the importance of distinguishing OCC from oropharyngeal cancers [1, 15]. HPV has been found to be uncommon in OCC [16, 17]. The incidence of oropharyngeal cancers was estimated globally to be around 93 000 cases in 2018 [8].

Squamous cell carcinoma (SCC) accounts for more than 90% of malignant neoplasms of the oral cavity [11, 15, 18], and is classified by the TNM system according to primary tumor size (T), regional lymph node spread (N), and distant metastasis (M) [3, 4].

Tobacco smoking, betel chewing, and excessive alcohol drinking are major risk factors, though the habit of betel nut chewing is a factor mainly in Asia [9, 18–20]. Poor dental health is also considered to be a risk factor [20–23]. Some patients experience recurrence or risk of second primary tumors [24–27].

Primary surgery is the preferred treatment for oral cavity squamous cell carcinoma (OCSCC) in most institutions when the tumor is regarded resectable, with or without reconstruction and neck dissection. Postoperative adjuvant radiation therapy (RT) is often necessary, whereas chemotherapy is seldom used, except sometimes for advanced stages [28–34]. The treatment should be decided by a multidisciplinary team (MDT) [35]. In lack of a national treatment protocol for HN cancer, management of OCSCC in Norway usually follows the protocol published by the Danish Head and Neck Cancer Group (DAHANCA) [34].

Five-year survival rate for OCSCC is approximately 50% for most countries [9, 11, 36]. Despite earlier detection and more treatment options, survival rate has not improved more than three to five percent over the last decades [11, 15]. The Surveillance, Epidemiology, and End Result program (SEER) database has published five-year relative survival rate of 66% for tongue, and 53% for the floor of mouth in the period 2009–2015 [37].

The epidemiological and survival data for OCC are hampered with uncertainty as many studies report results from small patient cohorts, often selected from a single or a referral hospital, or a small region. Furthermore, some studies include only patients treated with curative intention, or unfortunately merge patients with cancers of various subsites of the HN region [5–7, 12, 16].

The aim of this retrospective study was to present a large cohort of OCSCC, from all four health regions of Norway, with descriptive clinicopathological characteristics and five-year survival outcomes. All patients were diagnosed with primary treatment-naïve OCSCC in the period 2005–2009, and the results were evaluated against comparable cohorts.

Materials and methods

Data collection process

The Norwegian Oral Cancer (NOROC) study is a retrospective study that includes patients diagnosed with primary treatment-naïve OCSCC in the four university hospitals in Norway between January 1st 2005 through December 31st 2009. In Norway, management of OCC is centralized to university hospitals of Rikshospitalet (Oslo), Haukeland (Bergen), St. Olavs (Trondheim) and North Norway (Tromsø), where Rikshospitalet in Oslo (The National Hospital) also is regarded as a tertiary referral hospital. Patients were identified by searching for the relevant International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) codes in the electronic health records (EHR) of these hospitals, as well as by searching the pathology archives for cancers with topographic systematically organized computer-processable collection of medical terms providing codes (SNOMED) coding T51 and T53. The patients diagnosed during this period were classified according to TNM 5th Edition 2005 UICC [3].

We included patients with the relevant ICD-10 classification codes C02-C06 [38], which refer to cancers in the buccal and labial mucosa, upper and lower gingiva and alveolar mucosa, hard palate, mobile tongue, and floor of mouth. We excluded ICD-10 codes C05.1 and C05.2 which are regarded as oropharyngeal sites, and cancer of the external upper or lower lip (vermilion), because these almost exclusively arise in the lower lip and are more likely to act as skin cancer [15]. Tumors with different histopathology than SCC were also excluded, as well as patients with HN second primaries or previous cancer treatment. Approximately 27% of the patients had been incorrectly coded; the majority of these had oropharyngeal cancer, and were excluded.

Extracting clinical data

Anonymized clinical data were recorded in a web-based Case Report Form (CRF). The last day of follow-up was June 1st 2015 when all patients had been followed throughout a minimum of five years after end of treatment. The patient EHRs were screened from date of diagnosis until date of death, or from date of diagnosis until last day of follow-up. Recording was done by experienced clinicians (IHB, OJ, GK, EJ and ÁK). Relevant patient data, ICD-10 diagnosis, TNM classification, treatment, and follow-up were registered. Since these patients were diagnosed between 2005–2009, the stage classification was done according to AJCC 6th edition 2002 [15].

In the TNM classification system, the term TX, NX, and MX can be used in cases where the primary tumor, regional lymph nodes, or distant metastasis cannot be assessed [3, 4, 15]. For this reason, some tumors lacked T, N, or M status. However, a T4 tumor could be staged without knowledge of the N and M status, since it automatically classifies as a stage IV tumor. This

was also true with N status ≥N2, and M1. In this study we pooled stage IVA, IVB, and IVC into Stage IV.

The study was approved by the Institutional Review Board of Northern Norwegian Regional Committee for Medical Research Ethics (REK Nord) giving validated approval for all four hospitals (Protocol number REK Nord; 2013/1786 and 2015/1381). REK Nord waved the need for the patients still alive to have the opportunity to opt-out when they were informed about the project. The information-consent letter was approved by REK Nord before being sent out to the patients still alive. This study was planned retrospectively and approved in 2014, five to ten years after the patients had had their diagnosis and treatment. Patients were informed they could withdraw from the study without concern. The letter was sent to patients when the inclusion stage of research commenced between August 2015 and February 2017. The patient information-consent letter was sent to those still alive giving them the option to opt-out of the study. This was executed by the principal investigator, who received the list over patients still alive from one of the co-investigators who was the only one with access to the patient's identifying data. The address used was the latest address given in the EHR. No letters were returned from patients or postal services. Three patients contacted the principal investigator to confirm they were agreeing to participate, no one contacted to opt-out. Cause of death was acquired from Norwegian Cause of Death Registry.

Categorical grouping

Patients were divided into groups based on age at time of diagnosis; 51–60 years, 61–70 years, and 71–80 years. Those younger than 50 and older than 80 were few and thus pooled in a younger (≤50 years) and an older (≥81 years) age group. We also organized the patients according to an indicator called Integrated Risk Factor (IRF) based on extent of tobacco and alcohol consumption as previously described by Rikardsen et al. [16].

Patients with alcohol consumption recorded as seldom in the EHRs were classified as "light drinkers" (≤ 1 times weekly), whereas those with consumption denoted as current, moderate, heavy, or former alcoholic abuse were classified as "drinkers" (> 1 times weekly or daily) [39, 40]. Based on information from the EHR, dental status was categorized as good (no dental treatment needed), need of dental therapy before start of treatment, or edentulous [41]. Cancer treatment described in the EHR was categorized into different groups/combinations of treatment modalities. Palliative treatment and treatment vaguely described, were pooled. Level of education was poorly described in the EHRs and could not be used to describe socioeconomic status.

Statistical analyses

The correlation between gender and different variables was evaluated using Spearman bivariate correlation (2-tailed) and bootstrapping at 95% confidence interval (CI), as shown in Tables 1–3. For evaluating survival, Cox regression allowed us to report significance, hazard ratio (HR), and 95% CI after bootstrapping as shown in Tables 4 and 5. Results were considered to be significant at $p<0.05$. For survival the variables significant in univariate analysis were analyzed for multicollinearity (VIF), applying linear regression, testing independent variables against a dependent variable. VIF values <2 were regarded to indicate no multicollinearity. The variables with limited data (few in number), were excluded from calculations because of risk of sparse-data bias [42–44]. Kaplan-Meier (Log Rank) was used to construct survival analyses plot. For survival analysis the definitions used were overall survival (OS) and disease-specific survival (DSS); the latter was equivalent to cause-specific survival [45]. All statistical analyses were performed with IBM Statistical Package for the Social Sciences (SPSS) version 25.

Table 1. Clinicopathological characteristics of 535 primary oral cavity squamous cell carcinomas 2005–2009.

	Male n (%)	Female n (%)	(r_s)	(CI 95%)	p
Variable	294 (55)	241 (45)			
Age, median (range)	64 (25–101)	72 (24–96)			
Age groups					
≤50	31 (10.5)	19 (7.9)			
51–60	72 (24.5)	36 (14.9)			
61–70	108 (36.7)	55 (22.8)	0.245	(0.162–0.325)	<0.001
71–80	54 (18.4)	66 (27.4)			
>80	29 (9.9)	66 (27.0)			
Primary site					
Mobile tongue	142 (48.3)	98 (40.7)			
Gingival/alveolar	46 (15.6)	61 (25.3)			
Floor of mouth	69 (23.5)	33 (13.7)	0.062	(-0.026–0.147)	0.154
Cheek/bucca/retromolar	35 (11.9)	44 (18.3)			
Hard palate	2 (0.7)	5 (2.1)			
Tumor status					
T1	65 (22.1)	46 (19.1)			
T2	100 (34.0)	73 (30.3)			
T3	29 (9.9)	29 (12.0)	0.059	(-0.030–0.141)	0.180
T4	92 (31.3)	85 (35.3)			
Unknown*	8 (2.7)	8 (3.3)			
Lymph node status					
N0	186 (63.3)	143 (59.3)			
N1	34 (11.6)	23 (9.5)			
N2	53 (18.1)	56 (23.2)	0.050	(-0.039–0.136)	0.266
N3**	4 (1.4)	1 (0.4)			
Unknown*	17 (5.8)	18 (7.5)			
Stage of disease					
Stage I	61 (20.7)	40 (16.6)			
Stage II	75 (25.5)	51 (21.2)			
Stage III	35 (11.9)	28 (11.6)	0.089	(-0.004–0.172)	0.043
Stage IV	112 (38.1)	111 (46.1)			
Unknown*	11 (3.7)	11 (4.6)			

* Unknown data were not included in the calculations.

**Not included in calculations because of risk of sparse-data bias.

r_s = Spearman rank correlation, rho.

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Results

Our study identified 646 patients with cancer in the oral cavity, of which 111 were excluded as specified in the flowchart in Fig 1, giving a final cohort of 535 patients diagnosed with primary treatment-naïve OCSCC.

The male: female ratio was 1.2, and the median survival follow-up time from end of primary treatment till death or last day of follow-up was 48 months (range 0–125 months).

Clinicopathological characteristics

The clinicopathological characteristics are given in Table 1.

Median age at time of diagnosis for the whole cohort was 67 years (range 24–101 years) with few patients younger than 40 and older than 90 (13 and 11 cases, respectively). The median age at time of diagnosis for men was 64 years (range 25–101 years) compared to 72 years for women (range 24–96 years).

In 97% of the cases TNM staging was complete, and 93% of the cases were discussed in MDT meetings. There was no significant gender difference in either T or N status, and just slightly significant for stage.

T1 and T2 tumors constituted 53% of the cases, almost 11% were T3 tumors, and 33% were T4. According to the AJCC staging, 43% of the patients had stage I and II disease, 12% had stage III disease, and 42% had stage IV disease.

There was no significant gender difference in location of the primary tumor (Table 1). The mobile tongue was the most common tumor site, accounting for almost 50% of the cases for men and 40% for women. The second most common tumor location in men was floor of mouth while gingiva and alveolar mucosa were more frequent in women. Cancers in the mobile tongue were most often T1-T2 tumors, whereas gingiva and alveolar mucosa had more T4 tumors. Tumors of the floor of mouth were most often T2 and T4. There was no correlation between age group and location (p = 0.068, CI: 0.001–0.164). Only three patients had distant metastasis at time of diagnose, and no calculations were performed on this variable.

Risk factors

Risk factors are listed in Table 2.

Table 2. Risk factors for 535 patients with primary oral cavity squamous cell carcinoma in Norway 2005–2009.

	Male n (%)	Female n (%)	(r_s)	(CI 95%)	p
Variable	294 (55)	241 (45)			
Smoking					
Never	41 (13.9)	82 (34.0)			
Current	169 (57.5)	97 (40.2)	-0.230	(-0.317–0.137)	<0.001
Former	70 (23.8)	37 (15.4)			
Unknown*	14 (4.7)	25 (10.4)			
Alcohol comsumption					
Never (Non-drinker)	12 (4.1)	36 (14.9)			
≤1 times weekly (Light drinker)	42 (14.3)	55 (22.8)	-0.405	(-0.501–0.305)	<0.001
>1 times weekly or daily (Drinker)	155 (52.7)	49 (20.3)			
Unknown*	85 (28.9)	101 (41.9)			
Integrated Risk Factor					
Non-smoker/Non-drinker	12 (4.1)	32 (13.3)			
Non-smoker/Light drinker	39 (13.3)	41 (17.0)			
Smoker/Non-drinker**	1 (0.3)	5 (2.1)	-0.084	(-0.201–0.036)	0.119
Smoker/Light drinker	15 (5.1)	20 (8.3)			
Smoker/Drinker	138 (46.9)	42 (17.4)			
Unknown*	89 (30.3)	101 (41.9)			
Dental status					
Good	68 (21.4)	44 (18.3)			
Needs treatment	158 (53.7)	112 (46.5)	0.119	(0.024–0.206)	0.009
Edentulous	45 (15.3)	64 (26.6)			
Unknown*	28 (9.5)	21 (8.7)			

* Unknown data were not included in the calculations.

**Not included in calculations because of risk of sparse-data bias.

r_s = Spearman rank correlation, rho.

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Smoking habits were recorded for 93% of the patients. There was a significantly lower proportion of never-smokers among male compared to female patients (14% vs. 34%), and 58% of the male patients were current smokers compared to 40% of female patients. Only two patients were recorded consuming Scandinavian snuff, but both were former smokers and recorded as such. Current smoking neither correlated with site of primary cancer (p = 0.175, CI: -0.025–0.141), nor with T status (p = 0.909, CI: -0.093–0.085) or with N status (p = 0.628, CI: -0.064–0.109).

Men consumed significantly more alcohol than women, with 11% of the men being heavy drinkers compared to less than three percent (2.5%) of the women (Heavy drinkers were included in the "drinker"-group). Alcohol consumption neither associated with site of primary cancer (p = 0.858, CI: -0.068–0.094), nor with T status (p = 0.522, CI: -0.111–0.054) or N status (p = 0.770, CI: -0.084–0.069). Of note, 35% of the EHR lacked information of alcohol consumption, and were excluded when analyzing for known risk factors.

More men than women were classified as smokers and drinkers according to the IRF classification, but the difference was not statistically significant. IRF correlated with T status (p = 0.001, CI: 0.067–0.237), but not with site of primary cancer (p = 0.265, CI: -0.035–0.125) or with N status (p = 0.856, CI: -0.060–0.081).

Half of the patients needed some form of dental therapy before treatment, whereas 40% had no need of dental treatment, of whom 20% were edentulous. The remaining 10% lacked information on dental status. There were more edentulous patients in the older than younger age groups ($p<0.001$, CI: 0.178–0.348). There was a significant correlation between dental status and gender, but when adjusting for age this difference was no longer present (p = 0.708, CI: -0.071–0.104). Patients with tongue cancer had significantly better dental status than patients with cancer in other oral sites (p = 0.002, CI: 0.039–0.213). In the five-year follow-up time recurrence was found in 95 (17.8%) of the patients within three years. Second primaries, defined as a new OCC more than three years after first presence of OCC, was found in 56 (10.5%) of the patients.

Treatment

This study includes patients treated with both curative and palliative intention. Cancer treatment as described in the EHR are listed in Table 3. Palliative treatment was to some extent vaguely described and pooled.

For 69% (n = 386) of the patients the treatment was surgery, of whom 64% (n = 235) received postoperative RT as shown in Table 3. Very few of the patients had RT prior to surgery and there was no gender difference in this stratification of treatment (p = 0.215, CI: -0.171–0.026). Primary RT with or without chemotherapy was reported for 16%, and palliative treatment was effectuated for 11% (around six percent with RT, the rest with some debulking surgery or chemotherapy, vaguely described).

There was a significant difference in use of RT between age groups ($p<0.001$, CI: -0.334--0.173). Women seemed to receive significantly less RT than men, but when adjusting for age there was no difference in use of RT between the genders (p = 0.381, CI: -0.049–0.124). Use of RT was significantly associated with higher T status (p = 0.008, CI: 0.027–0.207) and positive N status (p = 0.031, CI: 0.007–0.171) but not with site of tumor (p = 0.683, CI: -0.070–0.100).

Survival

Five-year overall survival (OS) was 47% for the cohort, and disease-specific survival (DSS) was 52% (225 of 435 patients). Five-year DSS was 80% for stage I, 68% for stage II, 45% for stage III, and 33% for stage IV (Fig 2).

Table 3. Treatment of 535 primary oral cavity squamous cell carcinoma patients 2005–2009.

	All n (%)	Male n (%)	Female n (%)	(r_s)	(CI 95%)	p
Patients	535 (100)	294 (55.0)	241 (45.0)			
Treatment intention						
Curative	427 (79.8)	239 (81.3)	188 (78.0)			
Palliative	26 (4.9)	12 (4.1))	14 (5.8)	0.046	(-0.052–0.141)	0.329
Unknown*	82 (15.3)	43 (14.6)	39 (16.2)			
Given treatment						
Surgery						
Surgery alone	125 (23.4)	58 (19.7)	67 (27.8)			
Surgery + postop RT	235 (43.9)	147 (50.0)	88 (36.5)			
Preop RT + surgery**	4 (0.7)	2 (0.7)	2 (0.8)	-0.134	(-0.240–0.034)	0.009
Surgery + pre- and postop RT**	1 (0.2)	1 (0.3)	0			
Surgery with chemo**	0	0	0			
Surgery with RT and chemo	21 (3.9)	12 (4.1)	9 (3.7)			
Non-surgery						
RT alone	63 (11.8)	34 (11.6)	29 (12.0)			
Chemo alone**	0	0	0			
RT + chemo	23 (4.3)	12 (4.1)	11 (4.6)	0.016	(-0.194–0.241)	0.884
No treatment**	2 (0.4)	1 (0.3)	1 (0.4)			
Unknown*	3 (0.6)	2 (0.7)	1 (0.4)			
Palliative	58 (10.8)	25 (8.5)	33 (13.7)			
Neck surgery						
No neck surgery	255 (47.7)	133 (45.2)	122 (50.6)			
Elective neck	76 (14.2)	49 (16.7)	27 (11.2)			
Selective neck	52 (9.7)	30 (10.2)	22 (9.1)	-0.079	(-0.166–0.013)	0.094
Modified radical and radical neck	68 (12.7)	41 (13.9)	27 (11.2)			
Unknown neck surgery*	84 (15.7)	41 (14.0)	43 (17.8)			

* Unknown data were not included in the calculations.

**Not included in calculations because of risk of sparse-data bias.

r_s = Spearmans rank correlation, rho.

RT = Radiation therapy

Chemo = Chemotherapy

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When excluding patients given palliative treatment, the five-year OS and DSS for patients given treatment in curative intent, increased to 56% and 62%, respectively. The five-year DSS was then 80% for stage I, 68% for stage II, 51% for stage III, and 43% for stage IV ($p<0.001$, HR = 1.435, CI: 0.261–0.481). Tables 4 and 5 show calculations for five-year OS and DSS for the whole cohort compared to clinicopathologic characteristics and risk factors.

Age-groups, T status, N status, stage of disease, and dental status were all significantly associated with both OS and DSS, in univariate tests at p value <0.05 level (Tables 4 and 5). As stage is based on T status and N status, stage of disease was not included in multivariate analyses. Age-groups, T status, and N status were all independent predictors for OS in multivariate analyses (p = 0.001, HR; 1.487, CI: 0.288–0.517, p = 0.003, HR; 1.201, CI: 0.063–0.303 and p = 0.001, HR; 1.682, CI: 0.363–0.679). The same factors were independent predictors of DSS.

Table 4. Clinicopathological characteristics and five-year overall survival and disease-specific survival.

			OS				DSS	
	OS n (%)	HR	(CI 95%)	p	DSS n (%)	HR	(CI 95%)	p
Patients	251 (100)				225(100)			
Gender								
Male	137 (55)				122 (54)			
Female	114 (45)	1.022	(-0.206–0.248)	0.853	103 (46)	1.009	(-0.249–0.271)	0.929
Age groups								
≤50	35 (70.0)				35 (72.9)			
51–60	65 (57.4)				59 (60.2)			
61–70	88 (54.0)	1.435	(1.299–1.586)	<0.001	81 (61.4)	1.580	(0.338–0.594)	0.001
71–80	48 (40.0)				38 (41.3)			
>80	18 (19.1)				12 (18.5)			
Primary site								
Mobile tongue	117 (48.8)				106 (54.4)			
Gingiva/alveolar	49 (45.8)				44 (48.9)			
Floor of mouth	50 (40.9)	1.041	(-0.046–0.121)	0.338	43 (54.4)	1.058	(-0.041–0.152)	0.228
Cheek/bucca/retromolar	33 (41.8)				30 (45.5)			
Hard palate	2 (28.6)				2 (40.0)			
Tumor status								
T1	70 (63.1)				66 (72.5)			
T2	95 (54.9)				83 (61.9)			
T3	19 (32.8)	1.401	(0.232–0.437)	<0.001	16 (34.0)	1.531	(0.311–0.553)	<0.001
T4	60 (33.9)				53 (34.6)			
Unknown*	7 (38.9)				7 (70.0)			
Lymph node status								
N0	193 (58.7)				174 (65.2)			
N1	21 (36.8)				18 (37.5)			
N2	22 (20.2)	1.825	(0.469–0.741)	<0.001	22 (22.9)	1.929	(0.509–0.816)	<0.001
N3**	0 (0)				0 (0)			
Nx/Unknown*	7 (50.0)				3 (51.3)			
Stage of disease								
Stage I	69 (68.3)				65 (80.2)			
Stage II	77 (61.1)				67 (67.7)			
Stage III	29 (46.0)	1.435	(0.261–0.481)	0.001	24 (45.3)	1.665	(1.356–1.729)	0.001
Stage IV	68 (30.5)				62 (32.6)			
Unknown*	8 (36.4)				7 (58.3)			

* Unknown data were not included in the calculations.

**Not included in calculations because of risk of sparse-data bias.

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Discussion

Our study is large, and includes a substantial number of well characterized patients with primary treatment-naïve OCSCC compared to other publications in this field. Many epidemiological studies present merged data for OCC and oropharyngeal cancer [1, 15, 18]. In our study around a quarter of the cases recorded as oral cavity cancers in the pathology archives and EHRs were oropharyngeal cancers and thus excluded from the study population. This suggests that there is a need to raise the awareness among both clinicians and pathologists of the importance of a correct anatomical description of the cancer site in patient medical records.

Table 5. Risk factors and five-year overall survival and disease-specific survival.

			OS				DSS	
	OS n (%)	HR	(CI 95%)	p	DSS n (%)	HR	(CI 95%)	p
Patients	251 (100)				225 (100)			
Gender								
Male	137 (55)				122 (54)			
Female	114 (45)	1.022	(-0.206–0.248)	0.853	103 (46)	1.009	(-0.249–0.271)	0.929
Smoking								
Never	61 (49.6)				56 (52.8)			
Current	125 (47.0)	0.915	(-0.234–0.055)	0.222	111 (52.4)	0.926	(-0.254–0.110)	0.372
Former	51 (47.7)				45 (51.1)			
Unknown*	12 (32.4)				11 (40.7)			
Alcohol comsumption								
Never (Non-drinker)	18 (37.5)				14 (38.9)			
≤1 times weekly (Light- drinker)	49 (50.5)	0.929	(-0.203–0.052)	0.246	48 (55.8)	0.916	(-0.239–0.069)	0.242
>1 times weekly/daily (Drinker)	90 (44.1)				80 (48.5)			
Unknown*	94 (50.5)				83 (56.1)			
Integrated Risk Factor								
Non-smoker/Non-drinker	17 (38.6)				12 (37.5)			
Non-smoker/Light-drinker	46 (57.5)	0.969	(-0.067–0.006)	0.081	44 (62.0)	0.965	(-0.081–0.006)	0.091
Smoker/Non-drinker**	3 (50.0)				3 (60.0)			
Smoker/Light drinker	18 (51.4)				18 (56.3)			
Smoker/Drinker	70 (38.9)				63 (43.8)			
Unknown*	97 (51.1)				85 (56.3)			
Dental status								
Good	66 (61.7)				63 (68.5)			
Needs treatment	138 (51.1)	1.218	(0.030–0.382)	0.025	124 (54.6)	1.472	(0.177–0.613)	<0.001
Edentulous	31 (28.4)				24 (28.6)			
Unknown*	14 (30.4)				12 (41.4)			

* Unknown data were not included in the calculations.

**Not included in calculations because of risk of sparse-data bias.

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Correct coding is crucial for proper cancer statistics and treatment. One should avoid using the ICD-10 diagnose C02.4 (tongue tonsils) as this can easily be misinterpreted as an oral location. Tumors arising in the root of the tongue is best coded as C01 (base of the tongue) recognized as an oropharyngeal location [38]. Separating oral cavity and oropharyngeal cancers is important as they are associated with distinct risk factors and also differ in primary treatment protocols, response to treatment, and survival rates.

The five-year DSS for our cohort was approximately 52%, which is in accordance with the global report from the review in 2009 of Warnakulasuriya et al. [9]. The five-year OS in our Norwegian cohort was 47%, which is somewhat higher than reported in a Danish cohort for the period 1980–2014 (44%)[13], but lower than in a Finnish study (61%). However, the Finnish study included only patients with OSCC of the tongue, treated with curative intent [5]. When we excluded patients given palliative treatment, the five-year OS and DSS increased to 56% and 62%, respectively. Although the OCSCC treatment in Norway is centralized to four university hospitals, some patients with small T1 tumors may have been treated at local hospitals without referral to the HN cancer centers, and would be missed from our cohort. Patients with T1 tumors have significantly better survival rates than patients with more advanced

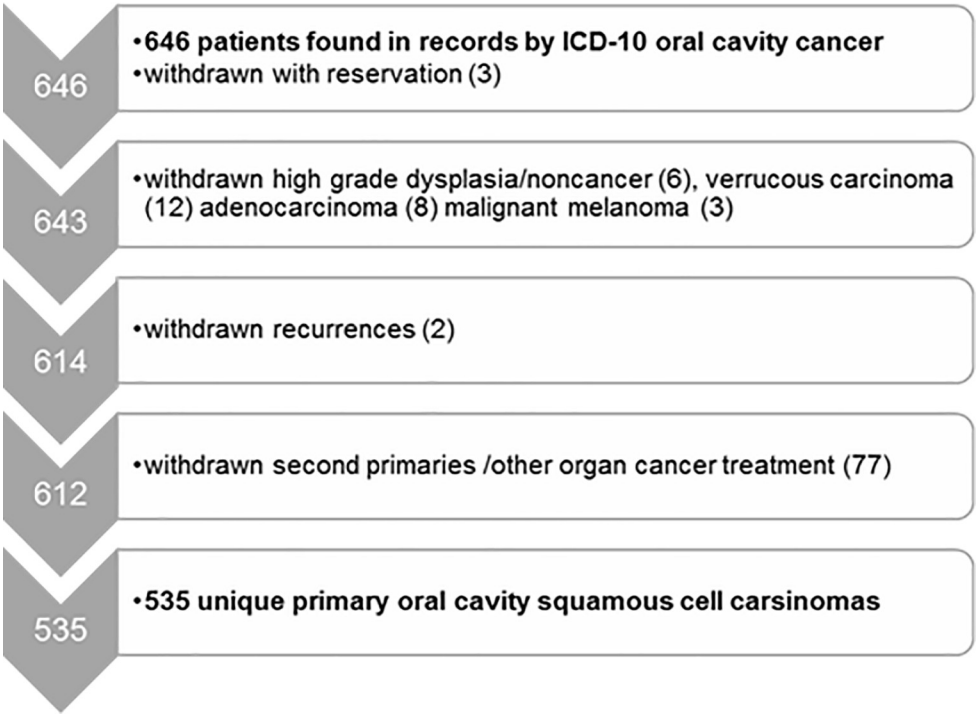

Fig 1. Flowchart outlining how we identified 535 unique primary treatment-naïve oral cavity squamous cell carsinomas in Norway in the time period 2005–2009.

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disease, and if some T1 tumors are missing in our cohort, this may have caused a negative shift in survival rate.

In the current study, 45% of the patients had cancer of the mobile tongue, which corresponds well to reports from previous studies [5–7]. The site and TNM classification are generally the most important prognostic factors for OCC [36]. In the present study, we found significantly higher survival for patients with low T status and stage, whereas the anatomical site of the tumor had no significant impact on survival. Number of recurrences or development of second primaries were in line with previous studies [5, 24].

There were more T1-T2 tumors in the tongue than in other locations, which may be caused by functions of the mobile tongue giving an earlier awareness of a tumor, along with the relative ease of self-inspection compared to other intraoral locations. The proportion of T4 tumors was higher in the gingiva and alveolar mucosa than in the other locations, which may reflect the short distance from the mucosa to the bone at these sites. Tumor involving the bone is classified as a T4 tumor irrespective of tumor size.

Stage I and II OCSCCs are often curable, thus early detection and treatment is of vital importance. In Norway, a large proportion of adults have regular dental examinations, and both dentists and dental hygienists are trained to examine the oral mucosa for malignant lesions. Still, we found 44% of the tumors to be size T3 and T4 at time of diagnosis, which could indicate a rapid growth of tumor. However, patients diagnosed with large tumors were more often edentulous or in need of dental treatment at time of diagnosis, suggesting that these patients did not seek dental care as frequently as those with smaller tumors. Older patients had larger T status, perhaps also reflecting later awareness of illness. It may also suggest that pain in the oral cavity, and symptoms such as changing diet and losing weight are regarded differently in elderly patients.

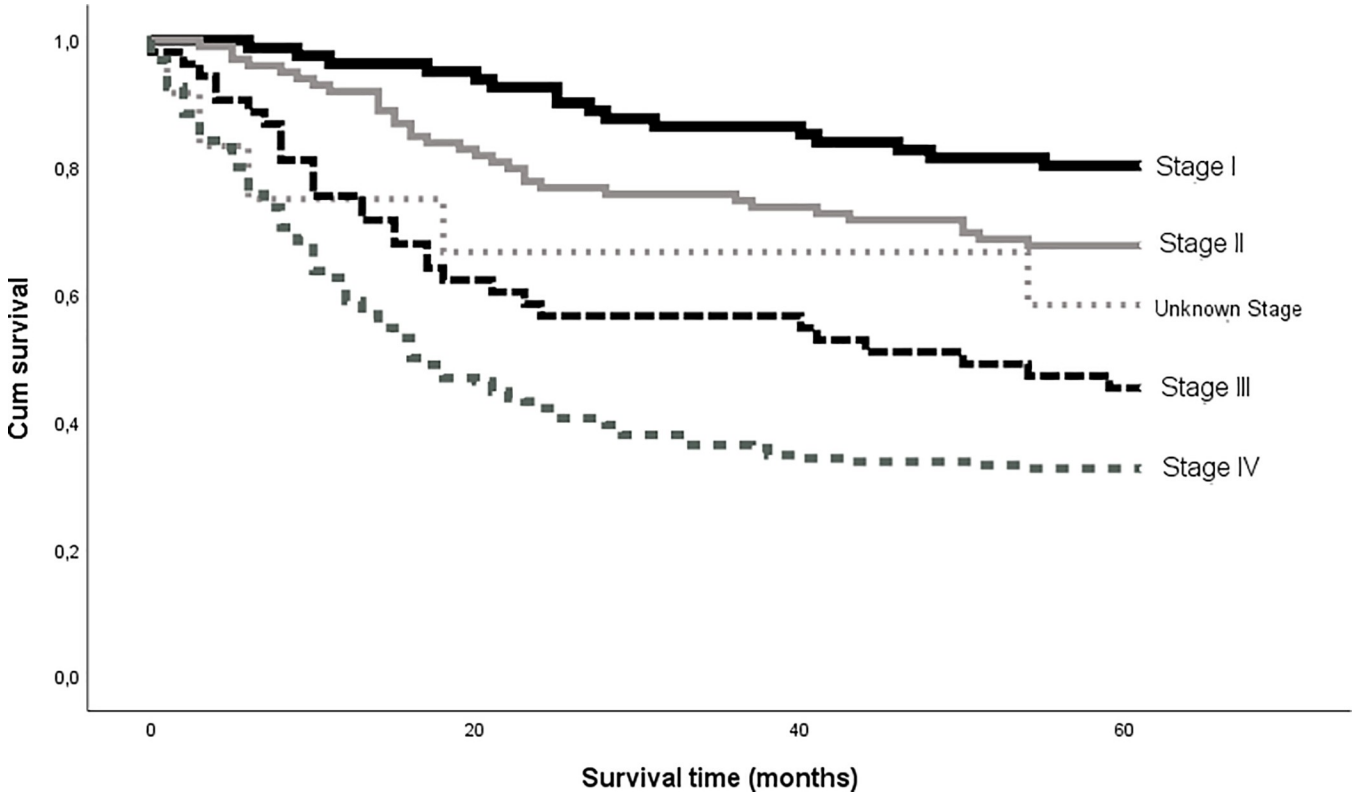

Fig 2. Kaplan-Meier curves show five-year disease-specific survival by stage in 435 patients diagnosed with primary treatment-naïve oral cavity squamous cell carcinoma in Norway in the years of 2005–2009.

https://doi.org/10.1371/journal.pone.0227738.g002

Globally, men have higher risk of OCC than women, and we found a male: female ratio of 1.2. This ratio is slightly lower than reported from a Danish and a US study (1.5 and 1.8 respectively) [13, 14], and slightly higher than reported in a Finnish study with 0.9 [5]. Tobacco and alcohol consumption have become more similar for men and women over the last three decades in Nordic countries compared to when the patients in our study were young [46]. The percentage of drinkers has also decreased both in Europe and in the US by approximately 10% since 2000 [47, 48].

Despite these changes in smoking and drinking habits, the incidence of OCC is rising. This suggests that other etiological factors are involved. For cancers arising in the oropharyngeal region, high-risk human papilloma virus (HPV) is considered to be an additional risk factor [49–51]. However, there is little scientific evidence to consider HPV as a risk factor for OCC, and the frequency of HPV positive SCC in the oral cavity is generally very low, with less than four percent in a Brazilian cohort [17] and less than 10% in a previous study from our group [16]. The use of Scandinavian snuff instead of cigarette smoking has increased tremendously over the last two decades in Norway, whereas cigarette smoking has decreased [52]. Future studies will reveal whether this influences the risk of OCC.

The choice of treatment was decided at MDT meetings for the vast majority of patients. This is according to current recommendations [35], and was a positive finding, as these patients were treated nine to 14 years ago when MDT meetings were less established than today. Cancer in the oral cavity is normally managed by surgical removal of the primary tumor, sometimes combined with neck dissection and/or RT, while chemotherapy is seldom used [28–34]. The same standard of treatment was also found in our cohort.

There are limitations to our study. It was not possible to specify the amount of tobacco use in pack-years or drinking units as this was a retrospective study. In as many as 35% of the EHR, the information of either smoking or drinking habits or both, were missing. Calculations must be evaluated with this perspective. The patient files stated present or past occupation, but not level of education. Level of education is interesting as a measure of cancer incidence in different socioeconomic classes. For a future prospective study one may recommend a systematic and accurate registration of socioeconomic status, smoking habits and alcohol consumption, as well as treatment modalities.

The Eighth Edition of the TNM classification has been introduced since this study was initiated, and in the new TNM classification tumor depth of invasion is included in the T classification of OCC, and this will influence determination of stage as well as prognosis [4].

Conclusion

We present a study of a large cohort of 535 primary treatment-naïve OCSCC. Five-year DSS for the whole cohort was near 52%, and included patients receiving curative as well as palliative treatment. When extracting patients given treatment with curative intent, the five-year DSS increased to 62%. There was no gender difference in survival even though men on average were eight years younger than the women at the time of diagnosis. Patients with the smaller tumors have better prognosis, and this emphasizes the importance of early detection.

Supporting information

S1 Dataset. Supplementary information for 535 patients included in the Norwegian Oral Cancer (NOROC) study.
(SAV)

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Paper II

High-risk human papilloma virus was not detected in a Norwegian cohort of oral squamous cell carcinoma of the mobile tongue.

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High-risk human papilloma virus was not detected in a Norwegian cohort of oral squamous cell carcinoma of the mobile tongue

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Abstract

Objectives: The presence of and the causative role of high-risk human papilloma virus (HPV) is a subject of controversy in oral squamous cell carcinoma (OSCC). The disagreement can be related to the misclassification of OSCC as oropharyngeal squamous cell carcinoma (OPSCC) and/or lack of standard detection methods. This study aimed to examine the presence of transcriptionally active high-risk HPV in a homogenous Norwegian cohort of primary and second primary OSCC of the mobile tongue (OTSCC).

Materials and Methods: Tissue microarrays containing formalin fixed and paraffin embedded cores of 146 OTSCC from the anterior 2/3 of the tongue (n=128 primary, and n=18 second primary) from a multicentric Norwegian cohort were examined for the presence of high-risk HPV by DNA- and RNA- in situ hybridization (ISH) assays and p16 immunohistochemistry (IHC).

Results: Transcriptionally active HPV (*E6/E7* mRNA) was not identified in any of the OTSCC specimens. In parallel, no tumors were positive for HPV by DNA ISH. Although, 61 (42%) OTSCC demonstrated p16 positivity with varying staining intensity and sub-cellular localization, only two cases demonstrated strong and uniform p16-staining (both cytoplasmic and nuclear) in > 70% of cancer cells.

Conclusions: The absence of transcriptionally active high-risk HPV in this cohort of OTSCC indicates that high-risk HPV is an unlikely causative factor in the present material.

Keywords: oral cancer, cancer of the head and neck, squamous cell carcinoma, tongue, human papillomavirus, human papillomavirus oncogene protein, immunohistochemistry, p16, *in situ* hybridization

Introduction

The oral cavity is considered to be a separate anatomical location from the oropharynx. Here, squamous cell carcinoma (SCC) accounts for the majority of malignant tumors [1]. The five-year overall survival for oral SCC (OSCC) is about 64% and is closely related to the tumor stage [2, 3]. In a recent systematic review, the mobile tongue is shown to be the most common site of oral cancer among the patients below 45 years of age [4]. This is of great concern since patients with oral tongue SCC (OTSCC) have a significantly more unfavorable prognosis than those at other oral cavity sites [5, 6].

High-risk human papilloma virus (HPV) is the primary etiological factor in oropharyngeal squamous cell carcinoma (OPSCC) in the Western world [7]. Accordingly, high-risk HPV subtypes have been shown to be present in more than 70% of OPSCC [8, 9]. The HPV positive OPSCC is considered to be a biologically distinct entity and is associated with a higher survival rate as compared to the conventional HPV negative (tobacco-induced) OPSCC [10]. In 1983, Sÿrjanen proposed that HPV could be a possible etiological factor for a subgroup of OSCC [11]. Since then, several studies have focused on HPV detection in OSCC, however, with conflicting results [12, 13]. Firstly, misclassification of OPSCC as OSCC makes it difficult to analyze the results [14]. Secondly, lack of standard methodological approach for HPV testing can significantly lead to over- or underestimation of HPV positivity [12, 15, 16]. In a systematic literature review of approximately 4000 oral cavity cancer specimens, the weighted prevalence of Polymerase Chain Reaction (PCR)-based HPV DNA detection was found to be 20.2 % [17]. However, the high sensitivity of DNA PCR analysis increases the risk of false positive results. Moreover, HPV DNA detection does not distinguish an active (driver) HPV infection from passenger/bystander infection [18, 19]. In recent years, the mRNA *E6/E7 in situ* hybridization (ISH) technique has become increasing popular and it allows direct visualization of viral transcripts in routinely processed tissues, thereby reflecting the active HPV infection [20]. To our knowledge, only a few studies using relatively limited numbers of OSCC have evaluated the presence of high-risk HPV in OSCC by this technique [12, 21, 22]. Their results indicate that high-risk HPV prevalence is very low in OSCC and challenge the view that HPV is a possible etiological factor in OSCC. This underscores the importance of studies aimed at identifying active HPV infection in a large and homogenous OSCC cohort.

The current work represents a sub-study of a joint initiative (Norwegian Oral Cancer (NOROC) multicenter study) between the four University hospitals in Norway treating

OSCC [23]. Here, 146 OTSCC diagnosed in Norway from 2005 until 2010 were included. The hypothesis of the current study was that high-risk HPV infection is uncommon in OTSCC in the Norwegian population. Here we compared three different and independent approaches for high-risk HPV detection (*E6/E7* mRNA- and DNA-ISH and p16 IHC) in OTSCC.

Material and methods

OTSCC selection and extraction of clinical data

The study was approved by the Northern Norwegian Regional Committee for Medical Research Ethics (REK Nord; 2013/1786 and 2015/1381). In this multicenter study, all OSCC cases diagnosed between January 1st , 2005 through December 31st , 2009 at the four Norwegian university hospitals treating head and neck cancer (Oslo, Bergen, Trondheim and Tromsø) were retrospectively identified. Using ICD-10 codes (C02-C06) except for codes C05.1 and C05.2 (oropharyngeal sites, and cancer of the external upper or lower lip/vermilion), a total of 608 OSCC patients were identified from the electronic health record. Two hundreds and seventy-three OTSCC with clinical data were identified. Of the 273 OTSCC, 146 (128 primary + 18 second primary) were included in the present study (for details of the exclusion criteria, see Figure 1). Unidentifiable clinical data were recorded in a web based Case Report Form (CRF). Relevant patient data, ICD-10 diagnosis, TNM classification, treatment received and minimum of five years follow-up (last follow-up date June 1st 2015) were registered. The patients were classified according to TNM 5th Edition 2005 UICC [24].

Tissue microarray generation

Tissue microarrays were constructed from formalin-fixed, paraffin-embedded (FFPE) tissue blocks in a fully automated tissue microarray machine (TMA Master II, 3DHISTECH). Two to four tissue cores (both invasive front and more superficial parts of the tumors) with a diameter of 2 mm were arrayed on the recipient paraffin blocks. The stained TMA-sections were scanned (Pannoramic® MIDI scanner, Thermo Fisher Scientific) and evaluated using the CaseViewer™ software (3dhistech.com). For scanned images with inadequate focus, the original glass slides were examined by a Leitz Aristoplan microscope.

p16 immunohistochemistry (IHC) and scoring

IHC was performed on TMAs on a Ventana Benchmark Ultra automated immunostainer (Ventana Medical Systems, VMS, Tucson AZ, USA), using a mouse monoclonal antibody clone E6H4 (CINtec® p16 Histology, VMS #805-4713). Bound antibody was detected by the biotin-free ultraView Universal DAB Detection Kit (VMS #760-500). A known p16-expressing OPSCC was used as a positive control. Sections incubated with phosphate buffer saline (instead of primary antibody) and with isotype-matched control (Mouse IgG2a, Sigma-

Aldrich, St. Louis, MO, # M9144) were used as negative controls. For details, see Appendix A, supplementary 1 (S1). Blinded for the clinicopathological data, the p16 stained TMA-sections were scored by TMS, DS, PJ and HL. p16 expression was evaluated as follows: score 0: no expression, score 1: positive staining in < 70% of the tumor cells, score 2: positive staining, either nuclear or cytoplasmic in >70% of the tumor cells score 3: Strong and uniform p16-staining (both cytoplasmic and nuclear) in > 70% of cancer cells [25].

HPV DNA ISH and scoring

Automated *in situ* hybridisations were carried out on TMAs on a Discovery XT (VMS) instrument using Research ISH UltraMap XT procedure and Ventana products (INFORM HPV III Family 16 Probe (B), #800-4295;). For details, see Appendix A, supplementary 2 (S2). Each TMA slide contained HeLa cells as positive staining controls. Additionally, sections of a known HPV positive OPSCC were also used as positive controls. A no probe control containing only RiboHybe and RiboWash served as a negative control.

Blinded for the clinicopathological data, the HPV DNA ISH results were scored by TMS and DS. The *in situ* results were interpreted following the manufacturer's guidelines (Interpretation Guide for Ventana INFORM HPV Probes ISH). Any blue nuclear dots in the tumor cells were regarded as positive staining and all of the samples were classified binary as either positive or negative.

HPV RNA ISH

Automated RNA *in situ* hybridisations were carried out on a Discovery Ultra (Ventana Medical Systems, Tucson, AZ, USA) using the fully automated RNAscope VS HRP assay (#323200 Advanced Cell Diagnostics Inc, Hayward, CA, USA). Standard protocols were used for the deparaffinization followed by heat pretreatement using Discovery CC1 and mRNA sample prep protease treatment. For details, see Appendix A supplementary 3 (S3). The RNA quality was controlled in some ramdomly selected specimens using a probe for the common housekeeping gene PPIB (#313909 ACD). Background signal was investigated with a negative control probe for the bacterial gene DapB (#312039 ACD). Both probes were incubated on full FFPE sections and evaluated according to the manufactures instructions. Sections of FFPE pellets of HeLa cells were used as positive controls.

Blinded for the clinicopathological data, the HPV RNA ISH results were scored by TMS and DS. A positive HPV test result was defined as punctate staining that localised in the cytoplasm and/or nucleus of malignant cells. The RNA ISH staining was scored according to

Advanced CELL diagnostics guidelines, as described in the Appendix A Supplementary 4 (S4).

Statistical analysis

Descriptive statistics (range, mean, median and frequencies), where applicable, were calculated for continuous and categorical variables using the Statistical Package for the Social Sciences (spss) 26.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

The present study adheres to the REMARK criteria [26]. Out of 146 OTSCC, cases with missing tissue cores or containing few/no malignant cells in the array were excluded from the analysis. Following these criteria, two primary cases were excluded from the analysis of p16 and DNA ISH, and three primary cases were omitted from the analysis of the RNA ISH.

The clinicopathological variables for 128 primary OTSCC are summarized in Table 1. In brief, primary OTSCC occurred in 77 males (60.2%) and in 51 females (39.8%). At the time of diagnosis, the median age for the cohort was 65.5 years (range: 25-90 years). The second primary OTSCC occurred in 10 males (55.6%) and 8 females (44.4%). The median age was 72.0 years (range: 42-91 years). Fifteen tumors (83.3%) were cT1/cT2, two were cT3/cT4 (11.1%) while T-classification was missing for one case.

p16 immunohistochemistry

Sixty one (42%) OTSCC showed p16 positivity with varying staining intensity and sub-cellular localization (Figure 2). Among the positives, only 2 (1%) OTSCC (both primary) demonstrated strong and uniform p16 staining, fulfilling the criteria for score 3 (Figure 2A). For details on distribution of p16 staining in OTSCC, see Table 2.

HPV DNA ISH

Based on the evaluation criteria described above, all tumors tested negative for HPV DNA (Figure 3A), including the two cases with p16 score 3. The positive controls, a known HPV positive OPSCC (Figure 3B) and HeLa cells (Figure 3C) showed positive staining. The no probe control was negative (Figure 3D).

HPV RNA ISH

No staining or less than one dot to every ten cells was observed in all of the OTSCC investigated (Figure 4A). The positive control, HeLa cells, was positive (Figure 4B) and the RNA controls with PPIB probes were positive (Figure 4C and D). The bacterial gene dapB was used as negative control (Figure 4F).

4.0 Discussion

The pathogenic role of HPV in OPSCC has been well established however, its role in OSCC carcinogenesis is a subject of a controversy. Although several tests are available for HPV detection, there is no consensus on the test method(s) for routine diagnostics of HPV-related oropharyngeal squamous cell carcinomas/head and neck carcinomas [16, 19]. Different methods have different detection targets including HPV DNA, RNA, viral oncoproteins like E6/E7, cellular proteins (e.g. p16 protein) or HPV-specific serum antibodies. Careful selection of the detection technique and viral target is extremely important to obtain reliable and clinically meaningful data. However, the commonly used assays such as p16 IHC and PCR have limitations. For example, p16 overexpression may be caused by molecular mechanisms independent of the presence of high-risk HPV. DNA or RNA extraction procedures in PCR techniques destroy the tumor tissue context of importance for morphological correlation [20]. Furthermore, the detection of HPV DNA (either PCR-based or by ISH) can not discern an incidental virus from a persistant viral oncogene expression [15, 16]. In contrast, RNA ISH probes complementary to *E6/E7* mRNA permit direct visualization of viral transcripts in routinely processed tissues [16, 20]. Unfortunately, only a handful of studies have used this approach to examine the HPV infection in OSCC. The current study consisted of a homogenous and a relatively large number of OTSCC specimens (both primary and second primary). Use of three different test methods enabled us to examine the presence of HPV DNA, its transcriptional active form (E6/E7 mRNA) and the HPV-surrogate marker, p16.

All of the OTSCC specimens in the current Norwegian cohort were negative for *E6/E7* mRNA and HPV DNA. This is in line with the observations reported by Lewis et al., where

all of the 45 OSCC examined were negative for HPV E6/*E7* RNA [12]. Similarly, only one of 107 OSCC contained transcriptionally active HPV in the study by Bishop et al., [22]. Due to the absence of HPV E6/E7 RNA positive carcinomas, we could not characterize their biological or clinical significance. The present HPV DNA results is in agreement with Jaber et al., [27]. Lewis et al., identified only one HPV DNA positive OSCC out of 45 in their study [12]. In contrast, a recent systematic review and meta-analysis reported a higher prevalence of HPV DNA positivity in South and Central America and Asia, as compared to that in North America and Europe [28].

In line with the DNA/RNA ISH results, only two OTSCC were p16 positive (score 3). However, both of the p16 positive OTSCC were negative for HPV DNA and RNA ISH. This suggests that the p16 expression in those cases might be related to non-HPV mechanisms and supports the view that p16 is not a reliable surrogate marker for HPV in OTSCC [22, 29]. One of the two OTSCC with p16 score 3 staining was a basaloid carcinoma (non-keratinizating). This is an interesting observation since p16 positive OPSCC usually are non-keratinizing. However, another non-keratinizing carcinoma included in the present study, was p16 negative.

A general increase in the incidence of OTSCC has been reported globaly with a shifting trend towards female and/or younger patients with OTSCC [30]. In the present study, such a trend was not obvious in Norway in the period 2005 - 2010. Here, the majority of the patients were males and 66% of the patients were 60 years or older. The current study benefitted from the use of a homogenous cohort of OSCC only including carcinomas from the anterior 2/3rd of the tongue. As the Norwegian population is homogeneous regarding ethnic origin, lifestyle and OSCC-related risk habits, the carcinomas can be considered similar with respect to etiology and biology, thereby minimizing the potential biases.

Additionally, to minimize the the possible bias caused by tumor heterogeneity, tissue cores representing both the invasive front and the more superficial parts of each tumor were included in the TMA block. From the majority of the OTSCC, four tissue cores were prepared. From the rest of the tumors, two tissue cores were made. Four cores should achieve a high degree of concordance when comparing results from whole sections with those of TMA cores [31]. A high concordance using triplicate TMA cores [32] and even when including only two cores is reported [33].

Conclusion

None of the 146 OTSCC (128 primary and 18 second primary) diagnosed from 2005 until 2010 were found to be positive for high-risk HPV. In parallel, only two OTSCC were p16 (score 3) positive. Our results suggest that high-risk HPV is an unlikely causative factor in the present material and will not influence future biomarker studies utilizing the current material.

Author contribution

T.M.S conceived and designed the study and supervised the course of the project, verified the pathological diagnosis in the CRF, evaluated and recorded histopathological features of the tumor tissue in the CRF, contributed to tissue microarray generation, participated in analysis and/or interpretation of the ISH and IHC results and in writing of the paper.

I-H. B contributed in structuring the patient data acquisition, descriptive analysis and interpretation, manuscript review and editing.

P.J collected tissue blocks and verified the pathological diagnosis in the CRF, contributed to tissue microarray generation, participated in analysis and/or interpretation of the IHC results.

H.L collected tissue blocks, verified the pathological diagnosis in the CRF, evaluated and recorded histopathological features of the tumor tissue in the CRF, participated in analysis and/or interpretation of the IHC results and manuscript editing and review.

O.S and D.S performed the DNA ISH. In addition, D.S participated in analysis and/or interpretation of the ISH and IHC results and writing of the paper. J.B.G performed the mRNA ISH and both O.S, and J.B.G contributed to manuscript editing and review.

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Conflicts of interest statement

None decleared.

Table 1. Clinicopathological variables in 128 patients with primary oral tongue squamous cell carcinoma.

	Patients (n=128) (%)
Gender	
Male	77 (60.2)
Female	51 (39.8)
Age at diagnosis, years	
0-59	43 (33.6)
≥ 60	85 (66.4)
Smoking history	
Never smoker	30 (23.4)
Former smoker	28 (21.9)
Current smoker	56 (43.8)
Unknown	14 (10.9)
Alcohol consumption	
Never drinker	12 (9.4)
Seldom (≤1 times weekly)	26 (20.3)
Moderately/heavy drinking (>1times weekly or daily)	39 (30.5)
Unknown	51 (39.9)
Tumor differentiation	
Well	23 (17.9)
Moderately	88 (68.8)
Poor	12(9.4)
Unknown	5 (3.9)
cT status	
cT1/cT2	101 (78.9)
cT3/cT4	25 (19.5)
Unknown	2 (1.6)
cN status	
N0	93 (72.7)
N+	31 (24.3)
Unknown	4 (3.2)

Table 2. Results of p16 immunohistochemical staining of 144 OTSCC

p16 IHC staining	Number (%)	Primary OTSCC	Second primary OTSCC
Score 0: no expression	83 (58)	72	11
Score 1: positive staining in < 70% of the tumor cells	47 (33)	42	5
Score 2: positive staining, either nuclear or cytoplasmic in >70% of the tumor cells	12 (8)	10	2
Score 3: Strong and uniform p16-staining (both cytoplasmic and nuclear) in > 70% of cancer cells	2 (1)	2	0

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Appendix A. Supplementary data

S1: p16 IHC

For the detection of p16 protein in TMA sections, IHC was performed on a Ventana Benchmark Ultra automated immunostainer (Ventana Medical Systems, VMS, Tucson AZ, USA), using a mouse monoclonal antibody clone E6H4 (CINtec® p16 Histology, VMS #805-4713) and the biotin-free ultraView Universal DAB Detection Kit (VMS #760-500). Briefly, the tissue sections were deparaffinised followed by heat epitope retrieval with Cell Conditioning 1 for 36 minutes at 96 ˚C (mild CC1, VMS #950-124) and quenching of endogenous peroxidase with 3 % hydrogen peroxide for 4 minutes. The slides where incubated with the p16 antibody or an isotype-matched control (Mouse IgG2a, Sigma-Aldrich, St. Louis, MO, # M9144) at the same concentration of 1 µg/ml for 16 minutes at 36 ˚C. Bound antibody was detected by an HRP-multimer labeled secondary antibody cocktail recognizing mouse and rabbit immunoglobulins for 8 min and visualized with 3,3'-diaminobenzidine tetrahydrochloride for 8 minutes before enhancement with copper sulfate for 4 minutes. A known p16- expressing head and neck squamous cell carcinoma was used as positive control. In addition to the isotype-matched control, sections incubated with phosphate buffer saline (instead of primary antibody) was included as a negative control.

S2: DNA in situ hybridization for HPV

Automated in situ hybridisations were performed on a Discovery XT (VMS) using the following Research ISH UltraMap XT procedure and Ventana products. Sections (4µm) were deparaffinised, followed by heat treatment for 16 minutes at 95 °C using a citrate-based acidic buffer (RiboCC, #760-107) and a protease treatment (Protease 3, #760-2020) for 4 minutes at 37 °C. 200µL Dinitrophenol-(DNP-) labelled probe (INFORM HPV III Family 16 Probe (B), #800-4295) that captures HPV genotypes 16, 18, 31, 33, 35, 45, 52, 56, 58 and 66 was diluted with 75 µL RiboHybe hybridisation buffer (#760-104) and 25 µL RiboWash (#760-105) and a total of 300 µL was added manually on each slide. Denaturation of the probe was performed for 8 minutes at 95 °C followed by a 2 hrs long hybridisation at 52 °C. After hybridisation, three stringency washes ensued with 2 x SSC (RiboWash, #760-105) for 8 min each, at hybridisation temperature. Sections were blocked for 4 minutes with Discovery Antibody Block (#760-4204) before bound probe was detected using a rabbit antibody detecting DNP (#780-4335) for 20 min, followed by an alkaline phosphatase-conjugated anti-rabbit antibody (UltraMap anti-Rb AP, #760-4314) for 16 minutes. Chromogenic signal detection was done by BCIP/NBT for 1 hr (ChromoMap Blue Kit, #760-161). Slides were counterstained manually with 0.1 % Nuclear Fast Red (Gurr, London) in a 5 % aqueous Aluminium sulphate (Sigma-Aldrich) solution for 2 min, washed, dehydrated and coverslips were applied using a xylene-based mounting medium (HistoKit, Assistant).

Each TMA slide contained HeLa cells as positive staining control. Additionally, sections of a known HPV positive OPSCC were also used as positive controls. A no probe control containing RiboHybe and RiboWash only served as a negative control.

S3: RNA in situ hybridization for HPV

HPV E6/E7 mRNA was examined using 5 µm FFPE TMA along with sections of FFPE pellets of human HeLa cells as positive control. The automated RNA in situ hybridisations were carried out on a Discovery Ultra (Ventana Medical Systems, Tucson, AZ, USA) using fully automated RNAscope VS HRP assay (#323200 Advanced Cell Diagnostics Inc, Hayward, CA, USA). Standard procedures were used for the deparaffinization followed by heat pretreatement at 100 °C for 32 minutes using Discovery CC1 and mRNA sample prep protease treatment at 37 °C for 16 minutes. Endogenous peroxidase was blocked with DAB inhibitor (#760-224 Ventana medical systems) for 4 minutes. The FFPE TMA slides were incubated with the HPV HR18 cocktail probe for detection of the HPV genotypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, & 82 (#312599 ACD) and hybridized at 43 °C for 2 hours. After the hybridization, the signals were amplified with AMP 5 for 1 hour and detected with the mRNA DAB kit (#760-224 Ventana medical systems). The sections were counterstained with Mayers Hematoxylin for 8 minutes.

To assess the RNA quality in the tissue prior to the HPV E6/E7 mRNA assay we used a positive probe for the common housekeeping gene PPIB (#313909 ACD) and to assess for background signal we used a negative control probe for the bacterial gene DapB (#312039 ACD). Both probes were incubated on FFPE full sections and evaluated according to the manufactures instructions.

S4: Scoring guideline HPV RNA ISH

Semi-quantitative scoring guideline utilizing the estimated number of punctate dots present within each cell boundary (Advanced Cell Diagnostics 2018).

The staining was categorized according to the following table:

Score 0: No staining or less than 1 dot for every 10 cells (40X magnification)

Score 1: 1–3 dots/cell (visible at 20–40X magnification)

Score 2: 4–10 dots/cell. No or very few dot clusters (visible at 20–40X magnification)

Score 3: <10 dots/cell. Less than 10% positive cells have dot clusters (visible at 20X magnification)

Score 4: >10 dots/cell. More than 10% positive cells have dot clusters (visible at 20X magnification)

Figure legends:

Figure 1. Flowchart illustrating the selection procedure for OTSCC used in the the present study. OSCC = oral squamous cell carcinoma, OTSCC = oral tongue squamous cell carcinoma, RT = radiation therapy, TMA = tissue microarray

Figure 2: p16 immunohistochemistry in OTSCC.

2A = Strong and uniform p16-staining (both cytoplasmic and nuclear) in > 70% of OTSCC cells, score 3. 2B = cytoplasmic p16 staining in >70% of the OTSCC cells, score 2, 2C= weak cytoplasmic staining in OTSCC cells, score 1, and 2D = p16 negative OTSCC cells, score 0.

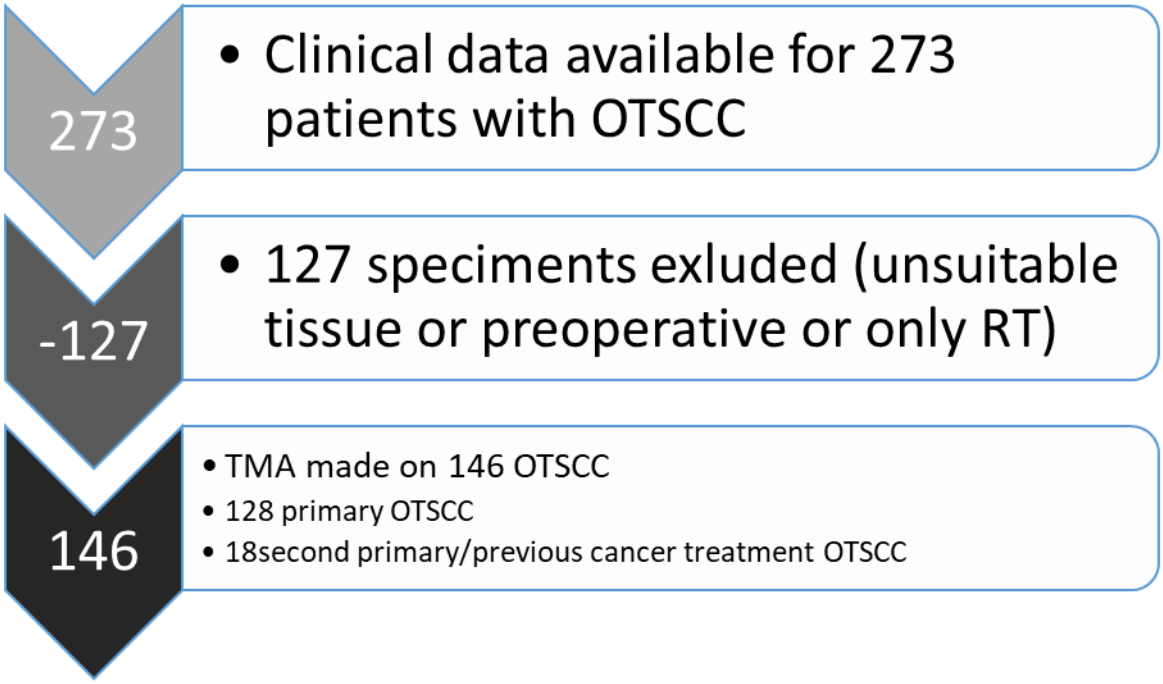
Figure 3: Representative figures for high-risk HPV DNA in situ hybridization in oral tongue squamous cell carcinoma (OTSCC)

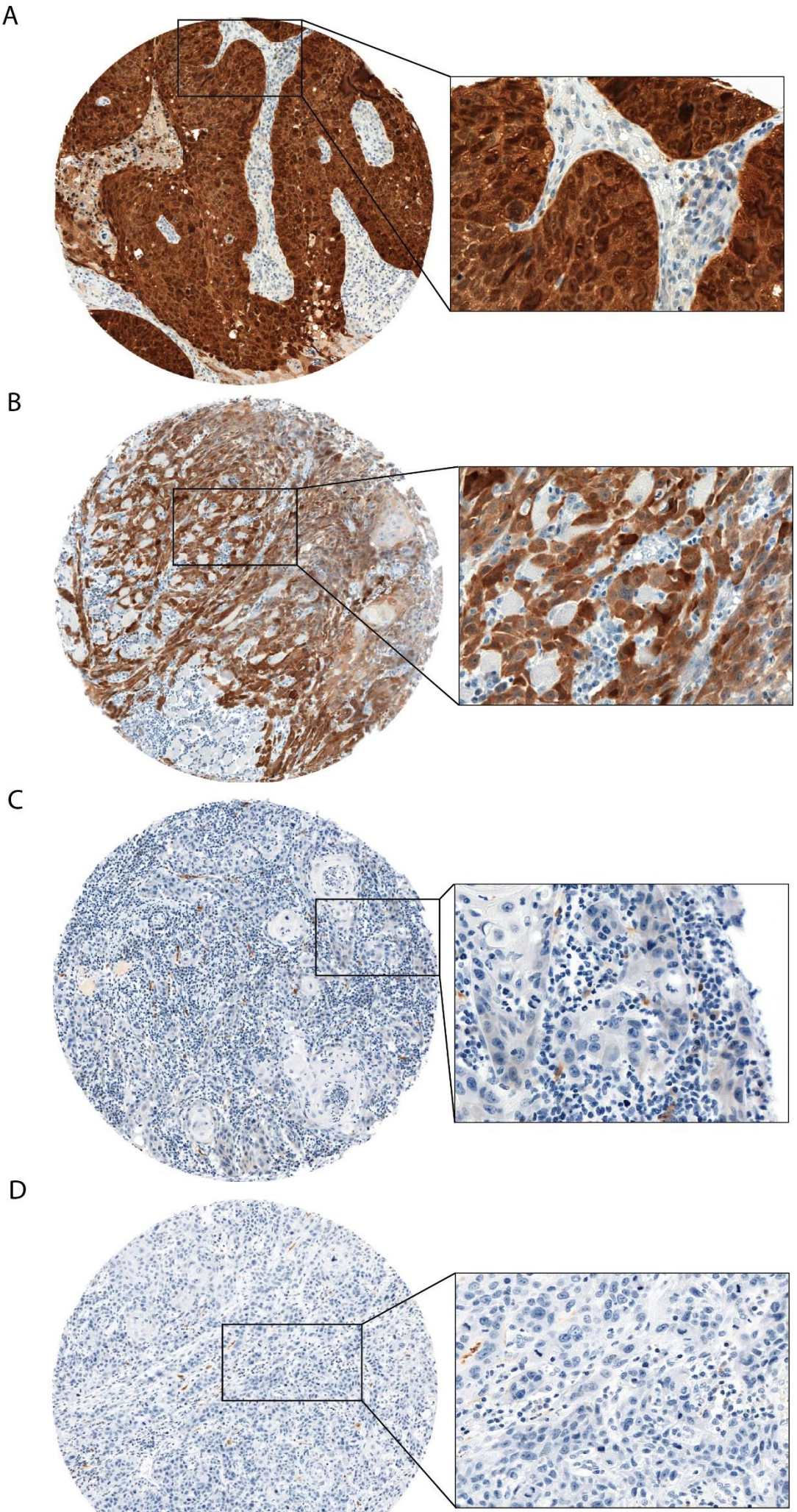
3A = HPV DNA negative OTSCC. 3B = HPV DNA positive OPSCC (positive control). 3C= HPV DNA positive HeLa-cells (positive control). 3D = negative no probe control (in situ without probe) in HeLa-cells.

OPSCC = oropharyngeal squamous cell carcinoma

Figure 4: Representative figures for high -risk HPV RNA in situ hybridization in oral tongue squamous cell carcinoma (OTSCC)

4A = No staining or less than one dot to every ten cells in a HPV mRNA negative OTSCC 4B = HPV mRNA positive HeLa cells. 4C and D = Two different OTSCC with PPIB probes were positive (RNA control). 4F = The bacterial gene dapB was used as negative control.


273
• Clinical data available for 273 patients with OTSCC
-127
• 127 speciments exluded (unsuitable tissue or preoperative or only RT)
146
• TMA made on 146 OTSCC
• 128 primary OTSCC
• 18second primary/previous cancer treatment OTSCC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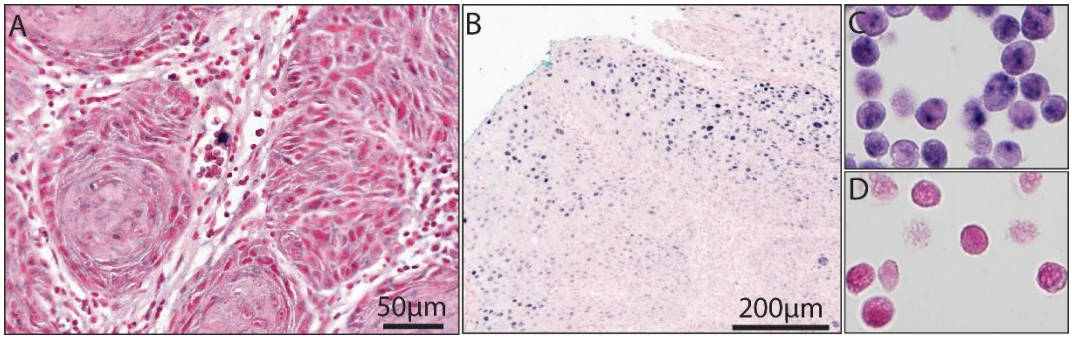
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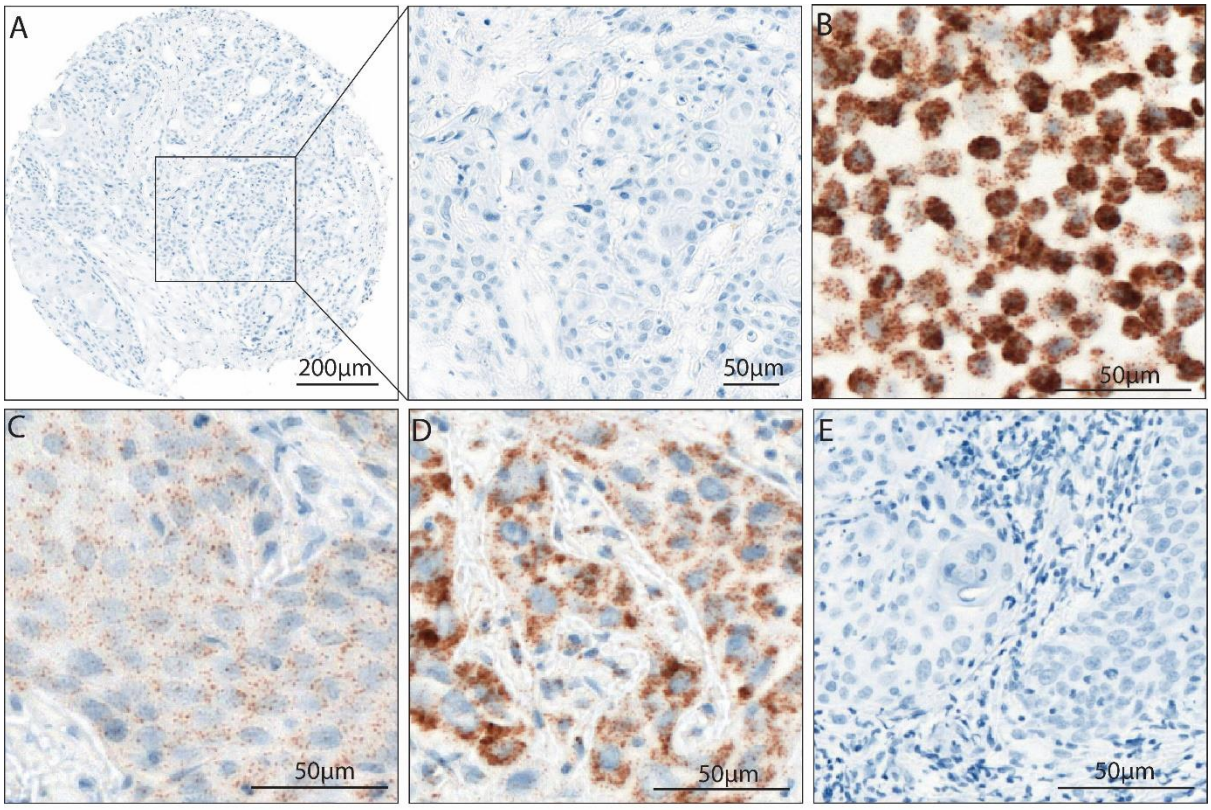
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Paper III

Tumor budding score predicts lymph node status in oral tongue squamous cell carcinoma and should be included in the pathology report.

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Tumor budding score predicts lymph node status in oral tongue squamous cell carcinoma and should be included in the pathology report

Short title: Tumor budding score predicts lymph node status in oral tongue squamous cell carcinoma

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Abstract

Background

The majority of oral cavity arises in the oral tongue. The aim of this study was to evaluate the prognostic impact of the newest classification of tumor size (T), where of depth of invasion (DOI) now supplements dimension, in oral tongue squamous cell carcinoma (OTSCC). We also assessed the prognostic value of tumor budding (TB) and DOI as separate variables, as well as a combination of TB/DOI score.

Methods

Patients diagnosed with primary oral tongue squamous cell were evaluated retrospectively. Spearman correlation with bivariate model including bootstrapping was used to identify correlation between parameters. Survival data were compared with clinical and histopathological data to evaluate their prognostic value using Log rank and Cox regression. All results were considered significant if $p \leq 0.05$.

Results

One-hundred and fifty patients had available material for microscopic evaluation on Hematoxylin and Eosin-stained slides. The newest T classification shifted more tumors to a higher level compared to previous models. High TB-score was associated with higher degree of lymph node metastases, as 22.5% of the patients with tumors with low score had lymph node metastases, compared with 42.8% in the group with a high score. In univariate analyses age groups, T-classification, lymph node status, tumor budding in 2-tier and 3-tier-scoring models, DOI in 3 tier scoring model, and the combined TB/DOI score were all

significant. In multivariate analyses the newest T-classification together with TB in a 3-tier score were independent prognosticators.

Conclusion

The new T classification shifted more patients to a higher stage, and also significantly influenced the prognosis. This indicates that DOI is an important contributor in the new T classification. Degree of TB was associated with lymph node metastases, and can be of important information for the clinicians as a supplement the decision of treatment.

Introduction

Oral cavity cancer is the most common subtype of head and neck cancer [1], and squamous cell carcinomas (SCC) account for about 90% of these [2, 3]. The majority of oral cavity cancers arise in the mobile, anterior two-thirds of the tongue called the oral tongue [2]. Recent studies report rising incidence of oral tongue (OT)SCC, especially in younger patients [4-6].

Patients with OTSCC have a high morbidity and poor prognosis even when tumors are small [7]. For low-stage tumors (T1-T2N0M0), as much as 20% of patients may develop neck metastasis and recurrence within 2 years [8, 9]. In Europe the five-year relative survival for oral cancer is around 50% [10]. Surgical removal of the tumor is the preferred choice of treatment for OTSCC in most institutions when the tumor is regarded resectable. In addition, neck dissection is performed when positive lymph nodes are detected clinically. For patients with clinically negative lymph node status, there is no established biomarker or method to predict whether they will benefit from neck dissection. Thereby, a neck dissection in these patients might result in overtreatment of patients. Postoperative radiation therapy is

recommended when the histopathological examination reveals short tumor margins and/or a positive lymph node status. Chemotherapy is mostly used for patients for palliative treatment [11, 12].

Squamous cell carcinomas are classified according to the tumor's greatest dimension and depth of invasion (T), regional lymph node metastases (N), and organ metastasis (M), according to the TNM system [2, 3]. The TNM classification is the most established predictor of patient survival in OTSCC, but it gives limited information about the aggressiveness of the tumor. The latest edition of the TNM classification (TNM8) has been in use since 2017. In this edition the depth of tumor invasion (DOI) supplements tumor greatest dimension when determining the T classification, in an attempt to increase its prognostic value [2, 13]. Prior to the TNM8 classification, only greatest dimension was included in the evaluation of T, and DOI was proposed as a separate prognostic variable [14]. Some report that the new T criteria in TNM8 lead to a shift to higher T-status compared with previous TNM editions, those who remains in a lower T-status have better survival outcomes when DOI is included [15].

Other histopathological characteristics than DOI have also been evaluated for prognostic value, such as tumor budding (TB). TB is defined as invading clusters of four or fewer tumor cells at the invasive front, and has been associated in many studies with lymph node metastasis, relapse, and accordingly poor prognosis [16-22]. In many studies, TB has been proposed to be a useful and significant prognostic marker that can be evaluated on hematoxylin and eosin (H&E)-stained sections, at low cost and with fair reproducibility [18, 23-25]. The International Tumor Budding Consensus Conference has made a scoring system for TB and recommends implementation of this marker in the classification of colorectal cancer [17]. Some propose that this is applicable also in OTSCC, and suggest that TB should

be included in the routine histopathological report [21, 22]. An additional prognostic factor, a combined score for TB and the score of DOI, was suggested for early stages of OTSCC before the DOI was implemented into the TNM8, and that these in a combination could be used as a prognostic model [14, 26].

The aim of this study was to evaluate the prognostic impact of the T classification after implementation of DOI as described in TNM8, in a large cohort of OTSCC. We also assessed the prognostic value of TB-scores and DOI as separate variables, as well as in combination as a TB/DOI score.

Methods

Identification of patients

In Norway, management of oral cavity cancer is centralized to the university hospitals of Rikshospitalet (Oslo), Haukeland (Bergen), St. Olavs (Trondheim) and North Norway (Tromsø). The Norwegian oral cancer (NOROC) study is a retrospective study that includes the majority of patients diagnosed with oral cavity SCC in Norway from January 1st 2005 through December 31st 2009 [27]. The patients diagnosed during this period were classified according to the fifth edition of the Union for International Cancer Control TNM Classification of Malignant Tumors [13]. Patients were identified by searching for the relevant ICD-10 codes in the electronic health record, and by searching the hospital's pathology archives for cancers with topographic SNOMED coding T51 and T53, which were subsequently matched with the relevant ICD-10 codes recorded in the electronic health records. In this sub-study, the relevant ICD-10 code was C02, which refers to cancers in the mobile tongue. Patients with cancers other than SCC were excluded, as well as patients with

second primaries or previous cancer treatment, and patients from whom formalin fixed, paraffin embedded tumor tissue was lacking.

Extracting clinical data

Relevant patient data including age, gender, ICD-10 diagnosis, TNM classification, treatment, and follow-up were registered as previously described [27]. Cause of death was acquired from Norwegian Cause of Death Registry. Patients were divided into ten-year interval groups (51-60 years, 61-70 years, and 71-80 years) based on age at time of diagnosis. Patients younger than 50 years and older than 80 years were pooled in younger (≤50 years) and older (≥81 years) age groups. Survival was measured from the date of diagnosis until death or last day of follow-up, which was June 1st 2015 ensuring a minimum of five years of follow-up for surviving patients.

Histological samples and categorical grouping

TB and DOI were scored on H&E-stained sections by calibrated and experienced pathologists (HL, ESN, TMS, DC, HH, LUH and SES). No special stains were provided. The scoring was done independently or by pairs of pathologists who were blinded for the patients` clinical outcomes. Biopsies or resections that were too fragmented, too shallow, too superficial, or with technical artefacts that rendered the histological evaluation impossible, were excluded; thus the number of cases with information of TB and DOI varies.

The TB was assessed after scanning 10 separate fields along the invasive front before counting number of buds in the single field (20x objective) with the highest density (hotspot) [17]. TB was categorized into a two-tier (2-tier) system where 0 through 4 buds were called low-Bd and ≥ 5 buds high-Bd, according to the work of Wang et al. and Xie et al. [28, 29] (Fig 1) Also, the three-tier (3-tier) system recommended by the International Tumor

Budding Consensus Conference was applied, where 0 through 4 buds is denoted Bd1, 5 through 9 buds as Bd2, and 10 and more buds as Bd3 [17].

DOI was measured in millimeters, and categorically divided into a DOI 2-tier system with cutoff ≥4mm according to Almangush et al. [23] as well as into a 3-tier system according to TNM8: ≤5 mm, 5.1-10.0 mm, and >10 mm [2].

The combined score of low or high number of buds and DOI was assigned according to Almangush et al. [14, 26]. In short, tumors with < 5 buds and thickness < 4 mm were given TB/DOI-score 0. Tumors with either < 5 buds and tumor thickness ≥4 mm, or ≥5 buds and thickness < 4 mm are given TB/DOI-score 1, whereas tumors with ≥5 buds and thickness ≥4 mm are given TB/DOI-score 2.

Ethics

The study was approved by the Institutional Review Board of the Northern Norwegian Regional Committee for Medical Research Ethics (REK Nord) (Protocol number REK Nord; 2013/1786 and 2015/1381), applicable to all four hospitals. A patient information-consent letter was sent to the patients still alive at the start of the retrospective study, giving them the opportunity to opt-out of the study.

Statistical analyses

Descriptive statistics with frequencies were used to describe the cohort. Spearman correlation with bivariate model including bootstrapping was used to identify correlation between parameters. Univariate survival analyses were conducted using the Log-rank test. Cox regression with bootstrapping was applied for calculating survival analyses, 95% confidence intervals and hazard ratio. Collinearity was evaluated with linear regression.

Multivariate survival analyses were conducted using forward-stepwise Cox regression. All results were considered significant if $p \leq 0.05$. Statistical analyses were performed with IBM Statistical Package for the Social Sciences (SPSS) statistics, version 26.

Results

Clinical characteristics

Altogether 239 patients with primary, treatment-naïve OTSCC were identified, and 200 (84%) of these were included in the study as they received treatment with curative intent. For the remaining 39 patients, palliative treatment was recorded for 16 (6.7%), and information was missing for 23 (9.6%); these patients were excluded. During the five-year follow-up time, 37 (18.5%) patients developed local recurrence and 23 (12%) patients developed a second primary head and neck cancer. H&E-stained tumor sections were available for 150 (75%) of the cases, of which 127 (84.7%) were resections, 18 (12%) were biopsies, and 5 (3.3%) lacked information.

When reclassifying T-status from the older TNM (T old) to the TNM8 edition (T 8 ed), 31 tumors shifted from T1 to T2, 17 from T1 to T3, and 10 from T2 to T3 (Table 1). Only two tumors shifted to a lower T status.

Correlation analyses

Correlation analyses were first performed on the whole cohort for the variables TB, DOI, T old, T 8 ed, and lymph node status (N). TB score (both 2-tier and 3-tier) correlated with lymph node status (Table 2), where 42.8% of tumors with a high TB-score had metastasized to lymph nodes, compared with 22.5% of the tumors with low TB-score. For

patients with low-stage disease (T1-T2N0M0), none of the analyzed variables were associated with TB-score.

DOI (2-tier and 3-tier) and the combined TB/DOI score were significantly correlated with T old (tumor dimension) and lymph node status (N) (Table 3), whereas TB/DOI-score was only significantly associated with lymph node status (N).

For low-stage disease, only DOI 3 tier was associated with tumor dimension ($p<0.001$, CI: 0.234-0.605, $r=0.431$).

Univariate survival analyses

In contrast to T-status according to the old TNM edition, the T-status in line with the TNM8 edition was significantly associated with DSS for the whole cohort as shown in Table 4. TB was significantly associated with 5-year DSS using both the 2-tier and 3-tier system.

DOI was a significant predictor of 5-year DSS only when assessed by the 3-tier scale. Furthermore, the combination of TB and DOI was significantly associated with 5-year DSS. Separate survival analyses for the low-stage disease patients showed similar results as analyses of the whole cohort, except that TB was not a significant prognosticator in this sub-group (Table 5).

Multivariate survival analyses

All variables with statistically significant results in the univariate survival, were potentially eligible for multivariate analyses. However, T 8 ed and DOI were highly collinear, and also TB 2-tier and TB 3-tier. Therefore, for the whole cohort, the histopathological variables implemented in the multivariate survival analyses were T 8 ed, TB 3-tier, and the combined TB/DOI-score. T 8 ed and TB 3-tier were independent prognosticators of 5-year

DSS (p=0.007 and 0.037 respectively). For the low-stage disease group, only T 8 ed and the combined TB/DOI-score were included in the equation, and none of them were significant independent prognostic factors.

Discussion

In this study the prognostic value of the T-status according to the TNM8 edition, where DOI is included, has been evaluated. Also, the prognostic value of TB, DOI, and combined TB/DOI scores has been assessed in a large cohort of primary treatment-naïve OTSCC. There are recent studies that support a prognostic value of these variables in OSCC and validation in a large, homogenous cohort can facilitate clinical implementation of the markers.

The TNM classification system of a cancer does not always provide adequate information for treatment stratification and prognostic outcome. A consequence of this is the risk of overtreatment or undertreatment. Therefore, it is important to find a reliable and reproducible method to distinguish between aggressive OTSCC needing more extensive treatment, such as neck dissection surgery and with postoperative radiotherapy, compared with less aggressive OTSCC, where the patients can be spared from the burden of the latter treatment modalities. Here, the use of simple prognostic markers or parameters that were easily assessed on H&E-stained histological sections from tumor biopsies or resection specimens was an ideal approach. In this way there was no need for expensive equipment, reagents, or extra laboratory procedures, and this is in accordance with other studies [14, 19, 21, 22, 24, 26].

The DOI 3-tier showed a significant positive correlation with T old and N-status, supporting the introduction of tumor DOI to the T-status. When we re-classified the T-status

according to TNM8 where DOI is included, there was a shift towards a higher number of T2 and T3 tumors, which is in line with a previous study [15]. The newest T-classification was a significant prognosticator for 5-year DSS, in contrast to T according to the old classification that only included dimension of the tumor.

Several studies have investigated TB in low-stage OTSCC (T1-T2N0M0), where TB has been correlated with lymph node metastasis and poor prognosis [28, 29]. In some studies, a low TB count correlated well with longer survival, and TB has been suggested to be a valuable prognostic marker for OTSCC that should be implemented in treatment decision-making [17]. However, there are also studies that have not found TB to be of significant prognostic value [24], showing the need for further studies in large and homogenous cohorts. We found that TB was an independent prognostic factor when assessing the whole patient cohort, but it did not reach statistical significance when analyzing the low-stage and high-stage disease groups separately. Interestingly, degree of budding was associated with lymph node metastases. This could be of clinical importance for patients where the clinicians have restrained from neck dissection. If the pathology report states a high degree of budding this might indicate a higher chance of lymph node metastases, implying that a tighter follow up is warranted. This could include new radiological imaging for evaluation of lymph nodes at short intervals [30] , or neck dissection when in doubt.

Conclusion

With the newest T-classification many tumors shifted toward a higher T-status compared with the older classification, and this influenced the prognostic value significantly. This indicates that DOI is an important contributor in the new T classification. Degree of TB was

associated with lymph node metastases, and thereby suggests that TB can provide important information for the treatment decision.

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Table 1 Number of cases with T1-T4 according to the old staging system (T old) and reclassified according to the new staging system (T 8 ed).

T old	T 8 ed	n
1	1	43
1	2	31
1	3	17
2	1	1
2	2	17
2	3	10
3	2	1
3	3	6
4	3	1

Table 2 Spearman correlation between TB and T and DOI

	T old	T 8 ed	DOI 2-tier	DOI 3-tier	N
	n=126	n=132	n=130	n=130	n=139
TB 2-tier	p=0.919 CI: -0.152-0.188 r=0.009	p=0.566 CI: -0.108-0.212 r=0.050	p=0.403 CI: -0.094-0.221 r=0.074	p=0.397 CI: -0.103-0.238 r=0.075	p=0.013* CI: 0.033-0.392 r=0.210
TB 3-tier	p=0.975 CI: -0.172-0.179 r=0.003	p=0.624 CI: -0.124-0.199 r=0.043	p=0.429 CI: -0.107-0.211 r=0.070	p=0.459 CI: -0.107-0.220 r=0.066	p=0.030* CI: -0.052-0.358 r=0.185

n= number of cases.

p= significant ≤0.05

CI= Confidence interval.

r= spearman's Rho.

Table 3 Spearman correlation between DOI, TB/DOI, and T and N

	T old	N0/N+
	n=124	n=131
DOI	p=0.015*	0.038*
2-tier	CI: 0.084-0.335	CI: 0.041-0.302
	r=0.218	r=0.181
	n=124	n=131
DOI	p<0.001*	p=0.024*
3-tier	CI: 0.282-0.549	CI: 0.100-0.384
	r=0.425	r=0.241
	n=122	n=129
TB/DOI score	p=0.105	p=0.003*
	CI: 0.002-0.297	CI: 0.104-0.419
	r=0.147	r=0.262

n= number of cases.

p= significant ≤0.05

CI= Confidence interval.

r= spearman's Rho.

Table 4 Characteristics of the patients (n=150) with OTSCC treated in curative intent, including number of cases, percent of patients with disease-specific survival (DSS) with p-value (p), 95% confidence interval (CI), and hazard ratio (HR)

Characteristic		No. of cases	5-year DSS %	p (CI) HR
Gender	Male	92	70.7	0.702 (-0.612-0.609) 1.105
	Female	58	69.0	
Age (year) group	≤ 50	29	79.3	
	51-60	29	65.5	
	61-70	44	70.5	0.015 (0.66-0.600) 1.382
	71-80	31	67.7	
	≥81	17	64.7	
T old classification	T1	91	70.3	
	T2	28	75.0	0.806 (-0.546-0.472) 1.063
	T3	8	75.0.	
	T4	2	50.0	
	T unknown	21		
T 8 ed classification	pT1	48	87.5	
	pT2	53	64.2	0.006 (0.117-0.961) 1.734
	pT3	34	64.7	
	pT unknown	15		
N	N0	108	82.4	
	N+	40	37.5	0.001 (0.951-2.139) 4.639
	Unknown	2		
TB 2-tier	Low (Bd1)	112	76.8	
	High (Bd2+Bd3)	29	51.7	0.016 (0.085-1.446) 2.269
	Unknown	9		
TB 3-tier	Bd1 <5	112	76.8	
	Bd2 ≥5 and<10	18	66.7	0.002 (0.140-0.999) 1.847
	Bd3 ≥ 10	11	27.3	
	Unknown	9		
DOI 2-tier	< 4 mm	29	86.2	
	≥4 mm	103	68.0	0.090 (-0.103-2.434) 2.172
	Unknown	18		
DOI 3-tier	< 5 mm	47	87.2	
	≥5 mm and<10 mm	52	61.5	0.015 (0.099-0.911) 1.634
	≥10 mm	33	66.7	
	Unknown	18		
Combined TB/DOI*-score	TB/DOI-0	25	88.2	
	TB/DOI-1	84	65.8	0.013 (0.165-1.202) 1.889
	TB/DOI-2	21	52.6	
	Unknown	20		

* Almangush et al 2014

Table 5 Low-stage and high-stage disease evaluated separately. Percentage of 5-year DSS and p-value for each group

		Low-stage		High-stage	
		DSS %	p		p
T old classification	T1	79.2		45.2	
	T2	90.9	0.260 (-3.491-0.599) 0.411	45.5	0.591 (-0.718-0.314) 0.887
	T3			66.7	
	T4			50.0	
T 8 ed classification	pT1	91.2	0.035 (0.014-4.381) 3.344	25.0	
	pT2	73.3		35.3	0.210 (-0.828-1.87) 0.732
	pT3			58.6	
N	**N0**		÷	70.0	0.021 (0.187-2.273) 2.862
	N+	÷		30.6	
TB 2-tier	Low (Bd1)	86.8	0.089 (-3.182-2.574) 2.872	48.6	
	High (Bd2+Bd3)	66.7		40.0	0.496 (-0.563-1.071) 1.305
TB 3-tier	Bd1 <5	86.8	0.091 (-0.2.501-1.654) 1.875	48.6	
	Bd2 ≥5 and<10	66.7		71.4	0.094 (-0.134-0.751) 1.427
	Bd3 ≥ 10	66.7		12.5	
DOI 2-tier	< 4 mm	94.4	0.098 ((-0.047-3.821) 4.415	0.0	0.016 (-1.581--0.276) 0.461
	≥4 mm	76.7		51.1	
DOI 3-tier	< 5 mm	90.9		25.0	
	≥5 mm and<10 mm	71.4	0.043 (-0.107-4.456) 3.513	33.3	0.119 (-0.929-0.126) 0.686
	≥10 mm	÷		60.7	
Combined TB/DOI*-score	TB/DOI-0	100	0.005 (0.347-1.968) 2.797	0.0	
	TB/DOI-1	77.5		50.0	0.609 (-0.869-553) 0.843
	TB/DOI-2	66.7		50.0	

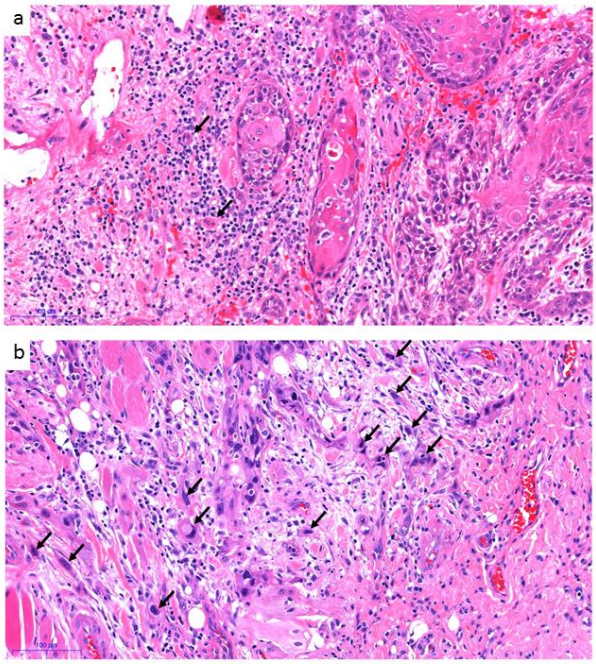

Fig 1. Tumor budding. Case with low number of buds (marked with black arrows) shown in a, and a case with high number of buds in b.

Paper IV

A combined histo-score based on tumor differentiation and lymphocytic infiltrate is a robust prognostic marker for mobile tongue cancer.

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A combined histo-score based on tumor differentiation and lymphocytic infiltrate is a robust prognostic marker for mobile tongue cancer

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ABSTRACT

We wanted to evaluate the prognostic value of common histopathological variables in a large cohort of patients with cancer in the mobile tongue as such information can be important for treatment stratification of the individual patient, especially for patients with low-stage disease. In addition we wanted to investigate whether an alternative scoring model with fewer options would compromise the prognostic value. 150 patients with oral tongue squamous cell carcinomas that were treated in curative intent and with available HE-stained tumor sections were included. We reclassified all tumors and performed univariate and multivariate survival analyses of histopathological and clinical variables. For the complete cohort, lymph node status, grade of differentiation, perineural infiltration, and lymphocytic infiltration were independent prognosticators. In the low-stage disease group independent prognostic factors were tumor size, grade of differentiation, and lymphocytic infiltrate. For patients with low stage disease, a histo-score combining the scores for tumor differentiation and lymphocytic infiltrate identified a group of patients with particularly low survival, as patients with moderately or poorly differentiated tumors and little lymphocytic infiltrate had a less favorable 5-year survival outcome than patients in the high-stage disease group. This study shows that a histo-score combining tumor differentiation and lymphocytic infiltration should be given special consideration in treatment planning. Our results also illustrate that many variables can be scored with fewer options than previously suggested to increase their reproducibility, and still maintain their prognostic value.

Keywords: Oral squamous cell carcinoma, low-stage, differentiation, lymphocyte infiltrate, histo-score, prognostic factors

DECLARATIONS

Funding:

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Conflict of interest

Elisabeth Sivy Nginamau has declared that she has been a consultant for MSD Norway

Availability of data and material:

Statistics file with generated data is provided

Code availability:

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Authors' contributions:

Inger-Heidi Bjerkli has been essential for building the database, conducting analyses, and writing the paper. Elin Hadler-Olsen and Lars Uhlin-Hansen have designed the research study, and contributed in scoring histopathological variables, critically evaluating results, and writing the paper. Elisabeth Sivy Nginamau, Helene Laurvik, Tine M. Søland and Daniela Elena Costea have performed scoring of histopathological variables, and contributed to the scientific content of the paper. Sonja E. Steigen has designed the research study, and contributed in scoring histopathological variables, conducting analyses, critically evaluating results, and writing the paper. All authors have read and commented on the final draft of the paper.

Ethics approval:

The study was approved by the Northern Norwegian Regional Committee for Medical Research Ethics (Protocol numbers REK Nord; 2013/1786 and 2015/1381).

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Not applicablle

INTRODUCTION

About half of all malignant tumors in the oral cavity arise in the mobile, anterior two-thirds of the tongue, and more than 90% of them are squamous cell carcinomas (SCC) [1]. The aggressiveness of oral cavity tongue (OT)SCC varies markedly, even for small tumors without lymph node metastases [2]. The search for morphological tumor traits that reliably predict the prognosis for the individual patient has been going on for decades [3-5]. Such prognostic markers could help clinicians select the optimal treatment for individual patients that could increase the chances of being cured of the disease, and at the same time minimize the side effects from overtreatment. The TNM system classifies tumors based on their size and depth of invasion (T), neck node involvement (N), and distant metastasis (M). Along with the International Union against cancer (UICC) staging, these factors are today the best survival prognosticators for cancers in the oral cavity [6]. On group level, patients with low-stage disease (stage I-II; T1-2, N0M0) have an estimated higher survival rate compared with patients with high-stage disease (stage III-IV) [7, 8]. However, there is a need to find markers that can differentiate between aggressive and more indolent tumors for individual patients within the same stage.

Various aspects of a tumor's morphology and growth pattern can be evaluated on hematoxylin and eosin (HE) stained tumor sections. Several of these characteristics have been proposed as prognostic markers in oral cancer [4, 9, 10]. However, despite some reports of prognostic usefulness, none of these markers has been implemented in clinical practice, mostly due to lack of coherence between studies. There are several putative explanations for the lack of consistency between prognostic studies. Many are based on small patient cohorts, and do not control for parameters known to affect prognosis, such as intraoral location and stage [11-14]. This biases the actual prognostic value of the markers in question. Furthermore, the evaluation of histopathological criteria is subjective, and different pathologists may interpret the same criterion differently [15, 16]. In a recent study, we found poor inter- and intra-observer agreement when

evaluating a selection of proposed histopathological prognostic markers in oral SCC, even though the observers had mutual training sessions and were experienced pathologists [17]. Improved agreement was obtained by reducing the number of scoring alternatives for each parameter. This suggests that fewer options for each parameter might increase the robustness of histopathological prognostic markers, provided that the reduction of scoring alternatives does not compromise the prognostic value. In the current study, we evaluated the prognostic value of a number of proposed histopathological variables as they were originally proposed, as well as with a reduced number of scoring alternatives, in a large, homogenous cohort of OTSCC. Our results show that some histopathological markers, individually and in combination, can add significant prognostic information for OTSCC. Our study further highlights the importance of controlling for known risk factors such as tumor size and lymph node metastasis when evaluating putative prognostic markers.

MATERIALS AND METHODS

Cohort of patients

The Norwegian Oral Cancer (NOROC) Study is a retrospective study that includes patients diagnosed with oral cavity SCC in Norway from January 1st 2005 through December 31st 2009.The NOROC study includes patients with strict oral cavity SCC [8]. In the present study, the relevant ICD-10 codes were C02, which refer to cancers in the mobile tongue. Of the original NOROC cohort, 273 patients (45%) had OTSCC. From them, we included only the primary, treatment-naïve patients who were treated in curative intent and from whom we had HE-stained sections from biopsies or resections available, altogether 150 patients.

Extracting clinical and histopathological data

Experienced head and neck surgeons retrieved clinical parameters from the electronic health records as previously described [8]. Of the 150 patients that underwent surgery, 72 patients had neck

surgery, and for them the N-status was based on histopathological evaluation. For the patients who did not have neck surgery, the N-status was based on clinical/radiological evaluation.
Senior pathologists re-evaluated the histopathological characteristics of the tumors, including WHO degree of differentiation, keratinization, nuclear polymorphism, perineural infiltration, lymphocyte infiltrate, and worst pattern of invasion [3, 4]. For several of these, a fairly elaborate grading system was originally suggested. In this study we have also applied alternative versions, as described in our previous paper [17] and summarized in *Table 1*. The pathologists were blinded for the patients' clinical information and outcome.

We calculated survival from the date of diagnosis until the date of death or last day of follow-up, which was June 1st 2015. At that time, all patients were followed up for a minimum of 5 years or until death. Cause of death was acquired from the Norwegian Cause of Death Registry.

The study was approved by the Northern Norwegian Regional Committee for Medical Research Ethics (Protocol numbers REK Nord; 2013/1786 and 2015/1381). Patients still alive were informed about the project and had the opportunity to opt-out.

Statistical analysis

Descriptive analyses and univariate survival analyses using Log Rank (Mantel Cox) giving Kaplan-Meier survival curves were performed. Variables significant in univariate calculations were tested for collinearity before entering them into multivariable equations. Multivariate survival analyses were performed using Cox regression model. Associations were investigated using Chi-square. All statistical analyses were performed using SPSS version 26. All survival analyzes were significant at 0.05 level.

RESULTS

One hundred and fifty patients with OTSCC were eligible for histopathological reclassification and included in the study. Of the tumor material available, 127 were resection specimens, 18 biopsies, and 5 unknown. Seventy-seven patients had low-stage disease (Stage I and II according to TNM 8th edition), 63 had high-stage (Stage III and IV) [6], and for 10 cases the information for stage was missing.

Supplementary table 1 presents the scores for each variable for the whole cohort and after separation into low-stage and high-stage disease. *Table 2* presents gender, age, TNM-status and stage, as well as calculation of five-year disease-specific survival (DSS).

Survival

The 5-year DSS was 64.8% for the whole group, and 82.8% and 44.6% for the low- and high-stage group, respectively.

Univariate analyses

In *Table 3* Five-year DSS from univariate analyses are listed for each variable, both with original and alternative versions of grading. For the whole cohort, the following variables were significantly associated with five-year DSS: degree of differentiation (1.0 and 1.1), keratinization of the whole tumor (3.0 and 3.1), keratinization at tumor front (4.0 and 4.1), perineural infiltration (7.0 and 7.1), lymphocytic infiltrate (8.0, 8.1, and 8.2), and worst pattern of infiltration (9.2).

For patients with low-stage disease, differentiation of the whole tumor (1.0 and 1.1), nuclear polymorphism whole tumor (5.0 and 5.1), nuclear polymorphism at tumor front (6.1), and lymphocytic infiltrate (8.0, 8.1, and 8.2), were significantly associated with DSS. For patients with

high-stage disease, differentiation of whole tumor (1.1), and perineural infiltration (7.0), were the only significant prognosticators of DSS.

Separate calculations were also performed for resection specimens only (biopsies excluded) with results similar to those for all tumors (resections and biopsies), and it is presented in *Supplementary table 2*.

Multivariate analyses

We performed multivariate analysis of the histopathological variables that were significant in univariate calculations, with separate analyses for original and alternative grading of the variables. Additionally, T and lymph node status was included in the equation for the whole cohort, and T for the low-stage disease group. For the whole cohort this included differentiation, keratinization, perineural infiltration, and lymphocytic infiltration for both original and alternative scoring gradings, and WPOI was additionally included in the alternative version. Keratinization of the whole tumor and keratinization of the tumor front were collinear, and only keratinization of the whole tumor (3.0/3.1) was included in multivariate analyses. For patients with low-stage disease differentiation, nuclear polymorphism and lymphocytic infiltration were included. Nuclear polymorphism of the whole tumor and in the tumor front were collinear, and only polymorphism of whole tumor (5.0/5.1) was included in the multivariate analyses. Independent prognosticators for the complete patient cohort were lymph node status (N, $p<0.001$), differentiation of whole tumor (1.1, $p=0.022$), perineural infiltration (7.0, $p=0.025$), and lymphocytic infiltration (8.2, $p=0.048$). In the low-stage group, T ($p=0.003$), differentiation of whole tumor (1.1, $p= 0.022$), and lymphocytic infiltrate (8.2, $p=0.003$), were all independent variables.

Combined histo-score

For the low-stage group, we created a combined score, called histo-score, based on tumor differentiation and lymphocytic infiltrate (*Figure 1*). The histo-score was calculated by summarizing the individual score of differentiation and lymphocyte infiltration (*Supplementary table 3*). Using the original grading, the lowest score was 2 and the highest was 6. There was a highly significant difference in survival between the groups (p<001), *Figure 2***.** Of the 48 patients with score 2, 3 or 4, only two patients died of the disease within 5 years (DSS = 95.8%). Of the 14 patients with a score of 5 or 6, eight patients died within 5 years (DSS 42.9%). The combined histo-score based on the alternative grading differentiation 1.1 and lymphocytic infiltration 8.2, showed the same significant prognostic power (p<0.001).

There were no common denominators for the patients with low-stage disease and low versus high histo-score who died with respect to age, gender, T-stage, keratinization, or worst pattern of infiltration. Additionally we explored whether there was a difference between different treatment options (with or without neck dissection, with or without postsurgical radiotherapy), but we could not find any associations.

DISCUSSION

Reliable, prognostic markers that can supplement tumor staging are lacking for oral cavity cancer. As tumors of the same stage can have different degree of aggressiveness, there is a need to find additional markers to assist the treatment planning and to predict the outcome of individual patients. Oral cancer is most prevalent in developing countries [18]. Thus, markers that do not require expensive equipment or reagents, such as histopathological traits that can be assessed on HE stained sections are especially valuable. In the present study, we have evaluated the prognostic power of a number of histopathological variables suggested for oral cancer, where results from previous studies are contradictory [19, 20]. We have tested them in a large, homogenous cohort of

patients with OTSCC, in which clinical and histopathological parameters are well controlled. Our hypothesis was that the lack of consistency of prognostic value in previous studies can be partly explained by small cohorts of patients and the inclusion of tumors from various intraoral locations. Furthermore, scoring of histopathological parameters is subjective, as reflected by the poor inter- and intra-rater agreement [17]. Several of the histological variables have been proposed with three to six options for scoring, sometimes with subtle differences between each alternative. Grouping categories and thereby reducing the number of scoring alternatives can make the scoring easier and more reproducible [17]. Therefore, we also tested the prognostic power of the variables as they were originally proposed, as well as with broader and fewer categories.

As expected, the well-established prognostic markers tumor size and lymph node metastases were independent predictors of survival. Additionally, we found that tumor differentiation was an independent prognosticator of survival both for the whole cohort and for patients with low-stage disease. WHO lists differentiation as a prognostic marker for oral cavity cancer, and degree of differentiation is usually described in pathology reports [21]. However, due to many studies reporting low prognostic value of differentiation for oral SCC, clinicians rarely give it much emphasis during treatment planning [22, 23]. Our results indicate that this marker has significant prognostic power.

A revised grading for lymphocytic infiltration where the categories for marked and moderate infiltration were combined, was also an independent prognostic marker for low-stage disease and for the whole patient cohort. Grouping categories generates larger groups for statistical analyses, and this can affect the significance level. However, the cutoff for dichotomizing the original three-tier variable was important. The variable only had independent prognostic power when separating the tumors with low lymphocytic infiltration from those with moderate and abundant infiltration. This suggests that lymphocyte infiltration is tightly related to the biology of the tumor, and that the tumors with little infiltration may take a more aggressive course. This is in line with previous studies showing that a rich lymphocyte infiltration is associated with favorable prognosis [24-26].

By incorporating degree of diffentiation and lymphocytic infiltration in a combined histo-score, we were able to define a subgroup of patients with low-stage disease that had a much lower survival rate than the rest of the low-stage disease patients. Interestingly, the survival in this subgroup was even less favorable than patients with high-stage disease (42.9 versus 46.6%). This indicates that patients with poorly differentiated tumors with a weak lymphocytic response should be regarded as high-risk patients who need special attention, even if the tumors are small and without lymph node metastases.

Perineural infiltration was a significant prognostic marker for the whole cohort and for patients with high-stage disease, but not for low-stage disease. One could assume that nerve bundles are more abundant in deeper parts of the oral mucosa, and tumors probably need to invade deeper than in T1 and T2 tumors for this to be a relevant marker. This illustrates the importance of evaluating prognostic markers in homogenous groups of tumors and controlling for known risk factors.

We found that alternative grading (fewer options) of histopathological variables only altered their prognostic value only to a minor extent. A previous study comparing inter- and intra-rater agreement showed significantly better agreement when using an alternative grading with fewer options, compared to the more elaborate original grading [17]. This supports the use of variables with fewer options as they improve the reproducibility of the scoring without reducing the prognostic power of the variables. A simplification of scoring models has been introduced for many cancers. The reproducibility for uterine endometrial endometrioid carcinoma was found to be higher with a binary tumor grading system [27]. In the latest WHO-classification of tumors in the GI tract, the adenocarcinomas are stratified into a two-tiered grading system; low-grade and high-grade, where grading is based on the least differentiated component [28].

The present study is retrospective, and this approach gives a larger risk of variation in how clinical variables are reported in the electronic health records compared with prospective studies.

When subgrouping, some groups became small, which increases the risk of underpowered statistical analyses and thereby of underestimating the prognostic power of some variables. Our cohort included some tumors from which we had only biopsy samples for histopathological evaluation, which makes evaluation less certain. Therefore, we performed separate statistical analyses excluding the grading of biopsies (supplementary tables), but this did not alter the results significantly.

CONCLUSION

Our study on a large, homogenous tumor cohort of OTSCC shows that a histo-score combining tumor differentiation and lymphocytic infiltration identified a subgroup among the low-stage disease patients that had lower DSS than the average patients with high-stage disease. This subgroup should be given special consideration in treatment planning. Our results also illustrate that many variables can be scored with fewer options than previously suggested to increase their reproducibility, and still maintain their prognostic value.

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Legends

Figure 1 Tumor differentiation and lymphocyte infiltration. Well, moderate and poorly differentiated tumor in a-c. Marked, moderate and little lymphocyte infiltration in d-f.

Figure 2 Kaplan-Meier curve showing the results after combining the variables of differentiation (1.0), and lymphocytic infiltrate (8.0). Scoring alternatives are shown in table 5. Patients with low score (2-4) had a statistically better survival than those with high score (5-6).

Table 1. Variables with original and alternative grading

	Tumor characteristics	Original variables	Alternative 1		Alternative 2	
1.0	Differentiation whole tumor	Well Moderate Poorly	1.1	Low-grade High-grade		
2.0	Differentiation worst pattern	Well Moderate Poorly	2.1	Low-grade High-grade		
3.0	Keratinization whole tumor	High Moderate Minimal None	3.1	Low-grade High-grade		
4.0	Keratinization tumor front*	High Moderate Minimal None	4.1	Low-grade High-grade		
5.0	Polymorphism whole tumor	Little/none Moderate Abundant Extreme	5.1	Low-grade High-grade		
6.0	Polymorphism tumor front*	Little/none Moderate Abundant Extreme	6.1	Low-grade High-grade		
7.0	Perineural infiltration	None Invasive front Tumor center	7.1	No Yes		
8.0	Lymphocyte infiltrate	Marked Moderate Little/none	8.1	Marked Not marked	8.2	Abundant Little
9.0	Worst pattern of invasion (WPO)**	Type 1 Type 2 Type 3 Type 4 Type 5	9.1	Low-grade High-grade	9.2	Low-grade High-grade

*according to Bryne et al. (1998).

**according to Brandwein-Gensler et al. (2005). Type 1 pushing borders; Type 2 finger-like growth pattern; Type 3 large separate islands, >15 cells per island; Type 4 small tumor islands, ≤15 cells per island; Type 5 tumor satellites, ≥1 mm from main tumor/satellite

Table 2. Clinicopathological characteristics related to five-year disease specific survival (DSS). Number and percent of patients in each group, and in addition percentage of patients with five-year DSS

Variable		n	%	DSS %	p *(DSS)
Gender	Male	92	61.3	70.7	0.702
	Female	58	38.7	69.0	
Age (year) group	≤ 50	29	19.3	79.3	0.015
	51-60	29	19.3	65.5	
	61-70	44	29.3	70.5	
	71-80	31	20.7	67.7	
	≥ 81	17	11.3	64.7	
pT 8th Edition	pT1	48	35.6	87.5	0.006
	pT2	53	39.3	64.2	
	pT3	34	25.2	64.7	
N**	N0	108	72.0	82.4	0.001
	N+	40	26.7	37.5	
	Nx/Unknown	2	1.3		
Stage	Low stage	77	55.0	82.8	<0.001
	High stage	63	42.0	44.6	
	Nx/Unknown	10	6.7		

* Significant at 0.05 level

** Combination of cN and pN. If neck dissection was performed the result on pN was superior to cN

Table 3. Variables (both original and alternative grading) and five-year disease-specific survival in univariate calculations for the whole cohort, and for low-stage disease and high-stage disease separately. The percentage of patients surviving according to different grading is specified under DSS%.

	Variables	Whole cohort (n=150)		Low-stage (n=78)		High-stage (n=63)	
			All		All		All
		DSS %	p (n)	DSS%	p (n)	DSS%	p (n)
1.0	**Differentiation, whole tumor**	**92.3/64.0/16.7**	**<0.001 (127)***	**94.7/84.6/40.0**	**0.002 (63)***	**75.0/47.7/0**	**0.055 (55)**
	1.1	70.4/16.7	<0.001*	87.7/40.0	0.001*	51.1/0	0.025 *
2.0	**Differentiation, worst pattern**	**100/72.0/56.7**	**0.074 (124)**	**100/88.9/76.9**	**0.248 (63)**	**100/50.0/40.0**	**0.646 (54)**
	2.1	75.4/56.7	0.053	90.9/76.7	0.121	52.6/40.0	0.676
3.0	**Keratinization whole tumor**	**85.0/68.0/53.6/47.6**	**0.038 (124)***	**82.4/91.3/83.3/66.7**	**0.402 (61)**	**83.3/46.2/30.8/22.2**	**0.137 (54)**
	3.1	73.3/51.0	0.010 *	87.5/76.2	0.238	53.1/27.3	0.078
4.0	**Keratinization, tumor front**	**/100/70.3/59.7**	**0.047 (120)***	**÷/100/89.5/75.8**	**0.133 (62)**	**÷/100/47.1/44.1**	**0.665 (52)**
	4.1	100/63.3	0.025*	100/80.8	0.146	100/45.1	0.374
5.0	**Nuclear polymorphism**	**84.0/64.4/60.0/52.0**	**0.147(125)**	**100/85.7/84.6/50.0**	**0.004 (62)***	**33.3/42.1/40.0/50.0**	**0.767 (54)**
	5.1	71.4/56.4	0.097	92.3/69.6	0.018*	40.0/44.8	0.673
6.0	**Nuclear polymorphism, tumor front**	**66.7/75.0/68.8/57.5**	**0.507 (120)**	**100/94.7/64.2/64.7**	**0.054 (63)**	**20.0/46.7/40.0/54.5**	**0.363 (52)**
	6.1	72.9/62.5	0.302	96.2/75.0	0.029*	40.0/50.0	0.343
7.0	**Perineural infiltration**	**70.0/52.9/28.6**	**0.003 (114)***	**84.0/71.4/50.0**	**0.250 (59)**	**52.8/33.3/20.0**	**0.046 (50)***
	7.1	70.0/45.8	0.020*	84.0/66.7	0.208	52.8/28.6	0.098
8.0	**Lymphocytic infiltration**	**87.5/67.2/50.0**	**0.008 (123)***	**100/87.5/56.3**	**0.003 (63)***	**62.5/40.0/47.6**	**0.711 (54)**
	8.1	87.5/60.0	0.021*	100/77.1	0.050*	62.5/43.5	0.409
	8.2	72.9/50.0	0.007*	91.5/56.3	0.001*	45.5/47.6	0.821
9.0	**WPOI**	**100/70.6/82.8/58.9/56.3**	**0.190 (120)**	**100/80.0/100/77.8/66.7**	**0.224 (62)**	**÷/50.0/50.0/42.3/50.0**	**0.997 (52)**
	9.1	73.7/65.3	0.461	83.3/84.0	0.999	50,0/45.7	0.908
	9.2	79.2/58.3	0.022*	93.1/75.8	0.061	50.0/44.4	0.902

* Significant at 0.05 level

Figure 1 Tumor differentiation and lymphocyte infiltration. Well, moderate and poorly differentiated tumor in a-c. Marked, moderate and little lymphocyte infiltration in d-f.

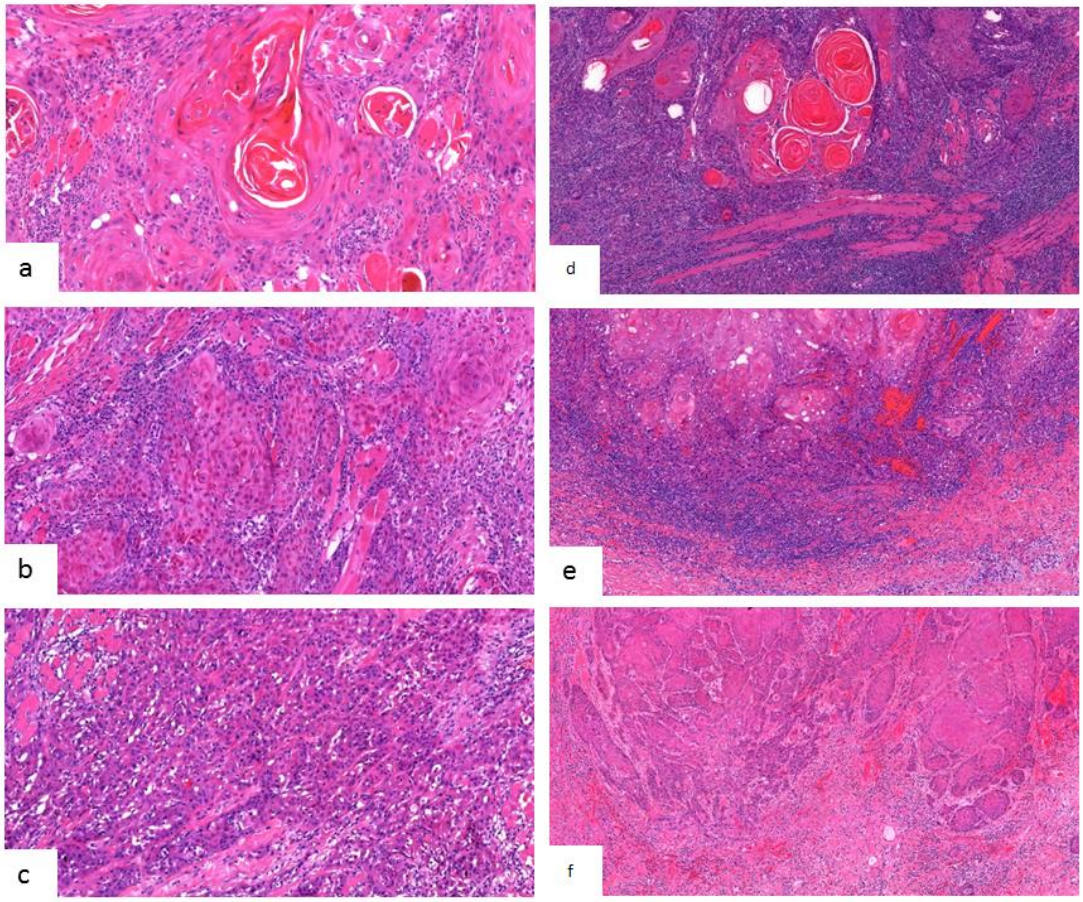
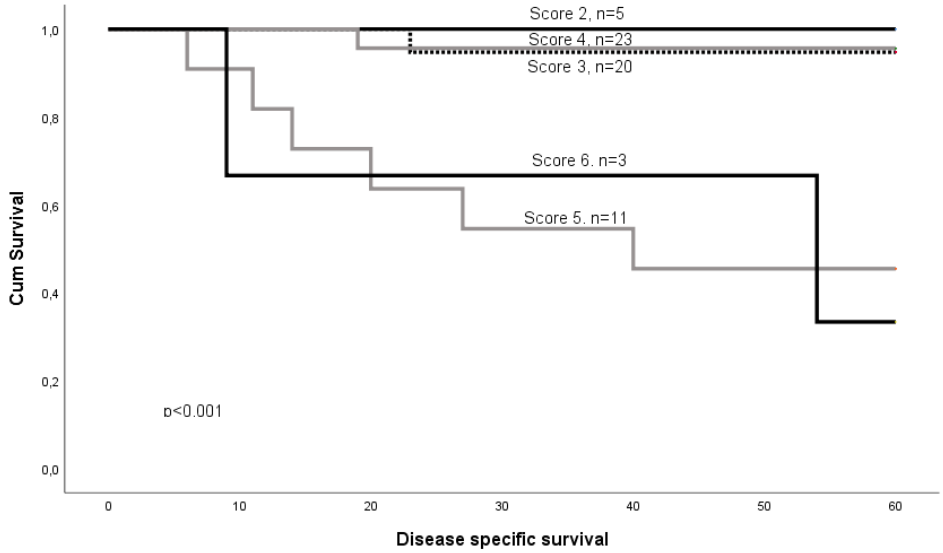


Figure 2 Kaplan-Meier curve showing survival of patients with low-stage disease stratified according to the histo-score



Supplementary table S1. Histopathological information for the whole cohort (n=150), the low-stage (n=77) and the high-stage group (n=63)

		Whole tumor		Low-stage		High-stage	
Variable		**n**	**%**	**n**	**%**	**n**	**%**
Differentiation whole tumor	Well	34	22.7	26	33.8	5	7.9
	Moderate	100	6.7	45	58.4	48	76.2
	Poorly	14	9.3	5	6.5	9	14.3
	Missing/Not evaluable	2	1.3	1	1.3	1	1.6
Differentiation worst pattern	Well	10	6.7	9	11.7	1	1.6
	Moderate	62	41.3	36	46.8	21	33.3
	Poorly	73	48.7	31	40.3	39	61.9
	Missing/Not evaluable	5	3.3	1	1.3	2	3.2
Keratinization whole tumor	High	33	22	23	29.9	8	12.7
	Moderate	60	40	29	37.7	30	47.6
	Minimal	29	19.3	12	15.6	13	20.6
	None	23	15.3	10	13.0	10	15.9
	Missing/Not evaluable	5	3.3	3	3.9	2	3.3
Keratinization tumor front*	High	0	0	0	0	0	0
	Moderate	15	10	14	18.7	1	1.6
	Minimal	41	27.3	23	29.9	17	27.0
	None	85	56.7	38	49.4	41	65.1
	Missing/Not evaluable	9	6	2	2.6	4	6.3
Nuclear polymorphism whole tumor	Little/none	31	20.7	23	29.9	7	11.1
	Moderate	51	34	26	33.8	19	30.2
	Abundant	35	23.3	15	19.5	18	28.6
	Extreme	29	19.3	11	14.3	17	27.9
	Missing/Not evaluable	4	2.7	2	2.6	2	3.2
Nuclear polymorphism tumor front*	Little/none	14	9.3	9	11.7	5	7.9
	Moderate	43	28.7	25	32.5	16	25.4
	Abundant	36	24	21	27.3	11	17.5
	Extreme	48	32	20	26.0	27	42.9
	Missing/Not evaluable	9	6	2	2.6	4	6.3
Perineural infiltration	None	106	70.7	60	77.9	42	66.7
	Invasive front	21	14	9	11.7	10	15.9
	Tumor center	7	4.7	2	2.6	5	7.9
	Missing/Not evaluable	16	10.7	6	7.8	6	9.5
Lymfocytic infiltrate	Marked	30	20	21	27.3	8	12.7
	Moderate	67	44.7	34	44.2	28	44.4
	Slight/none	46	30.7	21	27.3	24	38.1
	Missing/Not evaluable	7	4.7	1	1.3	3	4.8
Worst pattern of invasion (WPOI)	Type 1	3	2	2	2.6	1	1.6
	Type2	24	16	14	18.2	9	14.3
	Type 3	36	24	23	29.9	11	17.5
	Type 4	60	40	29	37.7	28	44.4
	Type 5	18	12	7	9.1	10	15.9
	Missing/Not evaluable	9	6	2	2.6	4	6.3

Supplementary Table S2. Variables and categorization and disease-specific survival for the whole cohort and for low-stage and high-stage disease separately. Separate columns for resection specimens only (biopsies excluded)

		Whole cohort (n=150)		Low-stage (n=77)		High-stage (n=63)	
		All	Resections	All	Resections	All	Resections
		p (n)	p (n)	p (n)	p (n)	p (n)	p (n)
1.0	**Differentiation whole tumor**	**<0.001 (126)***	**<0.001 (106)***	**0.003 (62)***	**0.002 (58)***	**0.055 (55)**	**0.035 (47)***
	Alternative 1.1	<0.001*	<0.001*	0.001*	0.008*	0.025 *	0.035
2.0	**Differentiation worst pattern**	**0.074 (123)**	**0.124 (106)**	**0.266 (62)**	**0.387 (58)**	**0.646 (54)**	**0.929 (47)**
	Alternative 2.1	0.061	0.081	0.134	0.219	0.676 (54)	0.929
3.0	**Keratinization**	**0.030 (123)***	**0.028 (104)***	**0.404 (60)**	**0.584 (56)**	**0.157 (54)**	**0.065 (46)**
	Alternative 3.1	0.007 *	0.004*	0.203	0.270	0.078	0.020*
4.0	**Keratinization tumor front**	**0.060 (119)**	**0.083 (106)**	**0.150 (61)**	**0.235 (58)**	**0.665 (52)**	**0.426 (47)**
	Alternative 4.1	0.033*	0.028*	0.168	0.179	0.374	0.350
5.0	**Nuclear polymorphism**	**0.176 (124)**	**0.423 (104)**	**0.005 (61)***	**0.023 (57)***	**0.767 (54)**	**0.514 (46)**
	Alternative 5.1	0.109	0.294	0.021*	0.057	0.673	0.185
6.0	**Nuclear polymorphism tumor front**	**0.548 (119)**	**0.822 (106)**	**0.061 (61)**	**0.134 (58)**	**0.363 (52)**	**0.290 (47)**
	Alternative 6.1	0.339	0.134	0.034*	0.050*	0.343	0.216
7.0	**Perineural infiltration**	**0.003 (113)***	**0.003 (101)***	**0.263 (58)**	**0.241 (55)**	**0.046 (50)***	**0.069 (45)**
	Alternative 7.1	0.023*	0.010*	0.221	0.200	0.098	0.080
8.0	**Lymphocytic infiltration**	**0.009 (122)***	**0.029 (106)***	**0.004 (62)***	**0.012 (58)***	**0.711 (54)**	**0.763 (47)**
	Alternative 8.1	0.019*	0.024*	0.047*	0.048*	0.409	0.492
	Alternative 8.2	0.008*	0.035*	0.001*	0.006*	0.921	0.908
9.0	**WPOI**	**0.218 (119)**	**0.323 (105)**	**0.248 (63)**	**0.249 (58)**	**0.997 (52)**	**0.833 (46)**
	Alternative 9.1	0.445	0.362	0.977	0.605	0.908	0.719
	Alternative 9.2	0.027*	0.050*	0.069	0.038*	0.902	0.734
	T (8th)	0.020 (113)*	0.005 (105)*	0.050 (63)*	0.019 (59)*	0.047 (50)	0.793 (46)
	cN0/pN0	<0.001 (128)*	<0.001 (107)*	Only N0	Only N0	0.016 (56)*	0.115 (47)

* Significant at 0.05 level

Supplementary table S3. Histo-score for patients with low-stage disease. Combination of differentiation of whole tumor (1.0) and lymphocyte infiltration (8.0)

Differentiation (score)	Lymphocytic infiltrate (score)	Sum of score (number of patients/dead*)
Well (1)	Marked (1)	2 (5/0)
Well (1)	Moderate (2)	3 (10/1)
Moderate (2)	Marked (1)	3 (10/0)
Well (1)	Slight/none (3)	4 (4/0)
Moderate (2)	Moderate (2)	4 (19/1)
Poor (3)	Marked (1)	4 (0/0)
Moderate (2)	Slight/none (3)	5 (9/5)
Poor (3)	Moderate (2)	5 (2/1)
Poor (3)	Slight/none (3)	6 (3/2)

*Dead within 5 years after diagnosis

Appendices

I. Letter of approval from REK-Nord (2013)

II. Letter of approval from REK-Nord (2015)

III. Informal and consent letter to the patients

IV. Letter with extension of time-period REK-Nord (2019)

V. Case Report Form

VI. Case Report Form; additional supplements histopathology

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord	Veronica Sørensen	77620758	25.06.2014	2013/1786/REK nord
			Deres dato:	**Deres referanse:**
			03.06.2014	

Vår referanse må oppgis ved alle henvendelser

Sonja E. Steigen
Postboks 46

2013/1786 Munnhulekreft,- en multisenterstudie for påvisning og verifisering av biomarkører som verktøy for mer individualisert behandling

Forskningsansvarlig institusjon: Oslo Universitetssykehus , St. Olavs Hospital, Oulu University Hospital
Prosjektleder: Sonja E. Steigen

Prosjektleders prosjektomtale
Antall personer som får munnhulekreft har økt de senere år. Kirurgi er førstevalg ved behandling og blir som regel kombinert med stråleterapi og eventuelt kjemoterapi. Ved små svulster uten holdepunkt for spredning prøver man å begrense kirurgien og mengden stråling, for å unngå overbehandling og plagsomme bivirkninger. Behandlingsstrategien ved munnhulekreft baseres i hovedsak på TNM-klassifisering (tumorstørrelse, metastasering til lymfeknuter eller andre organer). Generelt har pasienter med små svulster uten metastase (T1N0M0) best prognose, men det er store individuelle forskjeller i respons på behandling.Side 4 av 8Det finnes i dag ingen sikre markører som kan brukes til å forutsi hvilke tumorer som antas å ha et mer aggressivt forløp og hvilke som vil oppføre seg mer fredlig. Vi vil i denne studien undersøke markørmolekyler som kan være et supplement til TNM-klassifiseringen når man skal behandling av pasienten slik at den blir med individualisert.

Vurdering
Vi viser til tilbakemelding av 3.6.14

REK anser at tilbakemelding er i tråd med de merknader komiteen gav i sitt utsettelsevedtak av 15.5.14.

Etter fullmakt er det fattet slikt:

Vedtak
Med hjemmel i helseforskningsloven § 10 og forskningsetikkloven § 4 godkjennes prosjektet

Sluttmelding og søknad om prosjektendring
Prosjektleder skal sende sluttmelding til REK nord på eget skjema senest 01.10.2020, jf. hfl.
12. Prosjektleder skal sende søknad om prosjektendring til REK nord dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. hfl. § 11.

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningslovens § 28 flg. Klagen sendes til REK nord. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK nord, sendes klagen videre til

Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

May Britt Rossvoll

sekretariatsleder

Veronica Sørensen
rådgiver

Kopi til:kbj@ous-hf.no; trond.jacobsen@stolav.no; minna.makiniemi@ppshp.fi

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord	Veronica Sørensen	77620758	01.09.2015	2015/1381/REK nord
			Deres dato:	Deres referanse:
			27.08.2015	

Våreferanse må oppgis ved alle henvendelser

Lars Uhlin-Hansen
Institutt for medisinsk biologi

2015/1381 NOROC, en nasjonal multisenterstudie med formål å optimalisere behandlingen av pasienter med munnhulekreft.

Forskningsansvarlig institusjon: Universitetet i Tromsø, Universitetssykehuset Nord-Norge, OUS Rikshospitalet, Universitetet i Bergen, St. Olavs hospital, Haukeland universitetssykehus
Prosjektleder: Lars Uhlin-Hansen

Prosjektleders prosjektomtale
Munnhulekreft er forbundet med plagsomme, behandlingsrelaterte bivirkninger og relativt høy dødelighet. Det er imidlertid stor variasjon i aggressivitet i disse svulstene. Av og til vokser små svulster fort og fører til død innen 1-2 år, mens andre pasienter med svulster i samme stadium blir varig helbredet. Det er derfor nødvendig med bedre individtilpasset behandling som tar høyde for at svulstene oppfører seg forskjellig. Prosjektet er en nasjonal multisenterstudie basert på undersøkelse av svulstvev fra pasienter som munnhulekreft. Ved hjelp av mikroskopiske og molekylære metoder vil vi undersøke uttrykket av ulike molekyler i svulsten. Resultatene vil bli sammenholdt med opplysninger om bl.a. helbredelse, residiv og periode frem til sykdomsspesifikk død. Hovedmålet med prosjektet er å påvise molekyler i kreftcellene som kan brukes som grunnlag for valg av behandling, både for å begrense de behandlingsrelaterte bivirkningene og for å bedre sjansen for helbredelse.

Vurdering
Vi viser til skjema for tilbakemelding av 27.8.15, vedlagt revidert informasjonsskriv.

REK anser at tilbakemeldingen er i tråd med de merknader komiteen gav i sitt utsettelsesvedtak av 24.8.15.

Etter fullmakt er det fattet slikt:

Vedtak
Med hjemmel i helseforskningsloven §§ 2,9 10, samt forskningsetikkloven § 4 godkjennes prosjektet.

Sluttmelding og søknad om prosjektendring
Prosjektleder skal sende sluttmelding til REK nord på eget skjema senest 30.06.2021, jf. hfl. § 12. Prosjektleder skal sende søknad om prosjektendring til REK nord dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. hfl. § 11.

Klageadgang

Du kan klage på komiteens vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes til REK nord. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK nord, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Veronica Sørensen
Seniorrådgiver

Kopi til:terje.larsen@uit.no; gry.andersen@unn.no; teide@ous-hf.no; post@med.uib.no; harald.aarset@stolav.no; bjorn.inge.bertelsen@helse-bergen.no

UiT
NORGES
ARKTISKE
UNIVERSITET

INFORMASJON OM DELTAKELSE I FORSKNINGSPROSJEKTET

NOROC, -EN NASJONAL STUDIE OM MUNNHULEKREFT

Vi vil herved informere deg om dette forskningsprosjektet som omhandler pasienter som har blitt behandlet for kreft i munnhulen. Formålet med studien er å kartlegge molekyler i kreftcellene som kan benyttes til å gi fremtidige pasienter en best mulig behandling. I utgangspunktet vil alle pasienter som ble behandlet for munnhulekreft i Norge i perioden 2005-2009 bli innlemmet i studien. Universitetet i Tromsø er ansvarlig for gjennomføringen, men leger ved alle universitetssykehusene i landet er med som samarbeidspartnere.

HVA INNEBÆRER PROSJEKTET?

Når pasienter blir operert for kreft, blir kreftsvulsten undersøkt ved avdeling for patologi. Etter at diagnosen er stilt, vil deler av kreftsvulsten rutinemessig bli arkivert i en såkalt diagnostisk biobank ved sykehuset. Dette kreftvevet kan senere eventuelt bli benyttet til videre undersøkelser. I prosjektet vil vi benytte kreftvev fra alle pasienter som ble behandlet for munnhulekreft i Norge i perioden 2005-2009 til å studere forekomst av molekyler som har betydning for prognosen. For at man skal kunne gjøre det, er det også nødvendig å registrere opplysninger som er relevant for kreftsykdommen. Opplysningene hentes fra sykejournalen til den enkelte pasient av en overlege som er ansatt ved sykehuset.

HAR PROSJEKTET ULEMPER FOR DEG?

Det skal ikke tas noen nye prøver av deg og du trenger ikke svare på noen spørsmål. Det er kun opplysninger som allerede finnes i sykejournalen som vil bli benyttet. Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. Forskerne som deltar i prosjektet vil derfor ikke kunne gjenkjenne pasientene som er involvert i studien. Det vil være en kode som knytter deg til dine opplysninger gjennom en navneliste. Navnelisten oppbevares nedlåst i et arkivskap. Informasjonen som registreres om deg skal kun brukes slik som beskrevet i formålet med studien. Du har rett til innsyn i hvilke opplysninger som er registrert om deg og rett til å få korrigert eventuelle feil i de opplysningene som er registrert.

Prosjektleder Lars Uhlin-Hansen har ansvar for den daglige driften av forskningsprosjektet og at opplysninger om deg blir behandlet på en sikker måte. Informasjonen om deg vil bli slettet senest fem år etter prosjektslutt.

MULIGHET FOR Å RESERVERE SEG

Dersom du ikke ønsker at opplysninger fra din pasientjournal skal benyttes, eller at du ikke ønsker at det skal utføres undersøkelser på svulstvevet som ble fjernet i forbindelse med operasjonen, kan du reservere deg ved å kontakte professor Lars Uhlin-Hansen, Klinisk patologi, Universitetssykehuset Nord-Norge, 9038 Tromsø. Telefon 77627207, e-post: lars.uhlin.hansen@unn.no. Dette kan du gjøre uten å oppgi noen grunn og det vil selvfølgelig ikke få konsekvenser for din eventuelle videre behandling.

GODKJENNING

Prosjektet er godkjent av Regional komite for medisinsk og helsefaglig forskningsetikk (saksnr. 2015/1381)

UNIVERSITETET I TROMSØ

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord	Maren Melsbø	77620748	21.11.2019	28075
			Deres referanse:	

Sonja E. Steigen

28075 Munnhulekreft,- en multisenterstudie for påvisning og verifisering av biomarkører som verktøy for mer individualisert behandling

Forskningsansvarlig: Oslo universitetssykehus HF

Søker: Sonja E. Steigen

REKs vurdering

Vi viser til søknad om prosjektendring for ovennevnte forskningsprosjekt mottatt 21.11.2019. Søknaden er behandlet av sekretariatet i REK nord på delegert fullmakt fra komiteen, med hjemmel i forskningsetikkforskriften § 7, første ledd, tredje punktum. Søknaden er vurdert med hjemmel i helseforskningsloven § 11.

Prosjektleder opplyser i endringssøknaden at endringen gjelder forlengelse av prosjektslutt til 01.04.2025. Endringen er begrunnet med at *"Innsamling av materiale har tatt lenger tid enn forventet. Dette er nå fullført, og man har begynt analysering av data. Det gjenstår ennå mye arbeid med undersøkelse av markørmolekyler, og ber derfor om å få forlenget prosjektperioden."*

REK har ingen innvendinger til den omsøkte endringen.

Etter fullmakt er det fattet følgende

Vedtak

Godkjent

Med hjemmel i helseforskningsloven § 11 godkjennes prosjektendringen.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Maren Johannessen Melsbø
rådgiver

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes til REK nord. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK nord, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag (NEM) for endelig vurdering.

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: ID

ID

1. • **Gender**
   - ◎ Male
   - ◎ Female

2. • **Year of birth** *[integer]*

3. • **Date of diagnosis (dd.mm.yyyy)** *[date]*

4. • **County**
   - ◎ Østfold
   - ◎ Akershus
   - ◎ Oslo
   - ◎ Hedmark
   - ◎ Oppland
   - ◎ Buskerud
   - ◎ Vestfold
   - ◎ Telemark
   - ◎ Aust-Agder
   - ◎ Vest-Agder
   - ◎ Rogaland
   - ◎ Hordaland
   - ◎ Sogn og Fjordane
   - ◎ Møre og Romsdal
   - ◎ Sør-Trøndelag
   - ◎ Nord-Trøndelag
   - ◎ Nordland
   - ◎ Troms
   - ◎ Finnmark
   - ◎ Svalbard

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: ID

Signatures	CRF reporter / Researcher	Principal Investigator
Date (yyyy-mm-dd):	20 _ _ - _ _ - _ _	20 _ _ - _ _ - _ _
Place / Affiliation:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Name:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Signature:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
	(I hereby confirm that information provided in this form is a correct, accurate and complete representation of collected data.)	

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: Basic patient data

Basic patient data

1. **Patient alive?**
   - ◯ Yes
   - ◯ No

2. **Ev. time of death (dd.mm.yyyy)** *[date]*

3. **Level of education:**
   - ◯ Missing
   - ◯ No education
   - ◯ Finished primary school
   - ◯ Finished further school/high school
   - ◯ University degree

4. **If information about profession, please specify:** *[text]*

5. **Comorbidity:**
   - ☐ Missing
   - ☐ Heart and Coronary disease
   - ☐ COPD/Serious lung disease
   - ☐ Diabetic
   - ☐ Rheumatism
   - ☐ Transplanted
   - ☐ Previous cancer
   - ☐ Other
   - ☐ None

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: Basic patient data

6. **If previous cancer; please specify** *[text]*

7. **If other, please specify:** *[text]*

8. **Family history of cancer; 1.degree relatives, parent and/or siblings:**
   - ☐ Missing
   - ☐ Never
   - ☐ Others with head and neck cancer
   - ☐ Others with other types of cancer

9. **If others with other types of cancer please specify:** *[text]*

10. **Medication:**
    - ☐ Missing
    - ☐ None
    - ☐ Steroides
    - ☐ Immunosuppressiva
    - ☐ Antidiabetic insulin
    - ☐ Bisphosphonates
    - ☐ Others

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: Basic patient data

11. **If others, specify:** *[text]*

LIFESTYLE

12. **Smoking:**
- Missing
- Never
- Current
- Former

13. **If current, specify cigarettes/d** *[integer]*

14. **If former smoker; if possible please specify when stopped;**
- Stopped 0-1 year
- Stopped 1-9 year
- Stopped >=20 years
- Stopped 10-19 years

15. **Specify smoking if not clear above/Scandinavian snuff/use of illegal drugs if possible:** *[text]*

16. **Alcohol consumption**
- Missing
- Never
- Current
- Seldom
- Moderately
- Heavy
- Former alcoholic abuse

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: Basic patient data

17. **If current alcohol consumption, please specify: Beer intake (units/week)** *[integer]*

(please specify: one unit equals 1 normal beer, 1 glass of wine, 1 liquor/spiritus >= 40%)

18. **If current alcohol consumption, please specify: Wine intake (units/week)** *[integer]*

(please specify: one unit equals 1 normal beer, 1 glass of wine, 1 liquor/spiritus >= 40%)

19. **If current alcohol consumption, please specify: Liquor/spiritus >= 40% (units/week)** *[integer]*

20. **Height cm:** *[decimal 1]*

21. **Weight kg:** *[decimal 1]*

22. **Dental status (Good: needs no dental treatment before radiation) :**

- ◎ Missing
- ◎ Good
- ◎ Needs treatment
- ◎ Edentulous(toothless)

23. **Comments; please specify:** *[text]*

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: Basic patient data

Signatures	CRF reporter / Researcher	Principal Investigator
Date (yyyy-mm-dd):	20 _ _ - _ _ - _ _	20 _ _ - _ _ - _ _
Place / Affiliation:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Name:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Signature:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
	(I hereby confirm that information provided in this form is a correct, accurate and complete representation of collected data.)	

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: DIAGNOSE ORAL CAVITY TUMOR SITE (UICC)

DIAGNOSE ORAL CAVITY TUMOR SITE (UICC)

1. **Neoplasma malignum partis alterius et non specificatae linguae (tongue):**
   - ◎ CO2
   - ◎ CO2.0
   - ◎ CO2.1
   - ◎ C02.3
   - ◎ C02.8
   - ◎ C02.9

2. **Neoplasma malignum gingivae (alveolus and gingiva):**
   - ◎ CO3
   - ◎ CO3.0
   - ◎ CO3.1
   - ◎ CO3.9

3. **Neoplasma malignum basis cavi oris (floor of mouth):**
   - ◎ CO4
   - ◎ CO4.0
   - ◎ CO4.1
   - ◎ CO4.8
   - ◎ CO4.9

4. **Neoplasma malignum palati (hard palate):**
   - ◎ CO5
   - ◎ CO5.0
   - ◎ CO5.8
   - ◎ CO5.9

5. **Neoplasma malignum partis alterius et non specificatae oris (cheek mucosa, bucco-alveolar sulci, retromolar areas):**
   - ◎ CO6
   - ◎ CO6.0
   - ◎ CO6.1
   - ◎ CO6.2

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: DIAGNOSE ORAL CAVITY TUMOR SITE (UICC)

- CO6.8
- CO6.9

6. **Others/not classified:** *[text]*

7. • **Tumor stage TNM done?**
   - Yes
   - No
   - Only biopsi

cTNM

8. • **T**
   - T1
   - T2
   - T3
   - T4
   - T4a
   - T4b
   - Missing

9. • **N**
   - Nx
   - N0
   - N1
   - N2
   - N2a
   - N2b
   - N2c
   - N3
   - Missing

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: DIAGNOSE ORAL CAVITY TUMOR SITE (UICC)

10. **M**
- Mx
- M0
- M1
- Missing

11. **Recidive: (previous (primary)(could be diagnosed before 01.01.2005) (lesion occurring at a distance less than 2 cm from the index tumor and within 3 yrs) :**
- Yes
- No
- Not relevant

12. **Second primary: (lesion at a distance greater than 2 cm, synchronous: less than 6 months. Metachronous: greater than 6 months. Lesion at the same site or less than 2 cm more than 3 years):**
- Yes
- No
- Not relevant

Signatures	CRF reporter / Researcher	Principal Investigator
Date (yyyy-mm-dd):	20 _ _ - _ _ - _ _	20 _ _ - _ _ - _ _
Place / Affiliation:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Name:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Signature:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

(I hereby confirm that information provided in this form is a correct, accurate and complete representation of collected data.)

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: PATHOLOGICAL INFORMATION

PATHOLOGICAL INFORMATION

(to be submitted preferently by pathologist)

1. **Speciment**
   - ◎ Biopsy
   - ◎ Resection

2. **Tumor differentiation histology :**
   - ◎ Missing
   - ◎ Squamous cell carcinoma
   - ◎ Verrucous carcinoma
   - ◎ Others

3. **If others, please specify** *[text]*

4. **Differentiation, WHO classification, whole tumor**
   - ◎ Well
   - ◎ Moderate
   - ◎ Poor

5. **Differentiation, WHO classification, worst pattern**
   - ◎ Well
   - ◎ Moderate
   - ◎ Poor

6. **Degree of keratinization, whole tumor:**
   - ◎ Highly keratinized (>50 % of tumor areal)
   - ◎ Moderately keratinized (20-50% of tumor areal)
   - ◎ Minimal keratinisation (5-20% of tumor areal)
   - ◎ No keratinisation (0-5 % of tumor areal)

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: PATHOLOGICAL INFORMATION

7. **Nuclear polymorphism, whole tumor:**
   - ◎ Little nuclear polymorphism (in less than 25% of the cells)
   - ◎ Moderately abundant nuclear polymorphism (in 25-50% of the cells)
   - ◎ Abundant nuclear polymorphism (in 50-75% of the cells)
   - ◎ Extreme nuclear polymorphism (in 75-100% of cells)

8. **Worst pattern of invasion (WPOI) (Brandwein-Gensler 2005, 2012, POI 1-4 based on Bryne et al 1998)**
   - ◎ Pushing, well delineated infiltrating borders
   - ◎ Infiltrating, solid cords, bands and/or strands
   - ◎ Small grous of cords of infiltrating cells (n>15 cells)
   - ◎ Marked and widespread celleular dissociation in small groups and/or in single cells (n<15 cells)
   - ◎ Tumor satellites of any size with 1 mm or greater distance of intervening normal tissue (not fibrosis) at the tumor host interface
   - ◎ Missing/ not evaluable

9. **Tumor budding known?**
   - ◎ Yes
   - ◎ Missing/not evaluable

10. **Tumor budding: (Detached cluster of fewer than 5 cells at the invasive front of a tumor)** *[integer]*

    (Number of 'budded' groups in a 20X microscopic field)

11. **Degree of keratinization, within the lowest differentiated parts of the most invasive 2-3 cell layers at the advancing front of tumors (Bryne et al 1998):**
    - ◎ Highly keratinized (>50 % of the cells)
    - ◎ Moderately keratinized (20-50% of the cells)
    - ◎ Minimal keratinisation (5-20% of the cells)
    - ◎ No keratinisation (0-5 % of the cells)
    - ◎ Missing/ not evaluable

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: PATHOLOGICAL INFORMATION

12. **Nuclear polymorphism, within the lowest differentiated parts of the most invasive 2-3 cell layers at the advancing front of tumors (Bryne et al 1998):**
- ◎ Little nuclear polymorphism (In less than 25% of the cells)
- ◎ Moderately abundant nuclear polymorphism (In 25-50% of the cells)
- ◎ Abundant nuclear polymorphism (In 50-75% of the cells)
- ◎ Extreme nuclear polymorphism (In 75-100% of the cells)
- ◎ Missing/ not evaluable

13. **HPV status (p16 status, from the pathology reports):**
- ◎ Missing
- ◎ Positive
- ◎ Negative

14. **Status of surgical margins exist**
- ◎ Yes
- ◎ Missing

15. **Status of surgical margins (closest distance between tumor and surgical resection) mm** *[decimal 1]*

16. **Tumor thickness exist**
- ◎ Yes
- ◎ Missing/ not evaluable

17. **Tumor thickness mm, whole tumor (ad modum Woolgar 1995, figure 1, TA)** *[decimal 1]*

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: PATHOLOGICAL INFORMATION

18. **Depth of invasion exist**
- ◎ Yes
- ◎ Missing/ not evaluable

19. **Depth of invasion (ad modum Woolgar 1995, figure 1, TR)** *[decimal 1]*

20. **Tumor largest diameter**
- ◎ Macroscopic
- ◎ Microscopic in one slide (på ett snitt/oftest tverrsnitt)
- ◎ Microscopic estimate(ved uttak av hele tumor)
- ◎ Missing/not evaluable

21. **Tumor largest diameter mm** *[decimal 1]*

22. **Infiltration**
- ◎ Missing/ not evaluable
- ◎ Subepithelial tissue(submucosa/lamina propria)
- ◎ Muscle
- ◎ Bone

23. **Lymfocytic infiltrate**
- ◎ Marked
- ◎ Moderate
- ◎ Slight/none
- ◎ Missing/ not evaluable

24. **Perineural infiltration**
- ◎ None
- ◎ Nerves at invasive front
- ◎ Nerves in tumor center
- ◎ Missing/ not evaluable

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: PATHOLOGICAL INFORMATION

25. • **Vascular infiltration: (Presence of aggregates of tumor cells within endothelial-lined channels)**
- ◎ Not present
- ◎ Present

pTNM

26. • **T**
- ◎ T1
- ◎ T2
- ◎ T3
- ◎ T4
- ◎ Missing

27. • **N**
- ◎ N0
- ◎ N1
- ◎ N2
- ◎ N2a
- ◎ N2b
- ◎ N2c
- ◎ N3
- ◎ Missing

28. • **M**
- ◎ M0
- ◎ M1
- ◎ Missing

29. **Dysplasia in the surgical margins(reseksjonsrender)**
- ◎ No
- ◎ Low grade (grad 1-2)
- ◎ High grade (grad 3)
- ◎ Missing/not evaluable

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: PATHOLOGICAL INFORMATION

Signatures	CRF reporter / Researcher	Principal Investigator
Date (yyyy-mm-dd):	20 _ _ - _ _ - _ _	20 _ _ - _ _ - _ _
Place / Affiliation:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Name:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Signature:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
	(I hereby confirm that information provided in this form is a correct, accurate and complete representation of collected data.)	

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: TREATMENT

TREATMENT

1. **Treatment was discussed (before/under treatment) in multidisciplinary teams MDT (MDT with members from two or more departments or functional areas)**
   - ◎ Missing
   - ◎ Yes
   - ◎ No

2. **Please specify departments:** *[text]*

3. **Time for referral exist**
   - ◎ Yes
   - ◎ No

4. **Date for refferal if it exist: (dd.mm.yyyy)** *[date]*

5. **Time from primary referral to treatment (weeks)** *[integer]*

6. **Referred from**
   - ◎ General practitioner
   - ◎ Specialist
   - ◎ Missing

7. **Operation performed**
   - ◎ Curative
   - ◎ Palliative

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: TREATMENT

8. **Access:**
- ◎ Missing
- ◎ Oral
- ◎ Mandibular split
- ◎ Lip split
- ◎ Lip split over
- ◎ Lip split under

9. **Type of surgery:**
- ☐ Soft tissue resection
- ☐ with marginal mandible resection
- ☐ with segmental mandible resection
- ☐ with maxilla resection
- ☐ elective tracheostomy
- ☐ Other

10. **If other, describe:** *[text]*

11. **Neck surgery:**
- ◎ Missing
- ◎ No neck surgery
- ◎ Elective neck 1,2,3 (N0 neck)
- ◎ Selective neck 1,2,3 (N+ neck, supraomohyoidal hgd)
- ◎ Modified radical neck (saves one or more of v.j.i /n.accessory/m.scm)
- ◎ Radical neck

12. **Postoperative complication (first 30 days):**
- ☐ None
- ☐ Haemathoma/bleeding (needed secondary surgery)
- ☐ Infection
- ☐ Necrosis of area
- ☐ Incomplete healing
- ☐ Postoperative cardiac complications
- ☐ Postoperative pneumoni
- ☐ Postoperative emboli/tromboemboli

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: TREATMENT

- [ ] Death
- [ ] Other
- [ ] Missing

13. **If postoperative emboli/tromboemboli**
- ◉ Pulmonary
- ◉ Extremeties
- ◉ Cerebral

14. **If death peri-/postoperative:** *[text]*

15. **If other, describe:** *[text]*

16. **Type of reconstruction:**
- [ ] Missing
- [ ] None
- [ ] Nasolabial
- [ ] Pedickel flap
- [ ] Distant flap
- [ ] Free flap
- [ ] Split skin
- [ ] Primary closure
- [ ] Other

17. **Adjuvant radiation**
- [ ] Missing
- [ ] Primary (no surgery done)
- [ ] Pre-operative
- [ ] Post-operative
- [ ] Conventional
- [ ] IMRT
- [ ] None

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: TREATMENT

18. **Dosage Gy exist**
- ◎ Yes
- ◎ No

19. **Dosage Gy** *[integer]*

20. **Fractions per week** *[integer]*

21. **Target**
- ☐ Unilateral
- ☐ Bilateral
- ☐ Neck region 1,2,3,
- ☐ Whole neck
- ☐ Primary site
- ☐ Other

22. **If other, describe** *[text]*

23. **Chemotherapy**
- ◎ Missing
- ◎ Yes
- ◎ No

24. **If yes**
- ◎ Concomitant
- ◎ Neo-adjuvant
- ◎ Adjuvant

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: TREATMENT

25. **Type of cytostatica** *[text]*

26. **Dosage cytostatica given** *[text]*

27. • **Naxogin**
- ◉ Yes
- ◉ No

28. **Withdrawal from treatment, and why** *[text]*

Signatures	CRF reporter / Researcher	Principal Investigator
Date (yyyy-mm-dd):	20 _ _ - _ _ - _ _	20 _ _ - _ _ - _ _
Place / Affiliation:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Name:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Signature:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

(I hereby confirm that information provided in this form is a correct, accurate and complete representation of collected data.)

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: FOLLOW UP

FOLLOW UP

Time for follow up until 01.06.2015

1. **Follow up (months) :** *[integer]*

2. • **Has continued smoking**
   - ◎ Yes
   - ◎ No
   - ◎ Non smoker
   - ◎ Missing

3. • **Dental rehabilitation**
   - ◎ Yes
   - ◎ No
   - ◎ Missing

4. • **Local recurrence/Recidive: (lesion occurring at a distance less than 2 cm from the index tumor and within 3 yrs)**
   - ◎ Yes
   - ◎ No

5. **Eventual time of recidive local recurrence (dd.mm.yyyy)** *[date]*

6. • **Recidive regional metastases**
   - ◎ Yes
   - ◎ No

7. • **Recidive distant metastases**
   - ◎ Yes
   - ◎ No

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: FOLLOW UP

8. **Eventual time of recidive distant metastases (dd.mm.yyyy)** *[date]*

9. **If metastasis**
   - ☐ Lung
   - ☐ Bone
   - ☐ Liver
   - ☐ Brain

10. **Treatment of recidiv** *[text]*

11. **Persistent/Residual disease: (no disease control within 6 months after treatment)**
    - ◯ Yes
    - ◯ No

12. **Eventual time of local recurrence/recidive (dd.mm.yyyy)** *[date]*

13. **Second primary: (lesion at a distance greater than 2 cm, synchronous: less than 6 months. Metachronous: greater than 6 months. Lesion at the same site or less than 2 cm more than 3 yrs)**
    - ◯ Yes
    - ◯ No

14. **Treatment of second primary** *[text]*

Date (yyyy-mm-dd): 20 _ _ - _ _ - _ _ **Patient ID / Initials:** _ _ _ _ _ _ _ _ _ _ / _ _ _
Oralcancer register: FOLLOW UP

15. **Long term complications**

- ☐ Damage to accessory nerve/shoulder-syndrome
- ☐ Necrosis of soft tissue
- ☐ Osteoradionecrosis
- ☐ Serious dental problem
- ☐ Long term nasogastric tube
- ☐ Long time PEG
- ☐ Long time tracheostomy
- ☐ Severe trismus
- ☐ Serious speech problem
- ☐ Hyperbar 0^2
- ☐ Xerostomi
- ☐ Others
- ☐ None

16. **If others, please describe** *[text]*

Signatures	CRF reporter / Researcher	Principal Investigator
Date (yyyy-mm-dd):	20 _ _ - _ _ - _ _	20 _ _ - _ _ - _ _
Place / Affiliation:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Name:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Signature:	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

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NOROC
THE NORWEGIAN ORAL CANCER STUDY

REGISTRERING AV PATOLOGIPARAMETRE - WebCRF

PATHOLOGICAL INFORMATION

(to be submitted preferently by pathologist)

1. * Speciment
   - ○ Biopsy
   - ○ Resection

2. * Tumor differentiation histology :
   - ○ Missing
   - ○ Squamous cell carcinoma
   - ○ Verrucous carcinoma
   - ○ Others

3. If others, please specify

4. * Differentiation, WHO classification, whole tumor
   - ○ Well
   - ○ Moderate
   - ○ Poor

5. * Differentiation, WHO classification, worst pattern
   - ○ Well
   - ○ Moderate
   - ○ Poor

Squamous cell carcinoma, differentiation, WHO

Well differentiated	Moderately differentiated	Poorly differentiated
Cells are large and slightly fusiform, nuclei shows a moderate degree of pleomorphism, few mitoses are seen. Lamelled keratin masses (keratin pearls) characteristic feature.	Nuclear pleomorphism more distinct. Higher mitotic activity, including abnormal mitoses. Keratinization is less prominent.	Dominated by immature cells, numerous typical and atypical mitoses, high nucleus/cytoplasmic ratio. Keratin pearl formation not seen, though individual cell keratinization might be present.

6. * Degree of keratinization, whole tumor:
   - ○ Highly keratinized (>50 % of tumor areal)
   - ○ Moderately keratinized (20-50% of tumor areal)
   - ○ Minimal keratinisation (5-20% of tumor areal)
   - ○ No keratinisation (0-5 % of tumor areal)

7. * Nuclear polymorphism, whole tumor:
   - ○ Little nuclear polymorphism (in less than 25% of the cells)
   - ○ Moderately abundant nuclear polymorphism (in 25-50% of the cells)
   - ○ Abundant nuclear polymorphism (in 50-75% of the cells)
   - ○ Extreme nuclear polymorphism (in 75-100% of cells)

8. * Worst pattern of invasion (WPOI) (Brandwein-Gensler 2005, 2012, POI 1-4 based on Bryne et al 1998)

- ○ Pushing, well delineated infiltrating borders
- ○ Infiltrating, solid cords, bands and/or strands
- ○ Small grous of cords of infiltrating cells (n>15 cells)
- ○ Marked and widespread celleular dissociation in small groups and/or in single cells (n<15 cells)
- ○ Tumor satellites of any size with 1 mm or greater distance of intervening normal tissue (not fibrosis) at the tumor host interface
- ○ Missing/ not evaluable

9. Tumor budding known?

- ○ Yes
- ○ Missing/not evaluable

10. * Tumor budding: (Detached cluster of fewer than 5 cells at the invasive front of a tumor)

[ ]

Number of "budded" groups in a 20X microscopic field

Save

11. * Degree of keratinization, within the lowest differentiated parts of the most invasive 2-3 cell layers at the advancing front of tumors (Bryne et al 1998):

- ○ Highly keratinized (>50 % of the cells)
- ○ Moderately keratinized (20-50% of the cells)
- ○ Minimal keratinisation (5-20% of the cells)
- ○ No keratinisation (0-5 % of the cells)
- ○ Missing/ not evaluable

12. * Nuclear polymorphism, within the lowest differentiated parts of the most invasive 2-3 cell layers at the advancing front of tumors (Bryne et al 1998):

- ○ Little nuclear polymorphism (In less than 25% of the cells)
- ○ Moderately abundant nuclear polymorphism (In 25-50% of the cells)
- ○ Abundant nuclear polymorphism (In 50-75% of the cells)
- ○ Extreme nuclear polymorphism (In 75-100% of the cells)
- ○ Missing/ not evaluable

13. * HPV status (p16 status, from the pathology reports):

- ○ Missing
- ○ Positive
- ○ Negative

14. * Status of surgical margins exist

- ○ Yes
- ○ Missing

15. Status of surgical margins (closest distance between tumor and surgical resection) mm

[ ]

Alle mål er i millimeter, kan også bruke desimal

16. * Tumor thickness exist

- ○ Yes
- ○ Missing/ not evaluable

17. Tumor thickness mm, whole tumor (ad modum Woolgar 1995, figure 1, TA)

18. * Depth of invasion exist

- ○ Yes
- ○ Missing/ not evaluable

19. Depth of invasion (ad modum Woolgar 1995, figure 1, TR)

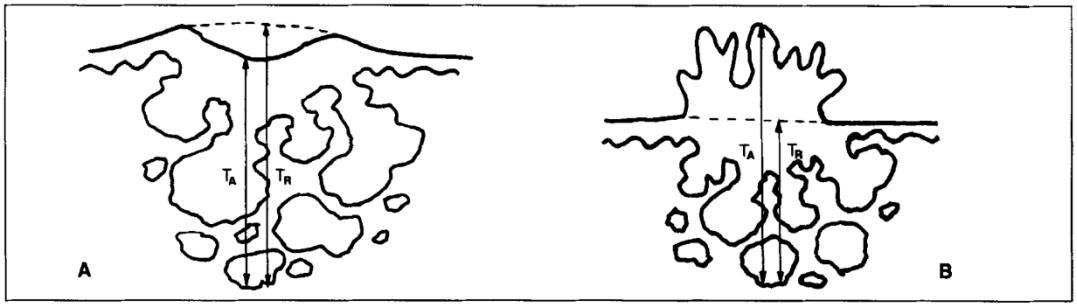

FIGURE 1. (A) Actual tumor thickness (T_A) and reconstructed tumor thickness (T_R) in an ulcerated, endophytic carcinoma. T_A was measured from the floor of the ulcer to the deepest extent of growth at the advancing tumor front. T_R was measured from a theoretically reconstructed normal mucosal line to the deepest extent of growth. **(B)** T_A and T_R in an exophytic carcinoma. T_A was measured from the papillary surface (excluding surface keratin and inflammatory exudate) to the deepest extent of growth at the advancing tumor front. T_R was measured from a theoretically reconstructed normal mucosal line to the deepest extent of growth.

20. Tumor largest diameter

- ○ Macroscopic
- ○ Microscopic in one slide (på ett snitt/oftest tverrsnitt)
- ○ Microscopic estimate(ved uttak av hele tumor)
- ○ Missing/not evaluable

Save

21. Tumor largest diameter mm

22. * Infiltration

- ○ Missing/ not evaluable
- ○ Subepithelial tissue(submucosa/lamina propria)
- ○ Muscle
- ○ Bone

23. * Lymfocytic infiltrate

- ○ Marked
- ○ Moderate
- ○ Slight/none
- ○ Missing/ not evaluable

Histologic variable	Marked	Moderate	Slight/none
Lymphocytic infiltrate at interface	Continous band	Large patches	Little or none

24. * Perineural infiltration

- ○ None
- ○ Nerves at invasive front
- ○ Nerves in tumor center
- ○ Missing/ not evaluable

25. * Vascular infiltration: (Presence of aggregates of tumor cells within endothelial-lined channels)

- ○ Not present
- ○ Present

pTNM

26. * T

- ○ T1
- ○ T2
- ○ T3
- ○ T4
- ○ Missing

27. * N

- ○ N0
- ○ N1
- ○ N2
- ○ N2a
- ○ N2b
- ○ N2c
- ○ N3
- ○ Missing

28. * M

- ○ M0
- ○ M1
- ○ Missing

29. Dysplasia in the surgical margins(reseksjonsrender)

- ○ No
- ○ Low grade (grad 1-2)
- ○ High grade (grad 3)
- ○ Missing/not evaluable

Andre opplysninger / Additional Information or Corrections

SES, november 2016