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Prognostic impact of the loss of E-cadherin and *de novo* expression of N-cadherin at the invasive front of primary and recurrent oral squamous cell carcinoma

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1. Abbreviations

CAFs	Cancer-Associated Fibroblasts
CBCT	Cone-beam computed tomography
CCI	Charlson Comorbidity Index
CI	Confidence interval
CT	Computed tomography
CTC	Circulating tumor cells
EBV	Epstein-Barr virus
ECE	Extracapsular extension
ECAD	E-cadherin
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
EMT	Epithelial–mesenchymal transition
EMT-TFs	EMT-promoting transcription factors
FISH	Fluorescence in-situ hybridization
GBM	Glioblastoma multiforme
GEKID	Gesellschaft der epidemiologischen Krebsregister in Deutschland
HIF-1	Hypoxia-inducible factor-1
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papillomavirus
HR	Hazard Ratio
IHC	Immunohistochemistry
miR-134	microRNA
MET	Mesenchymal–epithelial transition
MDSCs	Myeloid-derived suppressor cells
MRI	Magnetic resonance imaging
MAPK/ERK	mitogen-activated protein kinases/ extracellular signal-regulated kinases
mTOR	Mammalian target of rapamycin
NCAD	N-cadherin
OPL	Oral premalignant lesion
OSCC	Oral Squamous Cell Carcinoma
PET-CT	Positron emission tomography–computed tomography
PD-1	Programmed cell death protein 1
PDCD7	Programmed Cell Death 7
pr DFS	post-recurrence disease-free survival
pr OS	post-recurrence survival

pr OCSS	post-recurrence oral cancer-specific survival
PTEN	Phosphatase and tensin homolog
PKM2	pyruvate kinase M2
ROI	Region of interest
ROS	Reactive oxygen species
TAMS	Tumor-associated macrophages
TGF	Transforming growth factor
TMA	Tissue microarray
US	Ultrasound
RT	Radiotherapy
RCT	Radio-Chemotherapy
RKI	Robert Koch-Institut
WHO	World Health Organization
Wnt	Wingless-INT

2. Introduction

Oral cancer encompasses malignancies of the floor of the mouth, tongue, gum, lip, palate, and gingiva and is one of the most common kinds of squamous cell carcinoma of the head and neck. Oral squamous cell carcinoma accounts for 91% of all instances of oral cancer [1].

Head and neck squamous cell carcinomas (HNSCCs) are the most common malignancies of the head and neck, arising from the mucosal epithelium of the oral cavity, larynx and pharynx. The risk of HNSCC varies by nation and area, and it has been associated with exposure to tobacco-derived carcinogens, excessive alcohol use, or both. Previous infections with the carcinogenic strains of human papillomavirus (HPV), like HPV-16 and HPV-18, increase the risk of oropharyngeal tumors [2-4].

Successful vaccination campaigns might prevent HPV-positive HNSCC across the globe since the two most prevalent carcinogenic HPVs, HPV-16 and HPV-18, are covered by FDA-approved HPV vaccines. Smoking is still a significant risk factor for oral cavity and laryngeal HNSCCs, now known as HPV-negative HNSCCs. However, a comprehensive physical examination is still the best method for early OSCC identification since there hasn't yet been a reliable screening procedure. Even though only a small number of oral pre-malignant lesions (OPLs) that show up as leukoplakia (white patches) or erythroplakia (red patches) turn into invasive cancer, most people with advanced stage HNSCC do not have pre-cancerous lesions. Generally speaking, OSCC is often treated with surgical resection followed by adjuvant radiation (RT) or radiochemotherapy (RCT), depending on the stage of the malignancy [5].

2.1 Incidence and prevalence

With 890,000 new cases and 450,000 mortalities in 2018, HNSCC is the sixth most frequent malignancy worldwide [5]. The global cancer observatory predicts that the incidence of HNSCC would climb by 30% (or 1.08 million new cases per year) by 2030 [6,

[7]. The high prevalence of HNSCC in Southeast Asia and Australia is linked to the intake of specific carcinogen-containing substances, whereas in the United States and Western Europe is linked to growing rates of oropharyngeal infection with HPV [8-10]. In general, men are two to four times more likely than women to develop HNSCC. The median age of HNSCC patients without a viral association is 66 years, but the median ages of patients with HPV-associated oropharyngeal cancer and Epstein-Barr virus (EBV)-associated nasopharyngeal cancer are 53 years and 50 years, respectively [11, 12].

Each year, approximately 10,000 individuals in Germany develop malignant tumors of the oral cavity and pharynx. Men are affected by the disease at a higher rate than women, with an average of 7600 men and 2800 women developing it annually since 2000. Additionally, men tend to develop the disease at a younger age, with a median onset of 61 years compared to 65 years for women. Despite improved interdisciplinary diagnostic and therapeutic strategies, the 5-year relative survival rate in Germany for oral cavity and pharynx ranged from 44 to 50% for men and 55 to 72% for women according to RKI and GEKID in 2013.

2.2 Risk factors

Smoking and excessive alcohol use are major risk factors for developing oral cancer, these risk factors are generally avoidable. [13] , when such risk variables are discovered simultaneously, they may have an enhancing effect [14].

2.2.1 Tobacco

Cigarette smoke attenuates oral immunity by inducing gingivitis, periodontitis, and oral cancer [15]. Tumor growth is induced by suppressing the tumor suppressor genes, particularly p53 and PTEN (phosphatase and tensin homolog). Smokers have a threefold increased chance of acquiring mouth cancer compared to nonsmokers [16]. Further, passive or second-hand smokers have an 87% higher risk of oral cancer than never-smokers who had never been exposed [17]. All tobacco products have different amounts

of cancer-causing chemicals like polycyclic hydrocarbons and tobacco-specific N-nitrosamines, which are known to play a big role in the development of cancer. Nicotine may contribute to cancer development by promoting a number of essential processes, according to evidence from experimental in vitro research on cell cultures, in vivo studies on rodents, and human investigations, including epidemiological studies [18]. Nicotine binds with a greater affinity to nicotine acetylcholine receptors than acetylcholine does. Nicotine's interaction with nicotine acetylcholine receptors triggers signaling pathways, resulting in a variety of responses, including increased cell proliferation [19, 20]. Nicotine induces epithelial-to-mesenchymal transition, a crucial stage in the development of a malignant phenotype. This change enables the cell to develop migratory capabilities, which may promote cancer metastasis [21].

2.2.2 Alcohol

According to epidemiological research, drinking alcohol increases the incidence of HNSCC in a dose-dependent manner. Yet, ethanol consumption has two aspects that affect health outcomes: first, Time-related patterns of consumption, such as age at beginning of consumption and duration, may affect the association between drinking intensity and cancer risk, and second, the amount of alcohol consumed [22]. Although it is unclear how alcohol contributes to oral carcinogenesis, numerous pathways have been postulated. To begin, ethanol is converted into acetaldehyde, a recognized carcinogen. Because acetaldehyde is a tumor trigger, persistent alcohol drinking promotes the development of oral cancer [23]. According to earlier studies, alcohol makes the oral mucosa more permeable, which causes epithelial atrophy. In addition, alcohol degrades the lipid composition of the oral mucosa's epithelial cell membrane, enabling carcinogens to infiltrate [24]. Acetaldehyde production has been linked to some Streptococcus species, Neisseria species, and other bacteria. Such bacteria have been shown to proliferate in smokers and heavy drinkers. Because these bacteria may

convert ethanol to carcinogenic acetaldehyde, they are linked to an increased risk of HNSCC [25].

2.2.3 Areca Nuts

Areca-nut-containing betel quid is the fourth most commonly used addictive stimulant worldwide, behind alcohol, caffeine, and cigarettes. Researchers from King's College London were the first to identify a dependency syndrome associated with areca nut consumption [26]. A Taiwanese study revealed that holding and then ingesting betel-quid juice and putting unripened whole areca fruit in the quid appeared to increase the risk of oral cancer by 11-fold [27]. Chewing areca nuts is considered a risk factor for developing possibly malignant oral submucous fibrosis, which results in oral and esophageal squamous cell carcinoma [28]. Moreover, contact with its extract resulted in oral mucosa deterioration as well as genotoxic and cytotoxic consequences on oral keratinocytes and fibroblasts. The active form arecoline N-oxide is solely accountable for areca-related oral carcinogenesis [28]. Additionally, areca nut extract enhances the formation of prostaglandins and cyclooxygenase-2, which are essential inflammatory mediators during the process of tumorigenesis and metastasis [29]. In order to determine whether an educational intervention program resulted in the cessation of chewing and a decrease in incident leukoplakia, prospective cohort research was carried out in India. The key finding following a 10-year follow-up showed that the incidence of oral leukoplakia decreased significantly [30].

2.2.4 Viral Infection

It is generally known that HPV contributes to pharyngeal cancer. However, further research is still being done on its impact on the oral cavity [31].

2.3 Histopathology

OSCC development is fostered by the accumulation of genetic transformations and epigenetic aberrations in cancer-associated signaling pathways [32]. Histologically, the

lesion goes through many different stages (preneoplastic damage) before it turns into cancer [33].

Lesions that appear in the epithelium during the process of tumorigenesis can be categorized based on their histopathological presentation as reactive epithelial changes (such as hyperkeratosis, hyperplasia, and acanthosis) or preneoplastic alteration (including mild, moderate, and severe dysplasia, before the development of an invasive carcinoma). Figure 1 depicts the progression from dysplasia to invasive cancer. OSCC begins as an epithelial dysplasia and is distinguished by the abnormal proliferation of dysplastic squamous cells on the epithelial layer's surface, which affects the subepithelial basement membrane. Basement membrane deterioration eventually causes devastation and metastasis. Local invasion of the underlying tissue also develops through epithelial cell islets and cords. Tumor cells' propensity to metastasize is strongly tied to their differentiation grade, corresponding to the architecture of neoplastic tissue and normal epithelium [33-37].

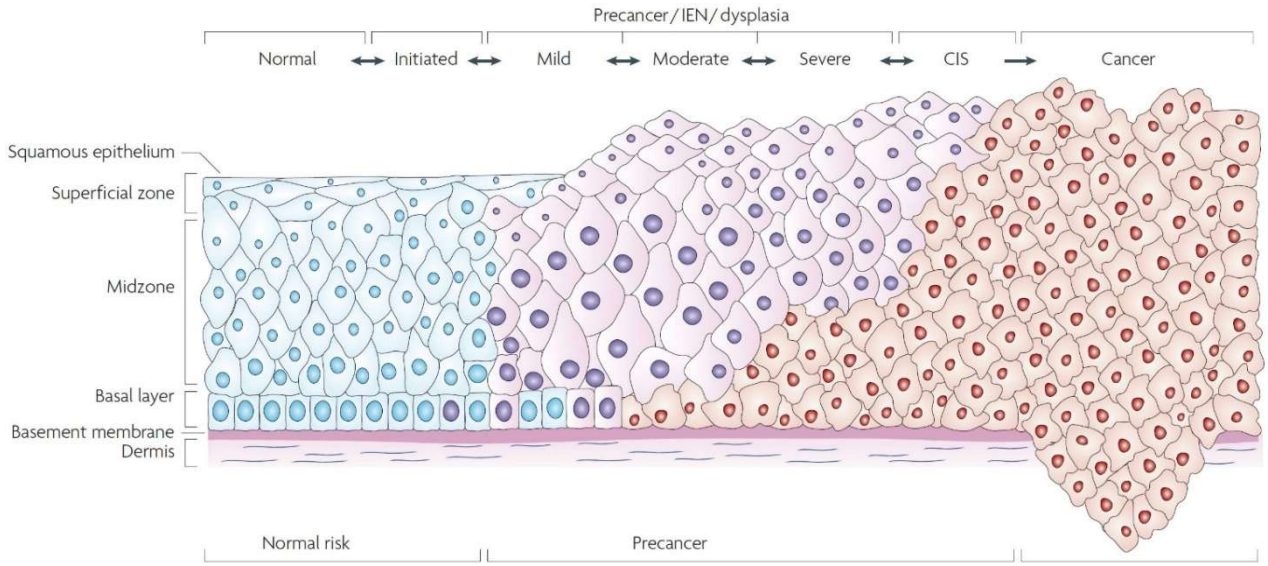

Figure 1: Schematic illustration of the development of epithelial cancer, modified according to Kelloff et al. [38]

2.4 Diagnosis and Staging

Despite advances in therapy over the last 20 years, there has been no significant increase in the 5-year survival rate in OSCC [39]. Even though there are novel methods for diagnosing oral cancer, biopsy and histopathologic analysis continues to be the gold standard to diagnose OSCC [40]. Premalignant or malignant oral lesions may be accurately diagnosed according to the standard of the biopsy, the availability of sufficient clinical data, and the analysis of the biopsy findings [41]. However, oral squamous cell carcinoma can form in intact oral mucosa, the vast majority of malignancies emerge from precursor lesions such as leukoplakia, erythroplakia, and erythroleukoplakia [42].

Positive lymph nodes are a substantial negative predictive indicator for survival [43]. Accordingly, a diagnostic evaluation prior to surgery is required for disease staging. To stage OSCC, computed tomography (CT) scans with contrast agent of the head, neck, and chest are routinely used to complete and optimize the staging of patients with OSCC. They are crucial in identifying tumor size, bone invasion, possible cervical node metastases, and pulmonary metastases [44].

Alternatively, ultrasound (US), magnetic resonance imaging (MRI) and in some cases, Positron emission tomography–computed tomography (PET-CT) can be employed. Especially for OSCC, an orthopantomogram and/or Cone-Beam CT (CBCT) are usually used to evaluate alveolar bone invasion and the degree of required bone resection [45]. PET-CT is a type of scan in which a radioactive substance is given intravenously to the patient and is taken up by cells with a high metabolic rate, which is a characteristic of many oral cancer types. In contrast, infection and inflammation may have comparable radiologic outcomes. As a result, it is commonly utilized in advanced diseases (stage 3 or 4), salvage/recurrent cases, and metastatic disease evaluations [46, 47].

One of the most distinguishing clinical aspects of OSCC is its ability to locally invade surrounding tissues and metastasis. As a result, it is critical to predicting the invasive

and metastatic potential of OSCC early in the therapy process. In addition, a variety of biological markers have been developed in recent years that may improve diagnosis and provide crucial prognostic information for the management of OSCC. Molecular markers that signify a high local recurrence rate in surgically treated patients could aid in identifying patients who would benefit from postoperative radiotherapy. Furthermore, markers that indicate local recurrence in patients who have received radiotherapy could help identify highly radioresistant cancer forms. Concurrent chemotherapy, radiosensitizing drugs, adjusted radiation fractionation regimens, and other treatments targeting specific tumor molecular aberrations may represent an adjuvant therapy in patients with such cancers [48].

2.5 Tumor Classification

Precise and reliable tumor staging is important for determining the type of treatment. According to the stage of the tumor, different aspects are decided, like curative or palliative treatment, the treatment planning (extent of resection in case of surgical treatment, or region of radiation therapy in case of primary or adjuvant radiation(chemo) therapy. The disease's staging also provides significant prognostic information to treating physicians and patients.

Staging of OSCC is performed according to the system developed by the Union for International Cancer Control (UICC) for malignant tumors" (Table 1). In addition to tumor size (T), this classification describes the existence of locoregional metastases (N) as well as distant metastasis (M) with organ involvement as seen in table 1 [49].

Table 1: The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging system for OSCC [50].

TNM classification of carcinomas of the lip and oral cavity	
T	Primary tumor
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor

Tis	Carcinoma in situ
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension
T4a (oral cavity)	Tumor invades through cortical bone, into deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), maxillary sinus, or skin of face
T4b (lip and oral cavity)	Tumor invades masticator space, pterygoid plates, or skull base; or encases internal carotid artery
Note: Superficial erosion alone of bone/tooth socket by gingival primary is not sufficient to classify a tumor as T4	
N - Regional Lymph Nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis as specified in N2a, 2b, 2c below
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension
T4b (lip and	Tumor invades masticator space, pterygoid plates, or skull base; or encases internal carotid artery
Note: Midline nodes are considered ipsilateral nodes.	

M - Distant metastasis	
M0	No distant metastasis
M1	Distant metastasis

Stage grouping		
Stage 0	Tis	N0
Stage I	T1	N0

Stage II	T2	N0
Stage III	T1, T2	N1
	T3	N0, N1
Stage IVA	T1, T2, T3	N2
	T4a	N0, N1, N2
Stage IVB	Any T	N3
	T4b	Any N
Stage IVC	Any T	Any N

2.6 Management of Oral Squamous Cell Carcinoma

The majority of oral cavity cancer treatment regimens include surgery. Invasive procedures such as surgery have various advantages, including the ability to harvest a specimen for histopathologic examination and the ability to remove the cancer with a single treatment method in a single session. Although primary radiation to T1 and T2 lesions may provide comparable disease management, the negative effects of radiation to the oral environment outweigh those of surgery in the majority of cases. Additionally, it is frequently preferable to postpone radiation whenever feasible in case it becomes necessary in the future for second primary malignancies among patients with head and neck cancer. The oral cancer patient population is vulnerable to the formation of second primary malignancies. Some believe that radiation for borderline indications should be preserved for future use if the need arises. [51].

Patients with advanced local disease T3-T4, advanced nodal disease N2-N3, or tumors with lymphovascular and perineural invasion may benefit from radiotherapy (RT) [52-54]. Combining RCT for patients with either extracapsular extension (ECE) or positive margins enhances locoregional control and overall survival, according to two randomized studies [55, 56]. In the past, all patients at high risk of locoregional failure were included in the commonly acknowledged indications for RT. Because it is widely believed that there is no significant disadvantage to the salvage strategy in this cohort, early T1-2 and N0-1 cases are generally treated with surgical resection followed by

observation, with RT reserved as part of salvage therapy for recurrent tumor cases [57, 58].

Immunotherapy is the fourth therapeutic option for HNSCC with promising outcomes. The average objective response rate for immune checkpoint inhibitors to far has only been around 15%, thus it is far from ideal. Activity levels are below expectations, with at least 80% of HNSCC patients showing no tumor size decrease. In both the CheckMate and KEYNOTE studies, there was no discernible difference between the immune checkpoint inhibitor and standard of care groups for progression-free survival. Anti-PD-1 (programmed cell death protein 1) and anti-PD-L1 therapy resulted in hyperprogression in 29% of patients with recurrent, metastatic HNSCC, shortening progression-free survival [51, 59].

2.7 Recurrence of Oral Squamous Cell Carcinoma

Recurrence rates of OSCC range from 18 to 76% in individuals who received standard treatment, and it is often thought to be the primary reason for low survival rates. The The previous investigations confirmed that the median time to recurrence after therapy is 7.5 months, and 86% of recurrences occur within 24 months [60-62]. The presence of cervical lymph node metastases is the major risk factor for death in individuals with OSCC. ECE is a highly predictive indicator for locoregional recurrence, distant metastasis, and disease-related death [63]. Local and regional recurrences are associated with post-surgery and radiation therapy failures [60, 64, 65]. Adjuvant Radiochemotherapy was found to reduce recurrence rates in this subset of patients compared to radiation alone [66]. Patients who are not candidates for salvage surgery or re-irradiation often get chemotherapy. However, even with the most cutting-edge medication combinations, the prognosis remains grim, and a cure is rare [67]. In terms of determining the most effective therapeutic options, patients with recurring carcinomas provide a clinical

dilemma. Salvage surgery is only appropriate for a tiny subset of patients, and roughly 30-45% of individuals have poor survival outcomes [65].

2.8 Tumor Microenvironment (TME)

Cancer has long been thought to be a cell-autonomous mechanism in which repeated mutations in the tumor suppressor and oncogene genes cause an endless growth of malignant cells [68]. As a result, cancer therapy approaches have been focused and constrained to such tumor cell alterations [14]. However, emerging data suggests that the tumor's genesis and growth are determined by tumor cells and a low TME [69]. Tumors are small organs made up of many distinct types of cells that interact to allow cancer cells to survive, develop, and spread [70]. The tumor's microenvironment, which includes immune cells (T and B lymphocytes), myeloid-derived suppressor cells (MDSCs), CAFs (or Cancer-Associated Fibroblasts), tumor-associated macrophages (TAMs), adipocytes, ECM (Extracellular Matrix) proteins, and mesenchymal stem cells, is one of the significant factors contributing to tumor development [71]. Another element with a pleiotropic impact is cytokines. They generate various reactions, usually on distinct cell types, and contribute to cancer cell growth, drug resistance, initiation of EMT in cancer cells, resistance to apoptosis, and amplification of chemokine impact on recruiting immunological suppressor cells. The growing evidence of the critical involvement of various stromal components in the regulation of HNSCC development points that the tumor microenvironment plays an essential role in providing a supportive habitat, hence significantly enhancing HNSCC development and metastasis [72].

2.8.1 Cadherins

Cadherins have been identified as Ca^{2+} -dependent cell-cell adhesion proteins in vertebrates. They connect cells by homophilic interactions. cadherins are essential for creating and maintaining intercellular connections in healthy epithelium. Cells with fewer cadherin molecules are typically less adherent [73]. The classical cadherins are

single-span transmembrane cadherins with five extracellular cadherins repeat domains that collaborate with members of the catenin family to bind to the actin cytoskeleton through their cytoplasmic domains [74, 75]. E- and N-cadherins belong to the family of classical cadherins [76]. E-cadherin (ECAD) is found on the cell surface of all epithelial cells, whereas N-cadherin (NCAD) is present in fibroblasts, skeletal, neural tissue, cardiac muscles, and endothelial cells [77].

2.8.2 E-cadherin

Epithelial cadherin is a 120 kDa transmembrane glycoprotein encoded by the CDH1/E-cadherin gene on chromosome 16q22.1, which is believed to be a tumor suppressor gene due to its ability to inhibit cell growth [78]. ECAD is an established member of the cadherin group and a potent tumor suppressor, since down-regulation of ECAD is typically detected in malignant epithelial tumors [79-81]. Due to its early identification and comprehensive characterization, ECAD is commonly considered as the model classical cadherin in mammals [82]. It is considered a critical cell-cell adhesion protein that plays crucial roles during development and is necessary for the homeostasis of multiple organs. Multiple pathways often disturb ECAD function in cancer, making it an appealing diagnostic and prognostic candidate protein in human medicine [80]. The loss of ECAD expression in tumor tissue triggers metastatic spreading and the amplification of several EMT transcription factors [83]. Some investigators have referred to the loss of ECAD expression in OSCC as a high-risk marker of malignancy [84, 85] since it has been linked to both clinical and histological characteristics of malignancy, such as metastasis, recurrence, poor tumor differentiation, and reduced survival [86-88].

2.8.3 N-cadherin

Neural cadherin, known as cadherin 2, was discovered as the antigen identified by the mouse brain tissue-specific monoclonal antibody NCD-1, which was first developed against mouse brain tissue [89]. According to research, the structural-adhesive function

of NCAD in adult tissues is necessary for the appropriate integrity of particular tissues. It also aids in cell communication by assisting neurons form functional synapses and forming a vascular wall that is important for stabilizing blood vessels [90-93]. The function of NCAD is diverse and varies depending on the cell environment. Any disruption in the functionality of NCAD may play a significant role in developing pathologic conditions since NCAD can influence the cytoskeleton, interact with other membrane receptors, and enhance cell attachment between cells of either the same or different types [93].

2.8.4 Epithelial-Mesenchymal Transition (EMT)

EMT is a cellular process in which epithelial cells obtain mesenchymal phenotypes and behavior in response to downregulated epithelial characteristics. Historically, Elizabeth Hay developed the term "epithelial-mesenchymal transformation" in 1968 to characterize the significant cell alterations that occur during embryogenesis; later, it was termed EMT to differentiate it from malignant transformation. [94-97]. The process is represented in figure 2.

EMT is induced when cells receive cues from their microenvironment. The epithelial state of cells starting EMT is denoted by stable epithelial cell–cell junctions, interactions with the basement membrane, and apical–basal polarity. These epithelial features are suppressed during EMT in favor of adopting mesenchymal traits due to modifications to gene expression and post-translational regulatory mechanisms. Cells exhibit morphology and cytoarchitecture similar to fibroblasts throughout this process and an enhanced ability to migrate. These migrating cells often have malignant characteristics [97].

EMT is commonly believed to happen in the beginning stages of embryonic growth to promote various morphogenetic processes later in development , and it also happens during the wound-healing process [98]. Furthermore, EMT has been linked to cancer etiology and tissue fibrosis. However, during development, the opposite process,

known as a mesenchymal-epithelial transition (MET), also happens. The transition from an epithelial to a mesenchymal state is usually imperfect in vivo, resulting in cells having both epithelial and mesenchymal traits; based on the biological environment, these transitional phases may appear in various ways [99, 100]. Because of the increasing complexity and diversity of the EMT literature, definitions of EMT and associated nomenclature have become unclear and frequently confusing [95]. Over the last two decades, the field of EMT research has expanded rapidly. In recent years, the vast majority of published studies on EMT have focused on EMT research in tumor biology [101-106].

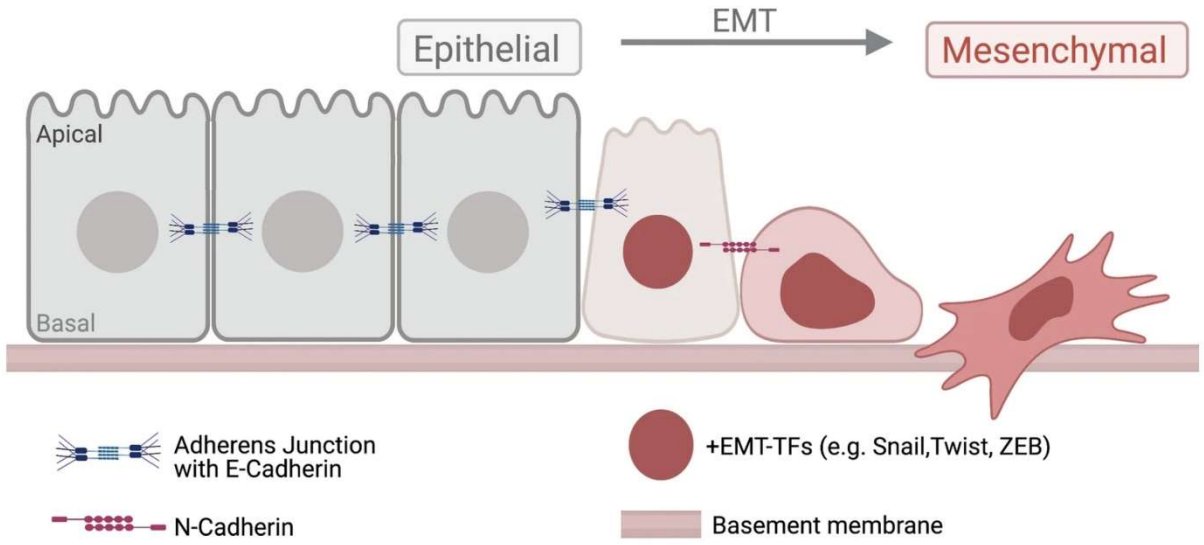

Figure 2: Schematic illustration of EMT. EMT is a complex process in which the activation of EMT-TFs causes the breakdown of cell-cell junctions, the loss of apical-basal polarity, and the upregulation of new cadherins. Modified from Amack [101].

2.8.5 Role of EMT in Cancer

The EMT provides epithelial cells with the characteristics necessary for infiltration and metastatic potentials, such as invasiveness and the capability to degrade extracellular matrix (ECM) proteins [94]. One of the challenges in understanding EMT is that the

transitions from epithelial to mesenchymal states are not binary. Instead, cancer cells frequently exhibit a mix of epithelial and mesenchymal features [107, 108].

Metastatic disease, rather than primary tumors, is responsible for around 90% of cancer-related mortalities [109]. According to literature, the most frequent epithelial markers are cytokeratins, ECAD, and occludins, whereas the most prominent mesenchymal markers are NCAD and vimentin. [110]. EMT has been shown to play critical roles in carcinogenesis and metastasis for decades. Most life-threatening human cancers arise from epithelial tissues, such as the liver, breast, ovary, kidney, prostate, pancreas, and colon [111].

Circulating tumor cells (CTCs) are cancer cells discharged from a solid tumor and enter the peripheral bloodstream; they are thought to be a biomarker of the metastatic process [112]. Many cancer cells may not undergo full EMT but instead develop these hybrid epithelial/mesenchymal characteristics over time. Over the full EMT phenotype, hybrid E/M phenotypes provide multiple advantages. Partial EMT or hybrid E/M phenotypes have some advantages over the full EMT phenotype. It is proposed that these migratory cells are cancer cell that separated from the original tumor, infiltrated surrounding tissues, and intravasated into lymphatic and blood arteries, eventually colonizing lymph nodes and distant organs [94, 107]. The majority of CTCs have hybrid E/M markers, indicating incomplete EMT. Furthermore, while EMT is critical in tumor progression, its reverse phase, MET, is also important in tumor dispersion. The final stage of the invasion-metastasis cascade is known as colonization, and it is heavily reliant on MET [113, 114].

2.9 Tissue microarray (TMA) as a method for histologic cancer studies

Kononen described the TMA in 1998 [115], as a high-throughput method for assessing histology-based laboratory procedures such as immunohistochemistry and fluorescence in-situ hybridization (FISH). Small cylindrical cores are taken from typical

formalin-fixed, paraffin-embedded tissue and organized in a matrix format within a recipient paraffin block, allowing a pathologist to quickly analyze hundreds of patient samples. TMAs have been used to investigate tumor biology, evaluate novel genetic biomarkers, and ensure laboratory quality assurance since their introduction. The TMA is also an ideal platform for validation and translation for other sorts of high-throughput molecular research. The TMA has proven indispensable for the study of tumor biology, the creation of diagnostic methods, and the analysis of oncological biomarkers [116].

TMAs have specific advantages over other molecular techniques, such as DNA microarrays and proteomics, in the field of molecular epidemiology. Tissues that have been formalin-fixed and paraffin-embedded serve as the basis for TMAs and are the most popular way to preserve surgical specimens. Since many hospitals must keep archival tissue blocks for at least 20 years, the source material for TMAs is widely accessible and frequently associated with long-term outcome information [116].

3. Aim of this study

The expression of ECAD and NCAD at the invasive front as a surrogate parameter for the epithelial-mesenchymal-transition process and its possible predictive value in terms of post-recurrence survival in recurrent OSCC have not been addressed in the existing research. In order to translate the EMT/cadherin-switch phenomenon as a histologic risk factor into a clinical setting, we needed a standardized evaluation and interpretation method of the EMT results, as well as an assignment of its degree to a specific risk profile and prognosis in an epidemiologic clinical setting.

We predicted that the cadherin switch in primary and re-OSCC specimens is an inherent characteristic of the tumor, influences its biological behavior, and further predicts post-recurrence survival outcomes in these patients.

To test this hypothesis, we analyzed the immunohistochemical expression of ECAD and NCAD in the primary and recurrent OSCC patients in a prospectively maintained, single-center cohort. We studied the post-recurrence survival of this high-risk group and correlated it with the standardized *h-score*-based immunohistochemical expression of both cadherin types with the clinical outcome (oral cancer-specific survival, overall survival, and post-recurrence disease-free survival).

To the best of our knowledge, this is the first study to investigate combined ECAD and NCAD expression as a predictor of EMT in relation to OSCC survival outcomes.

4. Material and methods

4.1 Study population

We identified all 1088 cancer patients who presented between 1992 and 2019 to the Department of Maxillofacial Surgery at the University Medical Centre of Lübeck, Germany, with primary non-metastatic OSCC. We included only patients with curative intent who underwent surgery alone or in combination with (chemo)-radiotherapy, as described in figure 3.

To ensure regular follow-up during the 5-year post-therapeutic period, all patients were enrolled in a strictly regulated recall system (every three months in the first two years and every six months after that). At the outset of the study and at each follow-up, data were accessible, including demographic information, risk factors, clinical tumor characteristics, and treatment decisions. The general condition of the patients, estimated using Charlson's comorbidity index (CCI) score [117], tumor stage, and other competing risk factors, were considered in the cohort analysis.

Inclusion Criteria

All OSCC patients with recurrence (N=94) were included in this study irrespective of age, sex, clinicopathological characteristics.

Exclusion Criteria

Patients with oropharyngeal carcinoma (N=87) or oral non-squamous cell carcinoma (N=131) were excluded from the study. Additionally, exclusion criteria included metastatic disease at diagnosis (N=87), patients who refused treatment or died prior to therapy (N=945), and patients who did not have a locoregional recurrence (N=815).

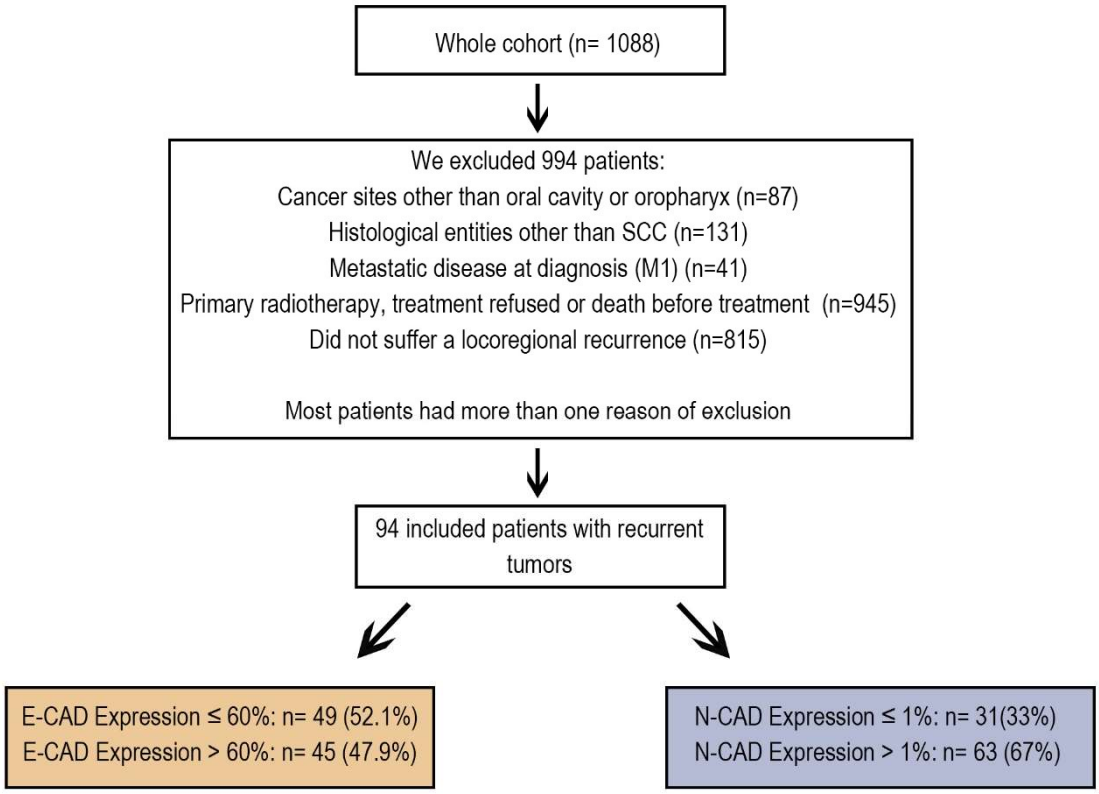

Figure 3: A flow chart depicts the inclusion/exclusion criteria for patients, as well as their assignment to the various cut-off groups for ECAD loss and NCAD de novo expression in primary and recurrent OSCC.

4.2 TMA construction and analysis

We obtained archived, formalin-fixed, paraffin-embedded tissue from surgically resected primary and recurrent oral cancer specimens from the Department of Pathology Bank at the University Hospital of Lübeck. The specimens contained tumor and adjacent normal tissues. The tissues were gathered between 1992 and 2019. For our tissue microarrays, we included 94 patients. The detailed clinical and pathologic information for these patients were available, including demographics, smoking history, clinical and pathologic TNM stage, overall survival duration, and time to recurrence.

All data concerning these patients were collected from our clinical patient database software (Agfa Orbis®). All data was re-evaluated and double-checked by two experienced maxillofacial surgeons and a pathologist.

Hematoxylin and Eosin slides and paraffin blocks of these patients were obtained from the archive. Tissue specimens were available from primary tumors, local recurrent tumors, and/or lymph node metastases (in cases with locoregional recurrence). Tissue samples were re-evaluated in order to categorize each case using the most recent TNM classification (8th edition) and UICC stages [50].

On Hematoxylin and Eosin slides, regions of interest (ROIs) were marked, and paraffin blocks were matched. Three 0.1 cm cores (triplets) were punched out of each tumor and organized in acceptor blocks as tissue microarrays (TMAs). Cores of patients that did not contain tumor tissue, had staining artifacts, or contained tissue folds were eliminated [118].

Prostate cancer tissues were used as a positive control for ECAD, while invasive lobular carcinoma specimens were used as a negative control. Positive controls for NCAD staining were renal cell carcinoma and high-grade ovarian cancer, and negative controls were prepared by omitting the primary antibodies from the staining procedure.

TMA sections were stained with the Ventana BenchMark staining system and detected with the IViewDAB detection kit (both available from Roche, Basel, Switzerland) [119], as mentioned in table 2.

For both ECAD and NCAD staining, heat-mediated antigen retrieval was done for 32 minutes at 92 °C with Cell Conditioning Solution 1 (CC1; #950-124, Ventana Medical Systems, Inc., Arizona, USA). The primary antibodies are mentioned in Table 1. ECAD and NCAD were used as membranous markers and cytoplasmatic markers.

Table 2: Antibodies used for the immunostaining of the Tissue microarray

Antibody	Isotype	Company	Concentration/Dilution	Clone
E-cadherin	Mouse monoclonal	Roche, Basel, Switzerland	0.314 µg/ ml	CDH1
N-cadherin	Rabbit polyclonal	Abcam, Cambridge, United Kingdom	1:100	CDH2

An excel sheet map was created before the TMA was built, as described in figure 4. This map aimed to direct assembly and subsequent scoring by designating a place within the TMA for each core sample. A tissue microarrayer was necessary for the physical fabrication of the TMA (Beecher Instruments, Sun Prairie, WI, USA). Each TMA held up to 180 tumor samples and 15 normal tissue samples as triplet cores of 1 mm2 diameter.

orientation	1	1	1	2	2	2
orientation	3	3	3	4	4	4
orientation	5	5	5	6	6	6
	7	7	7	8	8	8
	9	9	9	10	10	10

	11	11	11	12	12	12
	13	13	13	14	14	14
	15	15	15	16	16	16
	17	17	17	18	18	18
	19	19	19	20	20	20

	21	21	21	22	22	22
	23	23	23	24	24	24
	25	25	25	26	26	26
	27	27	27	28	28	28
	29	29	29	30	30	30

	31	31	31	32	32	32
	33	33	33	34	34	34
	35	35	35	36	36	36
	37	37	37	38	38	38
	39	39	39	40	40	40

	41	41	41	42	42	42
	43	43	43	44	44	44
	45	45	45	46	46	46
	47	47	47	48	48	48
	49	49	49	50	50	50

	51	51	51	52	52	52
	53	53	53	54	54	54
	55	55	55	56	56	56
	57	57	57	58	58	58
	59	59	59	60	60	60

Figure 4: yellow blocks: orientation cores, green blocks: normal tissues, white blocks: tumor

Figure 5: E-Cadherin tissue microarray cores under low magnification

4.3 Immunohistochemical analysis

The slides were visualized using the Ventana iScan HT scanner (Ventana, Tuscon, AZ, USA), as seen in figure 5. For digital evaluation of the slides, the image analysis software QuPath (University of Edinburgh, UK) was used. The file extension used in QuPath was ".tif" The data were analysed using ThinkPad P1 Mobile workstation, Intel® Xeon® processor, 32GB of RAM and OLED Screen with 4K resolution [120].

4.3.1 QuPath Software steps to analyze the antibodies:

1- First, a new project is created. Then, all images/data concerning this antibody are added to the selected folder. The cores are then identified using the TMA dearrayer feature. This step is essential to quickly identify, analyze, and export every core separately.

2- In the preprocessing phase, the stain vectors have to be estimated to improve stain separation.

3- Select all cores and execute the cell detection feature after adjusting all parameters based on the antibody (Threshold, Max background intensity). In our case we used the default setting for our antibodies.

4- The ROI are annotated and categorized into different groups: Tumor, Immune cells, Stroma, and Others

5- The Object Classifier feature is run to detect and analyze the number of different categories.

4.3.2 Evaluation and scoring of immunostaining results

The immunostaining evaluation based on the h-score was applied to a maximum of 300. Subsequently, this was formed by adding the percentage of strongly labeled cells (weighted 3), the percentage of moderately stained cells (weighted 2), and the percentage of weakly stained cells (weighted 1), resulting in a range of 0 to 300 [121]. Immunostaining was performed based on the proportion of positively stained tumor cells relative to the total investigated tumor area.

The assessment of NCAD immunostaining considered the proportion of tumor cells with positive staining in relation to the total evaluated tumor area. When cytoplasmic or membrane immunostaining was observed in epithelial tumor cells, NCAD expression was considered to be positive, as shown in figures 8 and 9.

Positive ECAD expression was determined when membranous immunostaining was observed in epithelial tumor cells, as seen in figures 6 and 7. For loss of ECAD staining in epithelial cells, an inverse estimation of the h-score-based evaluation was applied as 300 minus ECAD staining in order to normalize to NCAD values and corresponding vector development in both antibodies.

The ECAD and NCAD staining scores were entered as a continuous variable, and a cutoff was established for both categories depending on the available sample size.

The appropriate cut-offs for biomarkers were calculated using the R package 'Survminer,' which employs the maximum selected rank statistics in multivariate Cox regressions to offer the value of a cut-off that most strongly correlates with the outcome. Based on the *h-score*, a cut-off value of 60% was defined for ECAD loss of expression and 1% for NCAD expression. The significance level was set at p 0.05.

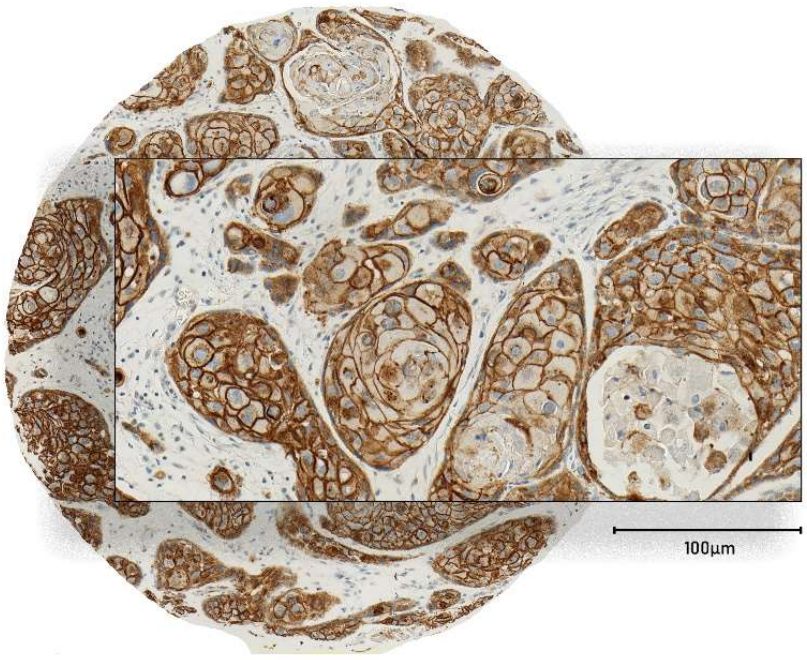

Figure 6: preserved expression of E-Cadherin in a representative section

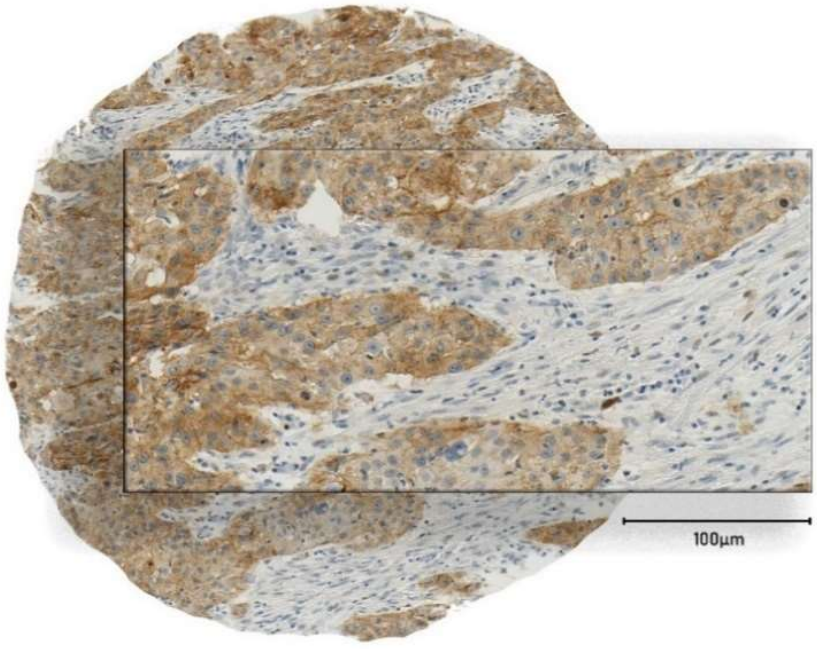

Figure 7: Loss of membrane staining in a representative section stained

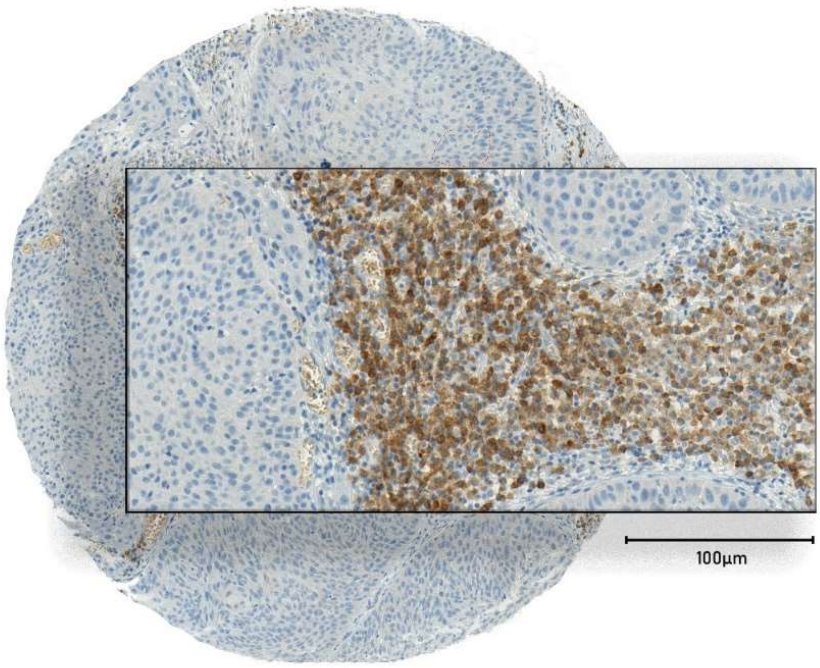

Figure 8: high expression of N-Cadherin in a representative section

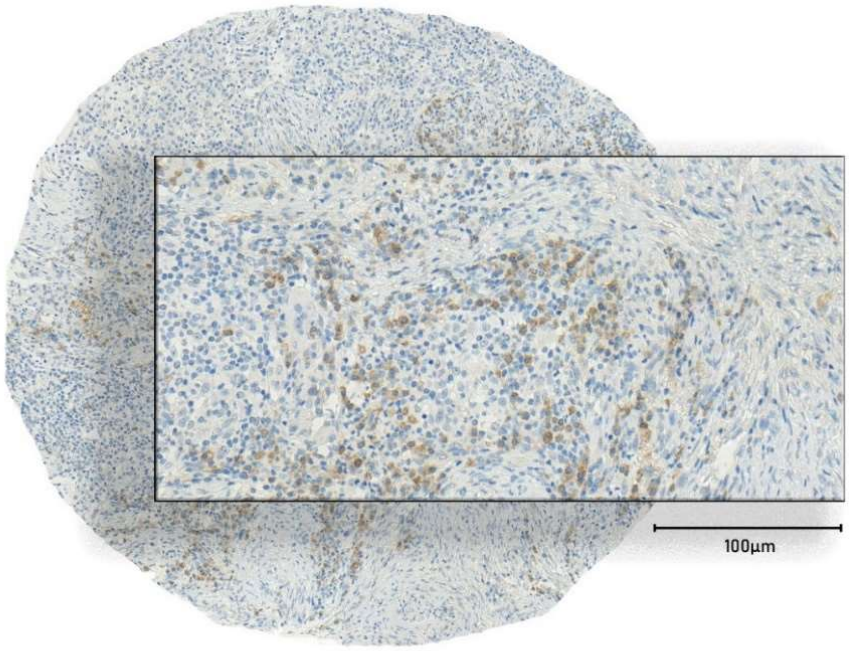

Figure 9: low expression of N-Cadherin in a representative section

4.4 Statistical Analysis

From the time of recurrence, all survival outcomes were calculated. Post-recurrence disease-free survival (pr DFS) was measured by the incidence of local or regional recurrence. Overall post-recurrence survival (pr OS) was measured by death from any cause. Post-recurrence oral cancer-specific survival (pr OCSS) was measured by oral cancer mortality. Patients were censored at the most recent follow-up.

R Statistical Software was utilized for all statistical studies (version 4.0.4; R Foundation for Statistical Computing, Vienna, Austria). The proportional hazards assumption was evaluated using Schoenfeld residual plots.

4.5 Ethics

All participants signed consent forms upon admission, allowing their data to be collected and used anonymously for academic research. The study was approved by the University of Lübeck's ethics review committee (ID: 12-079A).

5. Results

5.1 Characteristics of patients

Within the investigated cohort, 94 patients with recurrent OSCC were included. The recurrence age ranged between 53 and 72 years. 68% of local recurrences were associated with cervical lymph node metastasis, while only 8.8% were associated with distant metastasis. Eleven percent of patients received radiochemotherapy as an adjuvant treatment, while fifty percent solely received radiotherapy. The remaining patients were only surgically treated (Table 3).

The majority of patients reported a positive smoking history (n = 69, 78%), as well as excessive alcohol intake (n = 52, 60%). A safe resection margin (R0) was demonstrated in 19 cases (68%). Total resection (R1) was not achieved in 9 (32%) patients. Patients with distant metastases made up 8.8% of all patients (Table 3).

Tumors were categorized as rT1 (r for recurrent) in 24 patients (26%), rT2 in 16 (18%), rT3 in 10 (11%), and rT4 in 28 (31%). There were 63 (68%) patients with rN+ nodal status and 29 (32%), with no lymph node metastases (rN0). The most common diagnosis was moderately differentiated oral squamous cell carcinoma (G2), with 40 patients (59%) falling into this category according to Table 3.

The most commonly affected areas were the floor of the mouth (n= 39, 41%), the neck solely (n= 15, 16%), the cheek/ vestibule/ retromolar (n= 13, 14%), and the anterior tongue (n=11, 12%) (Table 3).

Based on the *h-score*, appropriate cut-offs for biomarkers were established; a cut-off value of 60% was defined for ECAD loss of expression and 1% for NCAD expression.

Males were predominant in the group of patients with ECAD expression loss of less than or equal to 60%, while sex distribution in the expression of NCAD was comparable, regardless of expression levels. The CCI score was similar regardless of both NCAD and

ECAD expression. In the ECAD group with expression loss less than or equal to 60%, excessive alcohol smoking (73%) was observed. The most common site of recurrence was the floor of the mouth ranging between 40-45%, when comparing the 4 subgroups of expression of both markers. In all subgroups, between 55 and 75% of patients showed local lymph node recurrences. The resection margins were clear in 75% of patients with ECAD expression loss less than or equal to 60% and 80% of patients with NCAD expression more than 1%. In all groups, the majority of patients presented demonstrated moderate OSCC grade (Table 3).

Irrespective of NCAD and ECAD expression levels, the vast majority of patients did not have distant metastases (Table 3).

ECAD and NCAD expression among the well-differentiated OSCC tended to maintain steady levels (Table 3). Forty-three percent of re-OSCCs categorized as G3 (poorly differentiated) exhibited an E-cadherin expression loss of less than or equal to 60%, while only 21% of patients with poorly differentiated OSCC showed an ECAD expression loss of more than 60% (Table 3). On the other hand, regardless of NCAD expression, most of the patients showed moderately differentiated OSCC (NCAD ≤ 1%: 62%, NCAD > 1%: 57%, Table 3).

Table 3: Clinical characteristics of patients, including tumor stage and IHC expression of E-cadherin and N-cadherin in recurrent oral squamous cell carcinoma, are included in this thorough descriptive analysis.

Variable	Overall N = 94[1]	strata by ECAD loss ≤ 60% N=49 (52%)[1]	strata by ECAD loss > 60% N=45 (48%)[1]	strata by NCAD[2] ≤ 1% N=31 (33%)[1]	strata by NCAD[2] > 1% N=63 (67%)[1]
Age at recurrence diagnosis	63 (53-72)	63 (51-71)	63 (55-73)	62 (52-73)	64 (54-72)
Sex					
Female	30 (32%)	10 (20%)	20 (44%)	10 (32%)	20 (32%)
Male	64 (68%)	39 (80%)	25 (56%)	21 (68%)	43 (68%)
CCI score					
0	59 (63%)	32 (67%)	27 (60%)	21 (68%)	38 (61%)
1 ≤	34 (37%)	16 (33%)	18 (40%)	10 (32%)	24 (39%)
Missing	1	1	0	0	1
Smoking					
Never	20 (22%)	8 (18%)	12 (27%)	5 (17%)	15 (25%)
Former or current	69 (78%)	37 (82%)	32 (73%)	24 (83%)	45 (75%)
Missing	5	4	1	2	3
Alcohol consumption					
None or moderate	35 (40%)	12 (27%)	23 (53%)	10 (34%)	25 (43%)
Excessive	52 (60%)	32 (73%)	20 (47%)	19 (66%)	33 (57%)
Missing	7	5	2	2	5
Site of recurrence					
Anterior tongue	11 (12%)	4 (8.2%)	7 (16%)	5 (16%)	6 (9.5%)
Cheek/vestibule/retromolar	13 (14%)	6 (12%)	7 (16%)	3 (9.7%)	10 (16%)
Floor of mouth	39 (41%)	20 (41%)	19 (42%)	14 (45%)	25 (40%)
Lip	2 (2.1%)	0 (0%)	2 (4.4%)	0 (0%)	2 (3.2%)
Neck only	15 (16%)	9 (18%)	6 (13%)	7 (23%)	8 (13%)
Oropharynx	11 (12%)	8 (16%)	3 (6.7%)	2 (6.5%)	9 (14%)
Palate	3 (3.2%)	2 (4.1%)	1 (2.2%)	0 (0%)	3 (4.8%)
rT					
rT1	24 (26%)	12 (26%)	12 (27%)	5 (17%)	19 (31%)
rT2	16 (18%)	8 (17%)	8 (18%)	8 (27%)	8 (13%)
rT3	10 (11%)	5 (11%)	5 (11%)	3 (10%)	7 (11%)
rT4	28 (31%)	16 (34%)	12 (27%)	13 (43%)	15 (25%)
rTx	13 (14%)	6 (13%)	7 (16%)	1 (3.3%)	12 (20%)
Missing	3	2	1	1	2
rN					
rN0	29 (32%)	15 (31%)	14 (32%)	14 (45%)	15 (25%)
rN+/x	63 (68%)	33 (69%)	30 (68%)	17 (55%)	46 (75%)
Missing	2	1	1	0	2
rM					
rM0/x	83 (91%)	46 (98%)	37 (84%)	28 (90%)	55 (92%)
rM1	8 (8.8%)	1 (2.1%)	7 (16%)	3 (9.7%)	5 (8.3%)
Missing	3	2	1	0	3

Variable	Overall N = 94[1]	strata by ECAD loss ≤ 60% N=49 (52%)[1]	strata by ECAD loss > 60% N=45 (48%)[1]	strata by NCAD[2] ≤ 1% N=31 (33%)[1]	strata by NCAD[2] > 1% N=63 (67%)[1]
Resection margins					
R0	19 (68%)	9 (75%)	10 (62%)	7 (54%)	12 (80%)
R1/2/x	9 (32%)	3 (25%)	6 (38%)	6 (46%)	3 (20%)
Missing	66	37	29	18	48
Grade					
Well	6 (8.8%)	2 (5.7%)	4 (12%)	2 (7.7%)	4 (9.5%)
Moderate	40 (59%)	18 (51%)	22 (67%)	16 (62%)	24 (57%)
Poor	22 (32%)	15 (43%)	7 (21%)	8 (31%)	14 (33%)
Missing	26	14	12	5	21

[1]Median (25%-75%); n (%), [2]NCAD expression, CCI: Charlson Comorbidity Index; rT: recurrent tumor; rN: recurrent regional lymph node; rM: recurrent distant metastasis.

5.2 Pattern of E-cadherin and N-cadherin immunohistological expression

Strong membranous ECAD expression was observed in intact epithelial regions close to the malignant epithelium (Figure 6), while degrees of reduced staining intensity and partial to complete absence of membranous labeling were observed in primary and re-OSCC (Figure 7). The suprabasal layer, epithelial cancer cells on the invasion front, and neoplastic epithelial nests in the stroma all showed evidence of this phenomenon.

Regardless of the tumor's degree of histological differentiation, NCAD was present in the cytoplasm and membrane of dispersed tumor cells within the invasion front and in the stroma at varying intensities (Figure 8, 9). ECAD and NCAD staining patterns were consistent across specimens and in primary and re-OSCC.

5.3 E-cadherin and N-cadherin expression in primary and recurrent malignancies

ECAD expression ranged from 43-62% (mean=53%) in primary tumors and was marginally higher in recurrent cases (mean = 57%) (Figure 10). A similar effect was seen for NCAD, which showed a non-significant increase from primary tumor tissues

(mean=2.88%, range: 0.29-4.09%) to recurrent tumor tissues (mean=5% range: 1-7%), as illustrated in Figure 10.

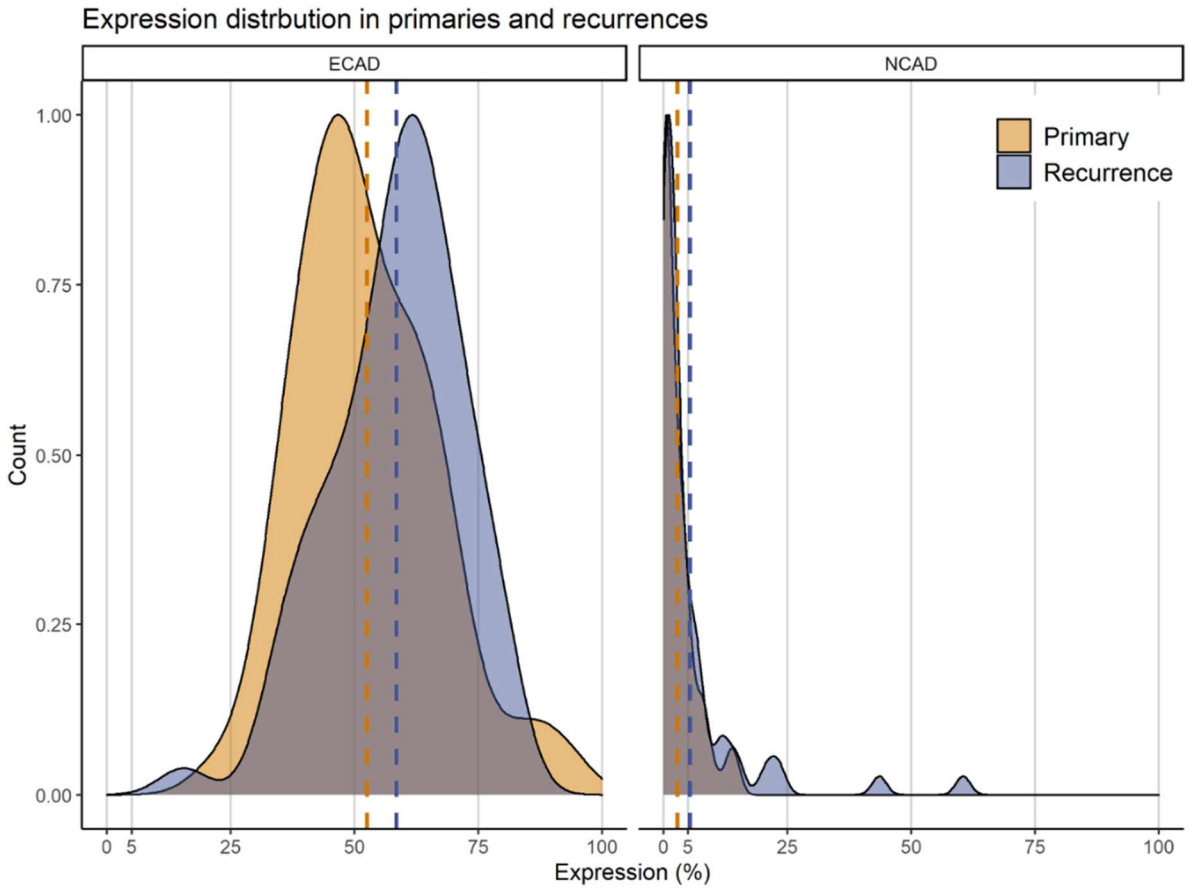

Figure 10: Histogram showing the comparison between the expression of ECAD and NCAD in primary and recurrent tumors (dotted lines represent mean value).

Overall, there was no significant difference in the expression of ECAD and NCAD between the primary and recurrent tumors, irrespective of treatment modality (Figure 11). Exceptionally, ECAD expression in the primary tumor was decreased compared to recurrent tumors in the irradiated group (Figure 11).

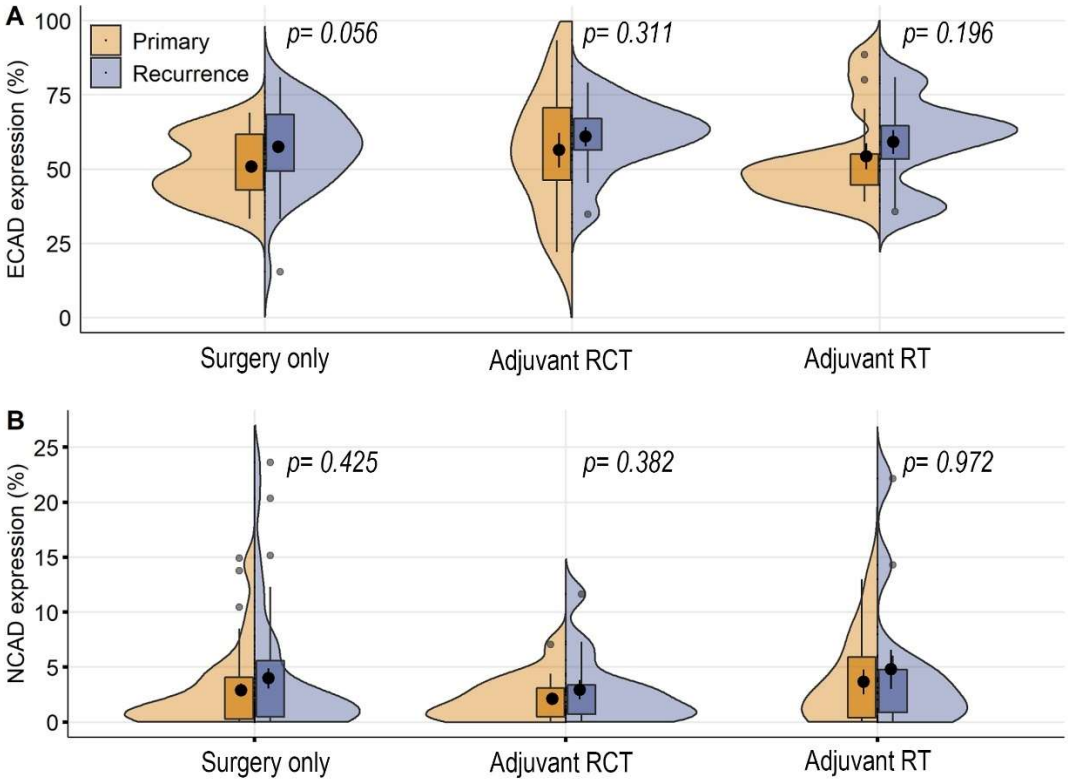

Figure 11: Distributions of the numerical data for ECAD (A) and NCAD (B) expression in primary and recurrent tumors are shown in violin plots divided into 3 subgroups (surgery only, radiotherapy, and radiochemotherapy).

These findings reveal a stable level of initial cadherin switch within the primary tumors, which does not change during the recurrence phase and appears to be irrespective of the type of adjuvant therapy (radio- or radiochemotherapy) given between the initial diagnosis and recurrence.

5.4 Survival outcomes for the E-cadherin and N-cadherin expression

Based on the *h-score* cutoff values presented in Table 5, the ECAD and NCAD survival events were evaluated. Thirty-six patients (80%) who passed away exhibited a loss of

ECAD expression greater than 60%, and of this group, 32 (74%) passed away due to complications of oral cancer. The remaining patients died from other reasons; in the group of patients with ECAD expression loss less than or equal to 60%, 31 patients (65%) died, with 25 (53%) of them died from complications related to the tumor (Table 5).

In the NCAD group, 43 (69%) of the deceased patients had an NCAD expression of more than 1%, while 24 (77%) of the deceased patients had an NCAD expression of less than or equal to 1%. Furthermore, 23 (77%) patients in the group with lower NCAD expression perished due to oral cancer, compared to 34 (57%) patients in the group with higher NCAD expression (Table 5).

Table 5: Mortality rates based on the *h-score* cutoff for E-cadherin and N-cadherin expression.

Variable	Events by ECAD loss		Events by NCAD[2]	
	≤ 60% N = 49 (52%)[1]	> 60% N = 45 (48%)[1]	≤ 1% N = 31 (33%)[1]	> 1% N = 63 (67%)[1]
Death from any cause				
Alive or censored	17 (35%)	9 (20%)	7 (23%)	19 (31%)
Dead	31 (65%)	36 (80%)	24 (77%)	43 (69%)
missing	1	0	0	1
Cause of death				
Alive or censored	17 (36%)	9 (21%)	7 (23%)	19 (32%)
Death from oral cancer	25 (53%)	32 (74%)	23 (77%)	34 (57%)
Death from other causes	5 (11%)	2 (4.7%)	0 (0%)	7 (12%)
missing	2	2	1	3

[1]n (%),[2]NCAD expression

The 2-year pr OS, pr OCSS, and pr DFS rates in the ECAD group less than or equal to 60% were 41% (29-57%), 46% (34-63%) and 34% (22-52%), respectively, whereas the rates in the group greater than 60% were 24% (15-41%), 72% (60-87%), and 52% (34-78%), respectively (Table 6). The 5-year pr OS, pr OCSS, and pr DFS rates in the ECAD group

less than or equal to 60% were 30% (20-47%), 55% (42-72%) and 41% (28-61%), respectively, whereas the rates in the group greater than 60% were 17% (8.9-33%), 79% (67-93%), and 61% (42-93%), respectively (Table 6).

The 2-year pr OS, pr OCSS, and pr DFS rates in the NCAD group ≤ 1% were 29% (17-50%), 70% (55-88%), and 39% (22-70%), respectively, whereas the rates in the group greater than 1% were 35% (25-49%), 53% (42-67%), and 40% (28-58%), respectively (Table 6). The 5-year pr OS, pr OCSS, and pr DFS rates in the NCAD group ≤ 1% were 19% (8.9-40%), 81% (67-96%), and 39% (22-70%), respectively, whereas the rates in the group greater than 1% were 27% (18-40%), 58% (47-72%), and 51% (37-69%), respectively (Table 6).

Table 6: Survival outcomes of patients based on of E-cadherin and N-cadherin expression.

	pr OS		pr OCSS		pr DFS	
Variable	at 2 years	at 5 years	at 2 years	at 5 years	at 2 years	at 5 years
Overall	33% (25%-44%)	24% (17%-35%)	59% (49%-70%)	66% (57%-77%)	40% (30%-55%)	48% (36%-64%)
ECAD loss						
≤ 60%	41% (29%-57%)	30% (20%-47%)	46% (34%-63%)	55% (42%-72%)	34% (22%-52%)	41% (28%-61%)
> 60%	24% (15%-41%)	17% (8.9%-33%)	72% (60%-87%)	79% (67%-93%)	52% (34%-78%)	61% (42%-91%)
NCAD[2]						
≤ 1%	29% (17%-50%)	19% (8.9%-40%)	70% (55%-88%)	81% (67%-96%)	39% (22%-70%)	39% (22%-70%)
> 1%	35% (25%-49%)	27% (18%-40%)	53% (42%-67%)	58% (47%-72%)	40% (28%-58%)	51% (37%-69%)

[2]NCAD expression, pr DFS: post-recurrence disease-free survival; pr OS: post recurrence survival; pr OCSS: post-recurrence oral cancer-specific survival.

5.5 Competing Risk Analysis and Hazard Ratio for E-cadherin and N-cadherin expression

Clinicopathological variables (age, sex, CCI, smoking, tumor size, alcohol, nodal metastasis) were analyzed in Table 7. Using Cox proportional hazard regression analysis, the hazard ratios (HR) and 95% confidence intervals (CI) for overall, oral cancer-specific, and post-recurrence disease-free survival were estimated. Multivariate analysis for pr OS and pr OCSS found no statistically significant differences for potential risk factors such as sex, CCI, smoking, and alcohol, with pr OS HR values of 0.97, 1.06, 1.74, and 1.28, respectively, and pr OCSS HR values of 0.93, 1.45, 1.50, and 1.30, respectively (Table7).

In tumors larger than rT1, pr OS as well as pr OCSS were both significantly decreased. The worst prognosis for pr OS and pr DFS was for rT2 tumors (HR= 6.24, CI 2.26-17.3, *p*= 0.001; HR= 4.11, CI: 1.45-11.6, *p*= 0.008), while the worst prognosis for pr OCSS was for rT3 (HR= 8.29, CI: 2.53-27.1, *p*=0.001). Both pr OS and pr OCSS were significant in patients with positive nodal status (pr OS HR= 2.48, CI: 1.29-4.75, *p*= 0.006 and pr OCSS HR= 2.39, CI: 1.15-4.99, *p*= 0.020) (Table7).

There was a substantial association between the ECAD loss more than 60% and pr OS as well as pr OCSS (HR=2.72, CI:1.50-4.95, *p*= 0.001, HR=3.84, CI:1.93-7.63, *p*= 0.001), respectively, when comparing expression loss more than 60% to low ECAD loss in tumor cells (Table 7, Figure 11).

There was no statistically significant association between the *de novo* expression of N-cadherin and pr OS, pr OCSS, or pr DFS. The results are presented in detail in Table 7. All survival outcomes in the analysis were assessed from the first recurrence, as seen in Kaplan-Meier curves (Figure 11).

Table 7: Hazards ratios for different prognostic factors in recurrence specimens using the h-score based cut-offs of E-cadherin (60%) and N-cadherin (1%).

	pr OS			pr OCSS			pr DFS		
Characteristic	HR[1]	95% CI[1]	*p*-value	HR[1]	95% CI[1]	*p*-value	HR[1]	95% CI[1]	*p*-value
Age at recurrence diagnosis	1.01	0.98-1.03	0.6						
Sex									
Female	—	—		—	—				
Male	0.97	0.47-1.96	>0.9	0.93	0.43-2.00	0.9			
CCI									
0	—	—		—	—				
1 ≤	1.06	0.61-1.85	0.8	1.45	0.79-2.63	0.2			
Smoking									
Never	—	—		—	—				
Former or current	1.74	0.74-4.07	0.2	1.50	0.57-3.92	0.4			
Alcohol									
None or moderate	—	—		—	—				
Excessive	1.28	0.66-2.47	0.5	1.30	0.64-2.66	0.5			
rT									
rT1	—	—		—	—		—	—	
rT2	6.24	2.26-17.3	**<0.001**	6.00	1.96-18.4	**0.002**	4.11	1.45-11.6	**0.008**
rT3	5.85	1.98-17.3	**0.001**	8.29	2.53-27.1	**<0.001**	1.60	0.41-6.25	0.5
rT4	4.72	1.97-11.3	**<0.001**	5.15	1.85-14.3	**0.002**	1.43	0.51-4.00	0.5
rTx	4.22	1.55-11.5	**0.005**	7.48	2.41-23.3	**<0.001**	0.99	0.26-3.81	>0.9
rN									
rN0	—	—		—	—		—	—	
rN+/x	2.48	1.29-4.75	**0.006**	2.39	1.15-4.99	**0.020**	1.27	0.56-2.89	0.6
ECAD loss									
≤ 60%	—	—		—	—		—	—	
> 60%	2.72	1.50-4.95	**0.001**	3.84	1.93-7.63	**<0.001**	1.45	0.70-3.04	0.3

	pr OS			pr OCSS			pr DFS		
Characteristic	HR[1]	95% CI[1]	*p*-value	HR[1]	95% CI[1]	*p*-value	HR[1]	95% CI[1]	*p*-value
NCAD[2]									
≤ 1%	—	—		—	—		—	—	
> 1%	1.23	0.68-2.21	0.5	0.90	0.47-1.73	0.8	1.60	0.69-3.73	0.3

[1]HR = Hazard Ratio, CI = Confidence Interval, [2]NCAD expression, pr DFS: post-recurrence disease-free survival; pr OS: post-recurrence survival; pr OCSS: post-recurrence oral cancer-specific survival; CCI: Charlson Comorbidity Index; rT: recurrent tumor; rN: recurrent regional lymph node; rM: recurrent distant metastasis.

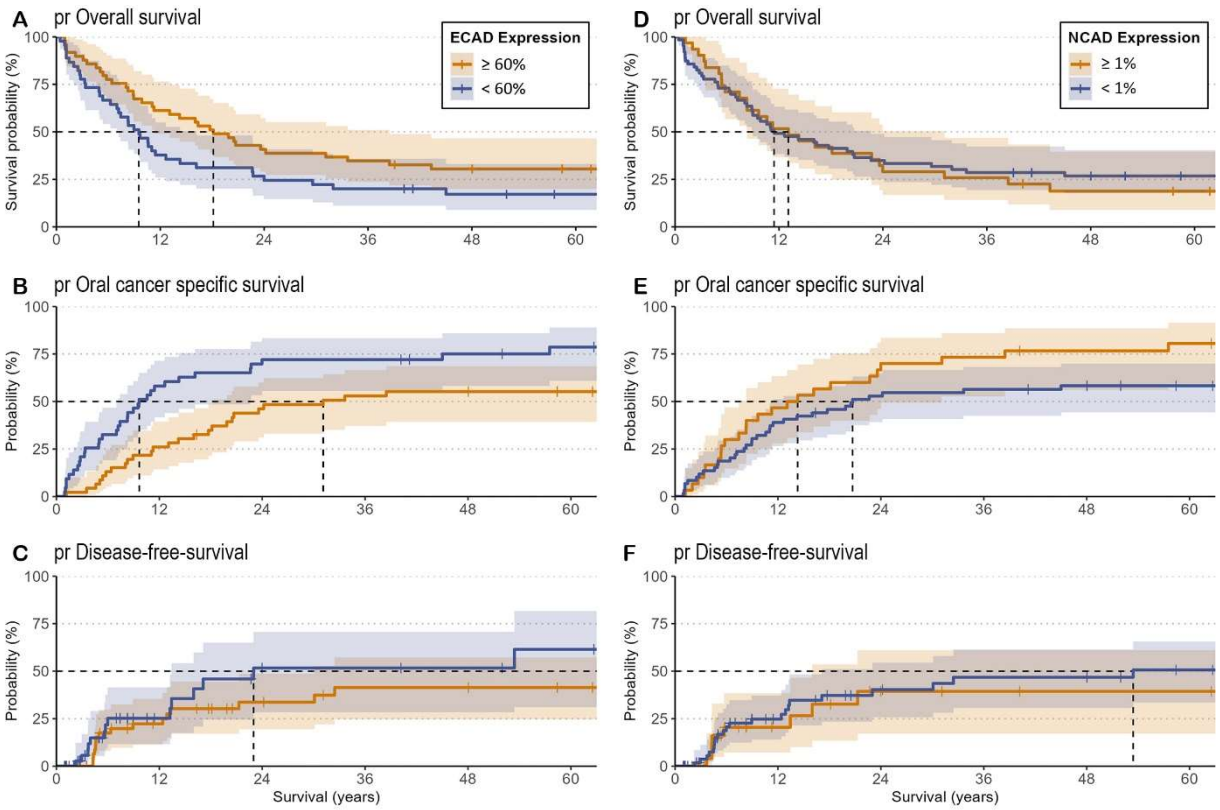

Figure 11: Separate analyses were carried out for ECAD (A-C) and NCAD (D-F). Based on the *h-score*, the cutoff was chosen at 60% for ECAD loss and 1% for N-cadherin *de novo* expression. All outcomes were assessed from the first recurrence. Kaplan-Meier curves for post-recurrence overall survival (pr OS), oral cancer-specific survival (pr OCSS), and post-recurrence disease-free survival (pr DFS) of patients with high ECAD loss and NCAD de novo expression (blue curves), as well as for low ECAD loss and negative NCAD expression (yellow curves).

6. Discussion

Nowadays, the way we think about malignant tumors has changed; the tumor is now seen as a complex subset of cancer cells that create TME to build a self-sufficient biological structure [72]. OSCC is a malignant tumor that arises from the oral mucosa's stratified squamous epithelium [122]. The lateral borders of the tongue, the floor of the mouth, as well as the lips are the most common sites for the malignant tumor [123]. Carcinogenesis starts with a cell being disrupted [32]. The treatment of re-OSCC is a challenging inter-disciplinary endeavor. Patients who have undergone first treatment for oral cancer suffer from a variety of sequela, including altered local anatomical structures and functional impairment due to food intake, masticatory and speech deficits, and xerostomia [124]. In the event of early chemotherapy, other general problems, such as impaired kidney and bone marrow function, may exacerbate these side effects and further restrict local and systemic treatment [125]. Therefore, it is necessary to adapt the available treatment options to the general condition of these patients and to stratify therapy based on their unique needs and survival probability.

6.1 E-cadherin

ECAD is believed to be a critical tumor-suppressor protein based on the characteristic loss of ECAD-mediated cell adhesion in epithelial malignancies and its role in suppressing tumor growth [126, 127]. The findings from a study conducted on glioblastoma multiforme (GBM) is in line with the theory that in a small percentage of high-grade gliomas, ECAD expression plays a significant role in tumor growth and invasiveness. According to this study, patients with GBM who have an epithelial or pseudo-epithelial morphology will have a worse prognosis than those who do not express ECAD [128].

Putzke et al. stated that ECAD overexpression is associated with aggressive disease in metastatic prostate cancer [129]. In another study, high ECAD expression was linked to

the development of ovarian epithelial cancers, as ECAD was only found in benign, borderline, and malignant ovarian epithelial tumors [130].

The above-mentioned studies are not consistent with our findings, as we detected an inverse connection between ECAD expression and the histological grade and survival outcome of patients with recurrent OSCC tumors in the present investigation. This might be attributed to the specific type of cancer on the one hand.

On the other hand, comparable studies that focused on OSCC came in line with our observations. Specifically, Pereira et al. observed that there was a reduction in the expression of ECAD at the invasive tumor front in the cytoplasmic membrane/nucleus [131]. Moreover, Peng et al. elucidated the mechanism behind ECAD loss in oral squamous cell carcinoma. They came to the conclusion that miR-134's (microRNA) oncogenic effect on oral cancer is caused by a decrease in PDCD7 (Programmed Cell Death 7) and ECAD expression [132]. Additionally, ECAD loss was detected in the precancerous stages, which suggests that loss of expression of ECAD is related to the development of OSCC from precursor lesions, as reported by Sharma et al [133].

As reported previously, we noticed a considerable degree of ECAD loss among all tumor specimens in our cohort. ECAD is largely involved in the deregulation of the extracellular matrix during EMT process, and its absence at the invasion front correlates with poor DFS and OS [134-137]. ECAD functionality is not considered an easy target for tumor therapy since it appears to be affected by a multitude of mechanisms, namely genetic alterations, transcriptional repression, and suppression of the ECAD adhesion complexes via several signaling pathways [126].

6.2 N-cadherin

The expression of NCAD is generally found in different cell types, including endothelial cells, neural cells, osteoblasts, and stromal cells [138, 139]. NCAD facilitates angiogenesis and mediates vascular stabilization [91].

NCAD acts as an indicator of the EMT process, and its expression has been associated with the development of numerous types of cancer [140-143]. In neuroblastoma, down-regulation of NCAD promotes metastasis [144]. There was an increase in pluripotency-associated markers in prostate cancer cells that overexpressed NCAD [140].

NCAD expression, as previously indicated in the literature, plays a vital function throughout the EMT process, when it is up-regulated [139, 145]. However, the NCAD expression in our tumor samples was less than 5%, and it did not change when comparing the expression of primary tumor to its recurrence. When we compare oral cancer to other tumor entities such as prostate cancer, we may conclude that high level of NCAD expression cannot be seen in oral cancer, which is reflective of the fact that NCAD is more frequent in non-epithelial tissues, and the increase of NCAD in normal epithelial cells implies that EMT and cancer growth are impending [106].

The discrepancy in ECAD loss compared to NCAD *de novo* expression raises the question of which of the Cadherins may indicate EMT and subsequently better correlates with post-recurrence survival outcome.

The key difference between the findings provided here and those in the relevant literature is the evaluation approach used for ECAD and NCAD expression. Predominantly, the threshold used to stratify patients is estimated differently. Previously, the cut-off was determined arbitrarily, mostly based on prior investigations, and was subject to multiple, not necessarily standardized immunohistological evaluation methodologies' interpretations. In addition, the available clinical data from the prospectively maintained cohort permit a valid risk-adjusted analysis, as all relevant parameters were collected at baseline and can be effectively incorporated into the applied regression model.

6.3 Effect of adjuvant treatment on the Epithelial-mesenchymal transition

Radiotherapy is used to treat more than fifty percent of cancer patients alone or in combination with other treatments. Current findings has demonstrated that RT could influence the immunobiological traits of OSCC cells. It typically works by either directly causing structural damage to DNA or indirectly causing damage via reactive oxygen species. Although RT is continuously advancing in recent years, most cancer patients continue to have poor prognosis. EMT is one of the most significant elements influencing radio-resistance in tumors, which has been recognized as a formidable hurdle to radiation efficacy [146, 147].

Our findings revealed that adjuvant RT didn't impact the expression of NCAD and ECAD. Yet, several studies have focused on the relationship between EMT and radioresistance. Lin et al suggested that X-Ray irradiation induced EMT through the smad signaling pathway in colorectal carcinoma [148]. Various signaling pathways, such as MAPK/ERK, TGF, HIF-1, Notch and Wnt, are extensively stimulated during carcinogenesis and development, and irradiation will further trigger these pathways, resulting in malignant features, including EMT and radio-resistance. Generally, RT can enhance the production of reactive oxygen species (ROS), which can activate signaling pathways and alter the TME. ROS has been connected to irradiation induced EMT via modulating a number of EMT markers and transcriptional factors [149].

One of the most popular chemotherapy drugs used now to treat HNSCC is Cisplatin. It can interact with RNA, DNA, and various proteins and induce apoptosis by triggering specific mechanisms [150, 151]. There are various disadvantages of utilizing Cisplatin in cancer treatment [152]. One of these is tumor resistance to therapy, several studies have demonstrated that the EMT process is critical not only for cancer cell development and malignancy, but it also causes chemotherapy resistance and decreases apoptotic cell death [103-105, 153]. Ashrafizadeh et al. described in their review the different pathways of

EMT-mediated cancer chemoresistance through Cisplatin therapy [102]. In contrast to the research cited, we did not find a variation in the expression of ECAD and NCAD after Cisplatin therapy, which could be attributed to our small sample size in the presented study.

6.4 Epithelial–mesenchymal transition and survival outcome

EMT is the complex cellular program through which epithelial cells turn into mesenchymal-like cells; this process facilitates the invasion and metastasis of epithelial cancer cells. EMT is distinguished by the decrease or loss of expression of adhesion molecules and the increase of expression of mesenchymal markers.

There are many things that could control EMT. Still, these were mostly studied in cell culture models and under standard conditions, which are very different from what happens when multiple organs or tissues of a patient interact with each other. Especially, this kind of setting makes it hard to figure out how much ECAD and NCAD changes affect how long patients with re-OSCC survive [154, 155].

EMT process is ruled by a range of factors. To this day, these factors were predominantly studied in cell culture models and under standard conditions, which are very different from what happens when different organs or tissues of a patient interact with each other [156]. It is especially difficult to understand how much changes in ECAD and NCAD could influence how long patients with re-OSCC survive in this environment. In this study, we investigated the predictive value of ECAD and NCAD expression as independent indicator of survival.

Our findings showed that evaluating combined protein expression of both Cadherins and correlating them to the clinical data were less accurate than evaluating single protein expression, since EMT is very complex and different regulatory components and signaling pathways are involved in the process. In our tumor samples the loss of ECAD and *de novo* expression of NCAD were detected at the tumor-stromal interface,

as mentioned above. Even the expression of the proteins in the recurrence tumor when comparing it to the primary of the same tumor didn't change. Pyo et al. investigated the relationship between the decreased ECAD expression and positive NCAD expression in OSCC, which in turn implies that cadherin switch most probably contributes significantly to the invasiveness and metastasis of oral squamous cell carcinoma [157].

Few clinical studies have been conducted on the relationship between ECAD and NCAD and survival in OSCC. These studies either studied the stage of primary disease as a variable [158], or compared tumor tissue with pre-cancerous lesions of oral mucosa as a control group [159].

According to recent research, this is the first study to look at how between ECAD and NCAD expression affects survival after recurrence. In the risk-adjusted hazard model, we found that the post-recurrence overall survival and the post-recurrence oral cancer-specific survival both dropped significantly when the *h-score* for ECAD loss was more than 60%. Therefore, in patients with re-OSCC, this IHC score serves as an independent risk factor for poor post-recurrence survival.

6.5 Outlook and perspectives for future studies

Notwithstanding these conclusions and the findings of the current study, drug-induced EMT inhibition may present a therapeutic possibility. Since EMT plays a crucial role in tumor aggressiveness and metastasis, various medications were developed to specifically target EMT-related signaling pathways. For example, Tangeretin and Rhamnetin were reported to induce radiosensitivity by interfering with Notch pathway [160, 161]. A combination therapy of radiotherapy and HIF-1-targeting agents such as Sorafeinib and Paclitaxel was shown to improve radio-sensitization [162, 163]. Furthermore, Also, By suppressing the EGFR signaling pathway, the anti-EGFR monoclonal antibody nimotuzumab was shown to increase the responsiveness of esophageal cancer KYSE-150R cells to radiation [164].

Metformin was also used to test this strategy, and the results showed that EMT in oral squamous cell carcinoma might be inhibited by the mTOR/HIF-1/PKM2/STAT3 pathway [165]. It is now necessary to conduct prospective, randomized clinical trials to determine whether this therapy is beneficial for patients with recurrent OSCC. It is becoming obvious that while previous research has mainly concentrated on alterations in gene expression and abnormal genetic and epigenetic mutations in cancer cells, examining different versions in the stromal structure of the HNSCC TME and their influence on tumorigenesis and progression may aim to grasp better the processes underlying different reactions to treatment [72].

6.6 Limitations of the present study

One of the study's shortcomings was its small sample size. Furthermore, the distribution of patients who received different treatments (surgery alone, surgery + radiotherapy, surgery + radiochemotherapy) was inhomogeneous.

Another point to note is that the EMT process extends far beyond the cadherin switch. Cell signaling, epigenetic modification, post-translational modifications, and transcriptional control all have an impact on the process [166]. Some studies showed that reduced ECAD expression is a signature of EMT. Still, some researchers contended that ECAD loss of expression is unnecessary during the EMT process and that restoring ECAD expression in ECAD-negative malignant cells did not affect the EMT [167]. It was discovered that the decreased expression of ECAD was insufficient to induce EMT in a non-malignant breast cell line [168]. The loss of ECAD expression, on the other hand, has long been associated with more aggressive, poorly differentiated malignant cells; additionally, ECAD loss has been linked to the activation of numerous EMT transcription factors. As a result, whether the loss of ECAD is a key cause or a consequence of EMT remains an open debate [169].

7. Summary

Previous research has indicated that the loss of ECAD and the overexpression of NCAD in tumor cells lead to metastatic dissemination and the triggering of different EMT transcription factors.

In this study the survival outcomes were determined by analyzing the immunohistochemistry expression of ECAD and NCAD in primary and recurrent OSCC patients as a marker of EMT in relation to OSCC. We correlated the post-recurrence survival of this high-risk group with the immunohistochemical expression of both cadherin types based on standardized *h-scores* and the clinical outcome (oral cancer-specific survival, overall survival, and post-recurrence disease-free survival). The patients' characteristics were scanned and digitally assessed. The evaluation of immunostaining was based on the *h-score*.

The current study reveals that Cadherin-switch appears to be an innate histological marker whose expression does not change between the primary tumor and its recurrence, regardless of the therapy used for the primary tumor. Crucial evidence was found of a proportionate correlation between ECAD loss, post-recurrence oral cancer-specific survival, and post-recurrence survival in re-OSCC using the automated evaluation of the *h-score* for IHC staining. ECAD loss of greater than 60% significantly raised the hazard ratio for post-recurrence survival outcome. Post-recurrence survival outcomes didn't correlate with the *de novo* expression of NCAD. Therefore, the loss of ECAD could be a potential biomarker for stratifying therapy and de-/escalating multimodal treatment as an independent risk factor for poor survival in patients with re-OSCC. In patients with re-OSCC, targeting EMT, may thus represent a potential adjuvant treatment option.

8. Zusammenfassung

Die epithelial-mesenchymale Transition (EMT) ist ein biologischer Mechanismus bei zahlreichen physiologischen und pathologischen Erkrankungen. Die damit verbundenen Veränderungen der Cadherin-Expression spielen eine entscheidende Rolle bei der Entstehung von Karzinomen, der Invasion und Metastasierung, der Angiogenese und der Immunantwort. EMT-Zellen weisen einen Übergang von einem epithelialen zu einem mesenchymalen Phänotyp auf (Cadherin-Switch). Dieser Prozess ist durch die *de novo*-Bildung von N-Cadherin (NCAD) gekennzeichnet, das E-Cadherin (ECAD) ersetzt und für eine erhöhte Migrationsfähigkeit und maligne Transformation der entarteten Zellen steht. Der Cadherin-Switch ist ein Charakteristikum der EMT und wurde bei verschiedenen Krebsarten beobachtet. Die vorliegende Arbeit untersucht als Hypothese den Cadherin-Switch im Gewebe von primären und rezidivierenden oralen Plattenepithelkarzinomen (re-OSCC) als inhärentes Merkmal des Tumors, das biologische Verhalten reguliert und darüber hinaus die Überlebensrate dieser Patienten nach dem Rezidiv beeinflusst. In der Methode wurden die Überlebensrate nach dem Rezidiv berechnet und die standardisierte *h-score* basierte IHC-Expression beider Cadherin-Typen mit dem klinischen Verlauf korreliert. In der zugrunde liegenden Kohorte wurden 94 Patienten mit einem Rezidiv eines OSCC im Rahmen der klinischen Routine kontrolliert. Es wurden Gewebeproben sowohl von Primär- als auch von Rezidivtumoren gesammelt und immunhistochemisch untersucht. Es wurde ein signifikanter Zusammenhang zwischen dem Verlust der ECAD-Expression und dem OSCC-spezifischen Überleben sowie dem Gesamtüberleben festgestellt (HR=2,72, CI:1,50-4,95, p=0,001) bzw. (HR=3,84, CI:1,93-7,63, p=0,001) bei einem Expressionsverlust von mehr als 60%. Es bestand kein statistisch signifikanter Zusammenhang zwischen der N-CAD *de-novo*-Expression und dem OSCC-spezifischen Überleben, dem Gesamtüberleben, oder dem krankheitsfreien Überleben nach Rezidiv.

Die aktuelle Studie zeigt, dass der Cadherin-Switch ein histologischer Marker zu sein scheint, dessen Expression sich zwischen dem Primärtumor und seinem Rezidiv nicht verändert, unabhängig von der Therapie des Primärtumors. Anhand der automatisierten Auswertung des *h-scores* für die IHC-Färbung wurde ein entscheidender Hinweis auf eine Korrelation zwischen ECAD-Verlust, OSCC-spezifischem Überleben nach Rezidiv und Gesamtüberleben nach Rezidiv gefunden. Ein ECAD -Verlust von mehr als 60 % erhöhte das Hazard Ratio für ein Sterben nach einem Rezidiv signifikant. Die Überlebensrate nach einem Rezidiv korrelierte nicht mit der *de-novo*-Expression von NCAD. Daher könnte der Verlust von ECAD ein potenzieller Biomarker für die Stratifizierung der Therapie und die De-/Eskalierung der multimodalen Behandlung sein, da er ein unabhängiger Risikofaktor für das Überleben bei Patienten mit re-OSCC ist. Bei Patienten mit re-OSCC könnte, neben der etablierten adjuvanten therapieverfahren, die gezielte Beeinflussung der EMT daher eine mögliche zusätzliche Behandlungsoption darstellen.

9. References

1. Roy, Monisha, Padmavathi, Lalhruaitluanga, Kumar, Singh, Bordoloi, Baruah, Ahmed, Longkumar, Arfuso, Kumar and Kunnumakkara, Isoform-Specific Role of Akt in Oral Squamous Cell Carcinoma, *Biomolecules*, 2019, **9**, 253.
2. A. P. Stein, S. Saha, J. L. Kraninger, A. D. Swick, M. Yu, P. F. Lambert and R. J. Kimple, Prevalence of Human Papillomavirus in Oropharyngeal Cancer: A Systematic Review, *Cancer J*, 2015, **21**, 138-146.
3. T. Isayeva, Y. Li, D. Maswahu and M. Brandwein-Gensler, Human Papillomavirus in Non-Oropharyngeal Head and Neck Cancers: A Systematic Literature Review, *Head and Neck Pathology*, 2012, **6**, 104-120.
4. D. S. Michaud, S. M. Langevin, M. Eliot, H. H. Nelson, M. Pawlita, M. D. McClean and K. T. Kelsey, High-risk HPV types and head and neck cancer, *International Journal of Cancer*, 2014, **135**, 1653-1661.
5. D. E. Johnson, B. Burtness, C. R. Leemans, V. W. Y. Lui, J. E. Bauman and J. R. Grandis, Head and neck squamous cell carcinoma, *Nature Reviews Disease Primers*, 2020, **6**.
6. J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D. M. Parkin, M. Piñeros, A. Znaor and F. Bray, Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, *International Journal of Cancer*, 2019, **144**, 1941-1953.
7. F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre and A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: A Cancer Journal for Clinicians*, 2018, **68**, 394-424.
8. M. Hashibe, P. Brennan, S. Benhamou, X. Castellsague, C. Chen, M. P. Curado, L. D. Maso, A. W. Daudt, E. Fabianova, V. Wunsch-Filho, S. Franceschi, R. B. Hayes, R. Herrero, S. Koifman, C. La Vecchia, P. Lazarus, F. Levi, D. Mates, E. Matos, A. Menezes, J. Muscat, J. Eluf-Neto, A. F. Olshan, P. Rudnai, S. M. Schwartz, E. Smith, E. M. Sturgis, N. Szeszenia-Dabrowska, R. Talamini, Q. Wei, D. M. Winn, D. Zaridze, W. Zatonski, Z. F. Zhang, J. Berthiller and P. Boffetta, Alcohol Drinking in Never Users of Tobacco, Cigarette Smoking in Never Drinkers, and the Risk of Head and Neck Cancer: Pooled Analysis in the International Head and Neck Cancer Epidemiology Consortium, *JNCI Journal of the National Cancer Institute*, 2007, **99**, 777-789.
9. H. Mehanna, T. Beech, T. Nicholson, I. El-Hariry, C. McConkey, V. Paleri and S. Roberts, Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region, *Head Neck*, 2013, **35**, 747-755.
10. H. Jiang, M. Livingston, R. Room, Y. Gan, D. English and R. Chenhall, Can public health policies on alcohol and tobacco reduce a cancer epidemic? Australia's experience, *BMC Medicine*, 2019, **17**.
11. M. J. Windon, G. D'Souza, E. M. Rettig, W. H. Westra, A. Van Zante, S. J. Wang, W. R. Ryan, W. K. Mydlarz, P. K. Ha, B. A. Miles, W. Koch, C. Gourin, D. W. Eisele and C. Fakhry, Increasing prevalence of human papillomavirus-positive oropharyngeal cancers among older adults, *Cancer*, 2018, **124**, 2993-2999.
12. S. Y. H. Fung, J. W. K. Lam and K. C. A. Chan, Clinical utility of circulating Epstein-Barr virus DNA analysis for the management of nasopharyngeal carcinoma, *Chinese Clinical Oncology*, 2016, **5**, 18-18.
13. W. L. Dissanayaka, G. Pitiyage, P. V. Kumarasiri, R. L. Liyanage, K. D. Dias and W. M. Tilakaratne, Clinical and histopathologic parameters in survival of oral squamous cell carcinoma, *Oral Surg Oral Med Oral Pathol Oral Radiol*, 2012, **113**, 518-525.

14. S. Koontongkaew, The Tumor Microenvironment Contribution to Development, Growth, Invasion and Metastasis of Head and Neck Squamous Cell Carcinomas, *Journal of Cancer*, 2013, **4**, 66-83.
15. J. Lee, V. Taneja and R. Vassallo, Cigarette Smoking and Inflammation, *Journal of Dental Research*, 2012, **91**, 142-149.
16. S. Gandini, E. Botteri, S. Iodice, M. Boniol, A. B. Lowenfels, P. Maisonneuve and P. Boyle, Tobacco smoking and cancer: A meta-analysis, *International Journal of Cancer*, 2008, **122**, 155-164.
17. Y.-C. A. Lee, M. Marron, S. Benhamou, C. Bouchardy, W. Ahrens, H. Pohlabeln, P. Lagiou, D. Trichopoulos, A. Agudo, X. Castellsague, V. Bencko, I. Holcatova, K. Kjaerheim, F. Merletti, L. Richiardi, G. J. Macfarlane, T. V. Macfarlane, R. Talamini, L. Barzan, C. Canova, L. Simonato, D. I. Conway, P. A. McKinney, R. J. Lowry, L. Sneddon, A. Znaor, C. M. Healy, B. E. McCartan, P. Brennan and M. Hashibe, Active and Involuntary Tobacco Smoking and Upper Aerodigestive Tract Cancer Risks in a Multicenter Case-Control Study, *Cancer Epidemiology Biomarkers & Prevention*, 2009, **18**, 3353-3361.
18. T. Sanner and T. K. Grimsrud, Nicotine: Carcinogenicity and Effects on Response to Cancer Treatment - A Review, *Front Oncol*, 2015, **5**, 196.
19. H. M. Schuller, Nitrosamines as nicotinic receptor ligands, *Life Sci*, 2007, **80**, 2274-2280.
20. S. Singh, S. Pillai and S. Chellappan, Nicotinic acetylcholine receptor signaling in tumor growth and metastasis, *J Oncol*, 2011, **2011**, 456743.
21. P. Dasgupta, W. Rizwani, S. Pillai, R. Kinkade, M. Kovacs, S. Rastogi, S. Banerjee, M. Carless, E. Kim, D. Coppola, E. Haura and S. Chellappan, Nicotine induces cell proliferation, invasion and epithelial-mesenchymal transition in a variety of human cancer cell lines, *Int J Cancer*, 2009, **124**, 36-45.
22. G. Di Credico, J. Polesel, L. Dal Maso, F. Pauli, N. Torelli, D. Luce, L. Radoï, K. Matsuo, D. Serraino, P. Brennan, I. Holcatova, W. Ahrens, P. Lagiou, C. Canova, L. Richiardi, C. M. Healy, K. Kjaerheim, D. I. Conway, G. J. Macfarlane, P. Thomson, A. Agudo, A. Znaor, S. Franceschi, R. Herrero, T. N. Toporcov, R. A. Moyses, J. Muscat, E. Negri, M. Vilensky, L. Fernandez, M. P. Curado, A. Menezes, A. W. Daudt, R. Koifman, V. Wunsch-Filho, A. F. Olshan, J. P. Zevallos, E. M. Sturgis, G. Li, F. Levi, Z.-F. Zhang, H. Morgenstern, E. Smith, P. Lazarus, C. La Vecchia, W. Garavello, C. Chen, S. M. Schwartz, T. Zheng, T. L. Vaughan, K. Kelsey, M. McClean, S. Benhamou, R. B. Hayes, M. P. Purdue, M. Gillison, S. Schantz, G.-P. Yu, S.-C. Chuang, P. Boffetta, M. Hashibe, A. L. Yuan-Chin and V. Edefonti, Alcohol drinking and head and neck cancer risk: the joint effect of intensity and duration, *British Journal of Cancer*, 2020, **123**, 1456-1463.
23. Z. Khan and P. S. Bisen, Oncoapoptotic signaling and deregulated target genes in cancers: special reference to oral cancer, *Biochim Biophys Acta*, 2013, **1836**, 123-145.
24. L. Feng and L. Wang, Effects of alcohol on the morphological and structural changes in oral mucosa, *Pak J Med Sci*, 2013, **29**, 1046-1049.
25. R. B. Hayes, J. Ahn, X. Fan, B. A. Peters, Y. Ma, L. Yang, I. Agalliu, R. D. Burk, I. Ganly, M. P. Purdue, N. D. Freedman, S. M. Gapstur and Z. Pei, Association of Oral Microbiome With Risk for Incident Head and Neck Squamous Cell Cancer, *JAMA Oncol*, 2018, **4**, 358-365.
26. A. R. Winstock, C. R. Trivedy, K. A. Warnakulasuriya and T. J. Peters, A dependency syndrome related to areca nut use: some medical and psychological aspects among areca nut users in the Gujarat community in the UK, *Addict Biol*, 2000, **5**, 173-179.
27. Y. C. Ko, Y. L. Huang, C. H. Lee, M. J. Chen, L. M. Lin and C. C. Tsai, Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan, *J Oral Pathol Med*, 1995, **24**, 450-453.

28. G. Arakeri, S. G. Patil, A. S. Aljabab, K. C. Lin, M. A. W. Merkx, S. Gao and P. A. Brennan, Oral submucous fibrosis: An update on pathophysiology of malignant transformation, *J Oral Pathol Med*, 2017, **46**, 413-417.
29. J. H. Jeng, Y. S. Ho, C. P. Chan, Y. J. Wang, L. J. Hahn, D. Lei, C. C. Hsu and M. C. Chang, Areca nut extract up-regulates prostaglandin production, cyclooxygenase-2 mRNA and protein expression of human oral keratinocytes, *Carcinogenesis*, 2000, **21**, 1365-1370.
30. P. C. Gupta, P. R. Murti, R. B. Bhonsle, F. S. Mehta and J. J. Pindborg, Effect of cessation of tobacco use on the incidence of oral mucosal lesions in a 10-yr follow-up study of 12,212 users, *Oral Dis*, 1995, **1**, 54-58.
31. B. Gupta, A. Ariyawardana and N. W. Johnson, Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives, *International dental journal*, 2013, **63**, 12-25.
32. D. Hanahan and R. A. Weinberg, The hallmarks of cancer, *Cell*, 2000, **100**, 57-70.
33. C. Rivera and B. Venegas, Histological and molecular aspects of oral squamous cell carcinoma (Review), *Oncol Lett*, 2014, **8**, 7-11.
34. Z. Wang, B. Zhang, L. Jiang, X. Zeng, Y. Chen, X. Feng, Y. Guo and Q. Chen, RACK1, an excellent predictor for poor clinical outcome in oral squamous carcinoma, similar to Ki67, *Eur J Cancer*, 2009, **45**, 490-496.
35. C. A. Rivera Martínez, 4NQO Carcinogenesis: A Model of Oral Squamous Cell Carcinoma, *International Journal of Morphology*, 2012, **30**, 309-314.
36. B. W. Neville and T. A. Day, Oral cancer and precancerous lesions, *CA Cancer J Clin*, 2002, **52**, 195-215.
37. B. Fuentes, J. Duaso, D. Droguett, C. Castillo, W. Donoso, C. Rivera, B. Venegas and U. Kemmerling, Progressive extracellular matrix disorganization in chemically induced murine oral squamous cell carcinoma, *International Scholarly Research Notices*, 2012, **2012**.
38. G. J. Kelloff and C. C. Sigman, Assessing intraepithelial neoplasia and drug safety in cancer-preventive drug development, *Nat Rev Cancer*, 2007, **7**, 508-518.
39. F. Bray, R. Sankila, J. Ferlay and D. M. Parkin, Estimates of cancer incidence and mortality in Europe in 1995, *Eur J Cancer*, 2002, **38**, 99-166.
40. S. M. Fati, E. M. Senan and Y. Javed, Early Diagnosis of Oral Squamous Cell Carcinoma Based on Histopathological Images Using Deep and Hybrid Learning Approaches, *Diagnostics (Basel)*, 2022, **12**.
41. C. F. Poh, S. Ng, K. W. Berean, P. M. Williams, M. P. Rosin and L. Zhang, Biopsy and histopathologic diagnosis of oral premalignant and malignant lesions, *J Can Dent Assoc*, 2008, **74**, 283-288.
42. C. Scheifele and P. A. Reichart, Is there a natural limit of the transformation rate of oral leukoplakia?, *Oral Oncol*, 2003, **39**, 470-475.
43. G. Mamelle, J. Pampurik, B. Luboinski, R. Lancar, A. Lusinchi and J. Bosq, Lymph node prognostic factors in head and neck squamous cell carcinomas, *Am J Surg*, 1994, **168**, 494-498.
44. P. S. Arunachalam, G. Putnam, P. Jennings, R. Messersmith and A. K. Robson, Role of computerized tomography (CT) scan of the chest in patients with newly diagnosed head and neck cancers, *Clin Otolaryngol Allied Sci*, 2002, **27**, 409-411.
45. A. Leslie, E. Fyfe, P. Guest, P. Goddard and J. E. Kabala, Staging of squamous cell carcinoma of the oral cavity and oropharynx: a comparison of MRI and CT in T- and N-staging, *J Comput Assist Tomogr*, 1999, **23**, 43-49.
46. T. Wong and D. Wiesenfeld, Oral Cancer, *Australian Dental Journal*, 2018, **63**, S91-S99.

47. G. W. Goerres, D. T. Schmid, B. Schuknecht and G. K. Eyrich, Bone invasion in patients with oral cavity cancer: comparison of conventional CT with PET/CT and SPECT/CT, *Radiology*, 2005, **237**, 281-287.
48. B. D. Smith and B. G. Haffty, Molecular markers as prognostic factors for local recurrence and radioresistance in head and neck squamous cell carcinoma, *Radiat Oncol Investig*, 1999, **7**, 125-144.
49. W. M. Lydiatt, S. G. Patel, B. O'Sullivan, M. S. Brandwein, J. A. Ridge, J. C. Migliacci, A. M. Loomis and J. P. Shah, Head and neck cancers-major changes in the American Joint Committee on cancer eighth edition cancer staging manual, *CA: A Cancer Journal for Clinicians*, 2017, **67**, 122-137.
50. S. H. Huang and B. O'Sullivan, Overview of the 8th Edition TNM Classification for Head and Neck Cancer, *Current Treatment Options in Oncology*, 2017, **18**, 40.
51. A. T. Meram and B. M. Woo, in *Peterson's Principles of Oral and Maxillofacial Surgery*, eds. M. Miloro, G. E. Ghali, P. E. Larsen and P. Waite, Springer International Publishing, Cham, 2022, DOI: 10.1007/978-3-030-91920-7_34, pp. 1009-1055.
52. G. Studer, K. Furrer, B. J. Davis, S. S. Stoeckli, R. A. Zwahlen, U. M. Luetolf and C. Glanzmann, Postoperative IMRT in head and neck cancer, *Radiation Oncology*, 2006, **1**, 40.
53. K. H. Fan, H. M. Wang, C. J. Kang, L. Y. Lee, S. F. Huang, C. Y. Lin, E. Y. Chen, I. H. Chen, C. T. Liao and J. T. Chang, Treatment results of postoperative radiotherapy on squamous cell carcinoma of the oral cavity: coexistence of multiple minor risk factors results in higher recurrence rates, *Int J Radiat Oncol Biol Phys*, 2010, **77**, 1024-1029.
54. E. Katsoulakis, J. E. Leeman, B. H. Lok, W. Shi, Z. Zhang, J. C. Tsai, S. M. McBride, E. J. Sherman, M. Cohen, R. Wong, I. Ganly, N. Y. Lee and N. Riaz, Long-term outcomes in oral cavity squamous cell carcinoma with adjuvant and salvage radiotherapy after surgery, *Laryngoscope*, 2018, **128**, 2539-2545.
55. J. S. Cooper, T. F. Pajak, A. A. Forastiere, J. Jacobs, B. H. Campbell, S. B. Saxman, J. A. Kish, H. E. Kim, A. J. Cmelak, M. Rotman, M. Machtay, J. F. Ensley, K. S. Chao, C. J. Schultz, N. Lee and K. K. Fu, Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck, *N Engl J Med*, 2004, **350**, 1937-1944.
56. J. Bernier, C. Domenge, M. Ozsahin, K. Matuszewska, J. L. Lefèbvre, R. H. Greiner, J. Giralt, P. Maingon, F. Rolland, M. Bolla, F. Cognetti, J. Bourhis, A. Kirkpatrick and M. van Glabbeke, Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer, *N Engl J Med*, 2004, **350**, 1945-1952.
57. S. R. Quinlan-Davidson, W. H. Morrison, J. N. Myers, G. B. Gunn, W. N. William, Jr., B. M. Beadle, H. D. Skinner, A. M. Gillenwater, S. J. Frank, J. Phan, F. M. Johnson, C. D. Fuller, M. E. Zafereo, D. I. Rosenthal and A. S. Garden, Recurrent oral cavity cancer: Patterns of failure after salvage multimodality therapy, *Head Neck*, 2017, **39**, 633-638.
58. S. Tam, R. Araslanova, T. H. Low, A. Warner, J. Yoo, K. Fung, S. D. MacNeil, D. A. Palma and A. C. Nichols, Estimating Survival After Salvage Surgery for Recurrent Oral Cavity Cancer, *JAMA Otolaryngol Head Neck Surg*, 2017, **143**, 685-690.
59. N. Denaro and M. C. Merlano, Immunotherapy in Head and Neck Squamous Cell Cancer, *Clin Exp Otorhinolaryngol*, 2018, **11**, 217-223.
60. A. L. Carvalho, L. P. Kowalski, I. M. Agra, E. Pontes, O. D. Campos and A. C. A. Pellizzon, Treatment results on advanced neck metastasis (N3) from head and neck squamous carcinoma, *Otolaryngology-Head and Neck Surgery*, 2005, **132**, 862-868.
61. L. P. Kowalski, A. L. Carvalho, A. V. M. Priante and J. Magrin, Predictive factors for distant metastasis from oral and oropharyngeal squamous cell carcinoma, *Oral oncology*, 2005, **41**, 534-541.

62. S. Fan, Q. l. Tang, Y. j. Lin, W. l. Chen, J. s. Li, Z. q. Huang, Z. h. Yang, Y. y. Wang, D. m. Zhang and H. j. Wang, A review of clinical and histological parameters associated with contralateral neck metastases in oral squamous cell carcinoma, *International journal of oral science*, 2011, **3**, 180-191.
63. J. S. Greenberg, A. K. El Naggar, V. Mo, D. Roberts and J. N. Myers, Disparity in pathologic and clinical lymph node staging in oral tongue carcinoma: Implications for therapeutic decision making, *Cancer: Interdisciplinary International Journal of the American Cancer Society*, 2003, **98**, 508-515.
64. C. R. Leemans, R. Tiwari, J. J. Nauta, I. V. D. Waal and G. B. Snow, Recurrence at the primary site in head and neck cancer and the significance of neck lymph node metastases as a prognostic factor, *Cancer*, 1994, **73**, 187-190.
65. I. M. G. Agra, J. G. Filho, E. P. Martins and L. P. Kowalski, Second salvage surgery for re-recurrent oral cavity and oropharynx carcinoma, *Head & Neck*, 2010, **32**, 997-1002.
66. J. Bernier, J. Bonner, J. Vermorken, R.-J. Bensadoun, R. Dummer, J. Giralt, G. Kornek, A. Hartley, R. Mesia and C. Robert, Consensus guidelines for the management of radiation dermatitis and coexisting acne-like rash in patients receiving radiotherapy plus EGFR inhibitors for the treatment of squamous cell carcinoma of the head and neck, *Annals of Oncology*, 2008, **19**, 142-149.
67. J. B. Vermorken, E. Remenar, C. Van Herpen, T. Gorlia, R. Mesia, M. Degardin, J. S. Stewart, S. Jelic, J. Betka and J. H. Preiss, Cisplatin, fluorouracil, and docetaxel in unresectable head and neck cancer, *New England Journal of Medicine*, 2007, **357**, 1695-1704.
68. P. A. Kenny, G. Y. Lee and M. J. Bissell, Targeting the tumor microenvironment, *Front Biosci*, 2007, **12**, 3468-3474.
69. J. Zhang and J. Liu, Tumor stroma as targets for cancer therapy, *Pharmacol Ther*, 2013, **137**, 200-215.
70. R. U. Jin and J. C. Mills, Tumor organoids to study gastroesophageal cancer: a primer, *J Mol Cell Biol*, 2020, **12**, 593-606.
71. B. Arneth, Tumor Microenvironment, *Medicina (Kaunas)*, 2019, **56**.
72. B. Peltanova, M. Raudenska and M. Masarik, Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review, *Molecular Cancer*, 2019, **18**, 63.
73. M. Takeichi, The cadherins: cell-cell adhesion molecules controlling animal morphogenesis, *Development*, 1988, **102**, 639-655.
74. N. Colás-Algora and J. Millán, How many cadherins do human endothelial cells express?, *Cellular and Molecular Life Sciences*, 2019, **76**, 1299-1317.
75. B. M. Gumbiner, in *The Cadherin Superfamily: Key Regulators of Animal Development and Physiology*, eds. S. T. Suzuki and S. Hirano, Springer Japan, Tokyo, 2016, DOI: 10.1007/978-4-431-56033-3_3, pp. 41-69.
76. S. Nakajima, R. Doi, E. Toyoda, S. Tsuji, M. Wada, M. Koizumi, S. S. Tulachan, D. Ito, K. Kami, T. Mori, Y. Kawaguchi, K. Fujimoto, R. Hosotani and M. Imamura, N-cadherin expression and epithelial-mesenchymal transition in pancreatic carcinoma, *Clin Cancer Res*, 2004, **10**, 4125-4133.
77. N. Matsuyoshi and S. Imamura, Multiple cadherins are expressed in human fibroblasts, *Biochem Biophys Res Commun*, 1997, **235**, 355-358.
78. M. T. Lau, C. Klausen and P. C. Leung, E-cadherin inhibits tumor cell growth by suppressing PI3K/Akt signaling via β-catenin-Egr1-mediated PTEN expression, *Oncogene*, 2011, **30**, 2753-2766.

79. W. Birchmeier and J. Behrens, Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness, *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 1994, **1198**, 11-26.
80. M. R. Schneider and F. T. Kolligs, E-cadherin's role in development, tissue homeostasis and disease: Insights from mouse models: Tissue-specific inactivation of the adhesion protein E-cadherin in mice reveals its functions in health and disease, *Bioessays*, 2015, **37**, 294-304.
81. M. Rosso, B. Majem, L. Devis, L. Lapyckyj, M. J. Besso, M. Llauradó, M. F. Abascal, M. L. Matos, L. Lanau, J. Castellví, J. L. Sánchez, A. Pérez Benavente, A. Gil-Moreno, J. Reventós, A. Santamaria Margalef, M. Rigau and M. H. Vazquez-Levin, E-cadherin: A determinant molecule associated with ovarian cancer progression, dissemination and aggressiveness, *PLoS One*, 2017, **12**, e0184439.
82. F. van Roy and G. Berx, The cell-cell adhesion molecule E-cadherin, *Cell Mol Life Sci*, 2008, **65**, 3756-3788.
83. T. T. Onder, P. B. Gupta, S. A. Mani, J. Yang, E. S. Lander and R. A. Weinberg, Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways, *Cancer Res*, 2008, **68**, 3645-3654.
84. J. Y. Wu, C. Yi, H. R. Chung, D. J. Wang, W. C. Chang, S. Y. Lee, C. T. Lin, Y. C. Yang and W. C. Yang, Potential biomarkers in saliva for oral squamous cell carcinoma, *Oral Oncol*, 2010, **46**, 226-231.
85. S. López-Verdín, M. L. Martínez-Fierro, I. Garza-Veloz, A. Zamora-Perez, J. Grajeda-Cruz, R. González-González, N. Molina-Frechero, M. Arocena-Sutz and R. Bologna-Molina, E-Cadherin gene expression in oral cancer: Clinical and prospective data, *Med Oral Patol Oral Cir Bucal*, 2019, **24**, e444-e451.
86. V. Mattijssen, H. M. Peters, L. Schalkwijk, J. J. Manni, B. van 't Hof-Grootenboer, P. H. de Mulder and D. J. Ruiter, E-cadherin expression in head and neck squamous-cell carcinoma is associated with clinical outcome, *Int J Cancer*, 1993, **55**, 580-585.
87. M. Diniz-Freitas, T. García-Caballero, J. Antúnez-López, J. M. Gándara-Rey and A. García-García, Reduced E-cadherin expression is an indicator of unfavourable prognosis in oral squamous cell carcinoma, *Oral Oncol*, 2006, **42**, 190-200.
88. Z. Zhao, J. Ge, Y. Sun, L. Tian, J. Lu, M. Liu and Y. Zhao, Is E-cadherin immunoexpression a prognostic factor for head and neck squamous cell carcinoma (HNSCC)? A systematic review and meta-analysis, *Oral Oncol*, 2012, **48**, 761-767.
89. B. M. Gumbiner, Regulation of cadherin-mediated adhesion in morphogenesis, *Nat Rev Mol Cell Biol*, 2005, **6**, 622-634.
90. H. Gerhardt, H. Wolburg and C. Redies, N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken, *Dev Dyn*, 2000, **218**, 472-479.
91. J. H. Paik, A. Skoura, S. S. Chae, A. E. Cowan, D. K. Han, R. L. Proia and T. Hla, Sphingosine 1-phosphate receptor regulation of N-cadherin mediates vascular stabilization, *Genes Dev*, 2004, **18**, 2392-2403.
92. O. W. Blaschuk, N-cadherin antagonists as oncology therapeutics, *Philos Trans R Soc Lond B Biol Sci*, 2015, **370**, 20140039.
93. A. Mariotti, A. Perotti, C. Sessa and C. Rüegg, N-cadherin as a therapeutic target in cancer, *Expert Opinion on Investigational Drugs*, 2007, **16**, 451-465.
94. M. A. Nieto, R. Y. Huang, R. A. Jackson and J. P. Thiery, EMT: 2016, *Cell*, 2016, **166**, 21-45.
95. J. Yang, P. Antin, G. Berx, C. Blanpain, T. Brabletz, M. Bronner, K. Campbell, A. Cano, J. Casanova, G. Christofori, S. Dedhar, R. Derynck, H. L. Ford, J. Fuxe, Antonio, G. J. Goodall, A.-K. Hadjantonakis, R. Y. J. Huang, C. Kalcheim, R. Kalluri, Y. Kang, Y. Khew-Goodall, H. Levine, J. Liu, G. D. Longmore, S. A. Mani, J. Massagué, R. Mayor, D. McClay, K. E. Mostov, D. F.

Newgreen, M. A. Nieto, A. Puisieux, R. Runyan, P. Savagner, B. Stanger, M. P. Stemmler, Y. Takahashi, M. Takeichi, E. Theveneau, J. P. Thiery, E. W. Thompson, R. A. Weinberg, E. D. Williams, J. Xing, B. P. Zhou and G. Sheng, Guidelines and definitions for research on epithelial–mesenchymal transition, *Nature Reviews Molecular Cell Biology*, 2020, **21**, 341-352.
96. J. M. Lee, S. Dedhar, R. Kalluri and E. W. Thompson, The epithelial-mesenchymal transition: new insights in signaling, development, and disease, *J Cell Biol*, 2006, **172**, 973-981.
97. E. D. Hay, An overview of epithelio-mesenchymal transformation, *Acta Anat (Basel)*, 1995, **154**, 8-20.
98. C. Yan, W. A. Grimm, W. L. Garner, L. Qin, T. Travis, N. Tan and Y.-P. Han, Epithelial to Mesenchymal Transition in Human Skin Wound Healing Is Induced by Tumor Necrosis Factor-α through Bone Morphogenic Protein-2, *The American Journal of Pathology*, 2010, **176**, 2247-2258.
99. R. Mayor and S. Etienne-Manneville, The front and rear of collective cell migration, *Nat Rev Mol Cell Biol*, 2016, **17**, 97-109.
100. D. Shook and R. Keller, Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development, *Mech Dev*, 2003, **120**, 1351-1383.
101. J. D. Amack, Cellular dynamics of EMT: lessons from live in vivo imaging of embryonic development, *Cell Communication and Signaling*, 2021, **19**, 79.
102. M. Ashrafizadeh, A. Zarrabi, K. Hushmandi, M. Kalantari, R. Mohammadinejad, T. Javaheri and G. Sethi, Association of the Epithelial–Mesenchymal Transition (EMT) with Cisplatin Resistance, *International Journal of Molecular Sciences*, 2020, **21**, 4002.
103. Y. Shi, J. Zhang, M. Liu, Y. Huang and L. Yin, SMAD3 inducing the transcription of STYK1 to promote the EMT process and improve the tolerance of ovarian carcinoma cells to paclitaxel, *Journal of Cellular Biochemistry*, 2019, **120**, 10796-10811.
104. M. J. Sale, K. Balmanno, J. Saxena, E. Ozono, K. Wojdyla, R. E. McIntyre, R. Gilley, A. Woroniuk, K. D. Howarth, G. Hughes, J. R. Dry, M. J. Arends, P. Caro, D. Oxley, S. Ashton, D. J. Adams, J. Saez-Rodriguez, P. D. Smith and S. J. Cook, MEK1/2 inhibitor withdrawal reverses acquired resistance driven by BRAFV600E amplification whereas KRASG13D amplification promotes EMT-chemoresistance, *Nature Communications*, 2019, **10**, 2030.
105. L. Jing, W. Bo, F. Yourong, W. Tian, W. Shixuan and W. Mingfu, Sema4C mediates EMT inducing chemotherapeutic resistance of miR-31-3p in cervical cancer cells, *Scientific Reports*, 2019, **9**, 17727.
106. J. W. Po, A. Roohullah, D. Lynch, A. DeFazio, M. Harrison, P. R. Harnett, C. Kennedy, C. Kennedy, P. de Souza and T. M. Becker, Improved ovarian cancer EMT-CTC isolation by immunomagnetic targeting of epithelial EpCAM and mesenchymal N-cadherin, *Journal of Circulating Biomarkers*, 2018, **7**.
107. M. K. Jolly, K. E. Ware, S. Gilja, J. A. Somarelli and H. Levine, EMT and MET: necessary or permissive for metastasis?, *Mol Oncol*, 2017, **11**, 755-769.
108. Y. Zhang and R. A. Weinberg, Epithelial-to-mesenchymal transition in cancer: complexity and opportunities, *Front Med*, 2018, **12**, 361-373.
109. A. W. Lambert, D. R. Pattabiraman and R. A. Weinberg, Emerging Biological Principles of Metastasis, *Cell*, 2017, **168**, 670-691.
110. J. P. Thiery, H. Acloque, R. Y. Huang and M. A. Nieto, Epithelial-mesenchymal transitions in development and disease, *Cell*, 2009, **139**, 871-890.
111. X. Ye and R. A. Weinberg, Epithelial-Mesenchymal Plasticity: A Central Regulator of Cancer Progression, *Trends Cell Biol*, 2015, **25**, 675-686.
112. M. H. Barcellos-Hoff, D. Lyden and T. C. Wang, The evolution of the cancer niche during multistage carcinogenesis, *Nat Rev Cancer*, 2013, **13**, 511-518.

113. A. Dongre and R. A. Weinberg, New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer, *Nat Rev Mol Cell Biol*, 2019, **20**, 69-84.
114. C. S. Scanlon, E. A. Van Tubergen, R. C. Inglehart and N. J. D'Silva, Biomarkers of epithelial-mesenchymal transition in squamous cell carcinoma, *J Dent Res*, 2013, **92**, 114-121.
115. J. Kononen, L. Bubendorf, A. Kallioniemi, M. Bärlund, P. Schraml, S. Leighton, J. Torhorst, M. J. Mihatsch, G. Sauter and O. P. Kallioniemi, Tissue microarrays for high-throughput molecular profiling of tumor specimens, *Nat Med*, 1998, **4**, 844-847.
116. D. Voduc, C. Kenney and T. O. Nielsen, Tissue microarrays in clinical oncology, *Semin Radiat Oncol*, 2008, **18**, 89-97.
117. M. E. Charlson, P. Pompei, K. L. Ales and C. R. MacKenzie, A new method of classifying prognostic comorbidity in longitudinal studies: development and validation, *J Chronic Dis*, 1987, **40**, 373-383.
118. J. Ribbat-Idel, S. Perner, P. Kuppler, L. Klapper, R. Krupar, C. Watermann, F. O. Paulsen, A. Offermann, K. L. Bruchhage, B. Wollenberg and C. Idel, Immunologic "Cold" Squamous Cell Carcinomas of the Head and Neck Are Associated With an Unfavorable Prognosis, *Front Med (Lausanne)*, 2021, **8**, 622330.
119. A. Queisser, S. Hagedorn, H. Wang, T. Schaefer, M. Konantz, S. Alavi, M. Deng, W. Vogel, A. Von Mässenhausen, G. Kristiansen, S. Duensing, J. Kirfel, C. Lengerke and S. Perner, Ecotropic viral integration site 1, a novel oncogene in prostate cancer, *Oncogene*, 2017, **36**, 1573-1584.
120. T. Jagomast, C. Idel, L. Klapper, P. Kuppler, L. Proppe, S. Beume, M. Falougy, D. Steller, S. G. Hakim, A. Offermann, M. C. Roesch, K. L. Bruchhage, S. Perner and J. Ribbat-Idel, Comparison of manual and automated digital image analysis systems for quantification of cellular protein expression, *Histol Histopathol*, 2022, DOI: 10.14670/hh-18-434, 18434.
121. D. K. Meyerholz and A. P. Beck, Principles and approaches for reproducible scoring of tissue stains in research, *Lab Invest*, 2018, **98**, 844-855.
122. V. Tumuluri, G. A. Thomas and I. S. Fraser, Analysis of the Ki-67 antigen at the invasive tumour front of human oral squamous cell carcinoma, *J Oral Pathol Med*, 2002, **31**, 598-604.
123. W. Jerjes, T. Upile, A. Petrie, A. Riskalla, Z. Hamdoon, M. Vourvachis, K. Karavidas, A. Jay, A. Sandison, G. J. Thomas, N. Kalavrezos and C. Hopper, Clinicopathological parameters, recurrence, locoregional and distant metastasis in 115 T1-T2 oral squamous cell carcinoma patients, *Head Neck Oncol*, 2010, **2**, 9.
124. K. L. Westgaard, H. Hynne, C. D. Amdal, A. Young, P. B. Singh, X. Chen, M. Rykke, L. H. Hove, L. A. Aqrawi, T. P. Utheim, B. B. Herlofson and J. L. Jensen, Oral and ocular late effects in head and neck cancer patients treated with radiotherapy, *Sci Rep*, 2021, **11**, 4026.
125. M. R. Trendowski, O. El Charif, P. C. Dinh, Jr., L. B. Travis and M. E. Dolan, Genetic and Modifiable Risk Factors Contributing to Cisplatin-induced Toxicities, *Clin Cancer Res*, 2019, **25**, 1147-1155.
126. G. Christofori and H. Semb, The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene, *Trends Biochem Sci*, 1999, **24**, 73-76.
127. B. P. Wijnhoven, W. N. Dinjens and M. Pignatelli, E-cadherin-catenin cell-cell adhesion complex and human cancer, *Br J Surg*, 2000, **87**, 992-1005.
128. L. J. Lewis-Tuffin, F. Rodriguez, C. Giannini, B. Scheithauer, B. M. Necela, J. N. Sarkaria and P. Z. Anastasiadis, Misregulated E-Cadherin Expression Associated with an Aggressive Brain Tumor Phenotype, *PLOS ONE*, 2010, **5**, e13665.
129. A. P. Putzke, A. P. Ventura, A. M. Bailey, C. Akture, J. Opoku-Ansah, M. Celiktaş, M. S. Hwang, D. S. Darling, I. M. Coleman, P. S. Nelson, H. M. Nguyen, E. Corey, M. Tewari, C. Morrissey, R. L. Vessella and B. S. Knudsen, Metastatic progression of prostate cancer and e-cadherin regulation by zeb1 and SRC family kinases, *Am J Pathol*, 2011, **179**, 400-410.

130. P. Reddy, L. Liu, C. Ren, P. Lindgren, K. Boman, Y. Shen, E. Lundin, U. Ottander, M. Rytinki and K. Liu, Formation of E-Cadherin-Mediated Cell-Cell Adhesion Activates Akt and Mitogen Activated Protein Kinase via Phosphatidylinositol 3 Kinase and Ligand-Independent Activation of Epidermal Growth Factor Receptor in Ovarian Cancer Cells, *Molecular Endocrinology*, 2005, **19**, 2564-2578.
131. C. H. Pereira, M. O. Morais, A. F. L. Martins, M. Q. S. Soares, R. d. C. G. Alencar, A. C. Batista, C. R. Leles and E. F. Mendonça, Expression of adhesion proteins (E-cadherin and β-catenin) and cell proliferation (Ki-67) at the invasive tumor front in conventional oral squamous cell and basaloid squamous cell carcinomas, *Archives of Oral Biology*, 2016, **61**, 8-15.
132. S.-Y. Peng, H.-F. Tu, C.-C. Yang, C.-H. Wu, C.-J. Liu, K.-W. Chang and S.-C. Lin, miR-134 targets PDCD7 to reduce E-cadherin expression and enhance oral cancer progression, *International Journal of Cancer*, 2018, **143**, 2892-2904.
133. J. Sharma, M. Bhargava, S. Aggarwal, A. Aggarwal, A. Varshney and D. Chopra, Immunohistochemical evaluation of E-cadherin in oral epithelial dysplasia and squamous cell carcinoma, *Indian J Pathol Microbiol*, 2022, **65**, 755-760.
134. S. V. Thangaraj, V. Shyamsundar, A. Krishnamurthy and V. Ramshankar, Deregulation of extracellular matrix modeling with molecular prognostic markers revealed by transcriptome sequencing and validations in Oral Tongue squamous cell carcinoma, *Scientific Reports*, 2021, **11**.
135. P. Balasundaram, M. K. Singh, A. K. Dinda, A. Thakar and R. Yadav, Study of beta-catenin, E-cadherin and vimentin in oral squamous cell carcinoma with and without lymph node metastases, *Diagn Pathol*, 2014, **9**, 145.
136. J. A. Hanemann, D. T. Oliveira, S. Nonogaki, I. N. Nishimoto, M. L. de Carli, G. Landman and L. P. Kowalski, Expression of E-cadherin and beta-catenin in basaloid and conventional squamous cell carcinoma of the oral cavity: are potential prognostic markers?, *BMC Cancer*, 2014, **14**, 395.
137. O. C. Ukpo, W. L. Thorstad, Q. Zhang and J. S. Lewis, Jr., Lack of association of cadherin expression and histopathologic type, metastasis, or patient outcome in oropharyngeal squamous cell carcinoma: a tissue microarray study, *Head Neck Pathol*, 2012, **6**, 38-47.
138. F. van Roy, Beyond E-cadherin: roles of other cadherin superfamily members in cancer, *Nat Rev Cancer*, 2014, **14**, 121-134.
139. K. M. Mrozik, O. W. Blaschuk, C. M. Cheong, A. C. W. Zannettino and K. Vandyke, N-cadherin in cancer metastasis, its emerging role in haematological malignancies and potential as a therapeutic target in cancer, *BMC Cancer*, 2018, **18**, 939.
140. M. Wang, D. Ren, W. Guo, S. Huang, Z. Wang, Q. Li, H. Du, L. Song and X. Peng, N-cadherin promotes epithelial-mesenchymal transition and cancer stem cell-like traits via ErbB signaling in prostate cancer cells, *Int J Oncol*, 2016, **48**, 595-606.
141. K. Jennbacken, T. Tesan, W. Wang, H. Gustavsson, J. E. Damber and K. Welén, N-cadherin increases after androgen deprivation and is associated with metastasis in prostate cancer, *Endocr Relat Cancer*, 2010, **17**, 469-479.
142. J. Hulit, K. Suyama, S. Chung, R. Keren, G. Agiostratidou, W. Shan, X. Dong, T. M. Williams, M. P. Lisanti, K. Knudsen and R. B. Hazan, N-cadherin signaling potentiates mammary tumor metastasis via enhanced extracellular signal-regulated kinase activation, *Cancer Res*, 2007, **67**, 3106-3116.
143. L. Hui, S. Zhang, X. Dong, D. Tian, Z. Cui and X. Qiu, Prognostic significance of twist and N-cadherin expression in NSCLC, *PLoS One*, 2013, **8**, e62171.
144. T. Lammens, K. Swerts, L. Derycke, A. De Craemer, S. De Brouwer, K. De Preter, N. Van Roy, J. Vandesompele, F. Speleman, J. Philippé, Y. Benoit, K. Beiske, M. Bracke and G. Laureys, N-

cadherin in neuroblastoma disease: expression and clinical significance, *PLoS One*, 2012, **7**, e31206.
145. K. Araki, T. Shimura, H. Suzuki, S. Tsutsumi, W. Wada, T. Yajima, T. Kobayahi, N. Kubo and H. Kuwano, E/N-cadherin switch mediates cancer progression via TGF-β-induced epithelial-to-mesenchymal transition in extrahepatic cholangiocarcinoma, *Br J Cancer*, 2011, **105**, 1885-1893.
146. J. Theys, B. Jutten, R. Habets, K. Paesmans, A. J. Groot, P. Lambin, B. G. Wouters, G. Lammering and M. Vooijs, E-Cadherin loss associated with EMT promotes radioresistance in human tumor cells, *Radiother Oncol*, 2011, **99**, 392-397.
147. D. Nantajit, D. Lin and J. J. Li, The network of epithelial-mesenchymal transition: potential new targets for tumor resistance, *J Cancer Res Clin Oncol*, 2015, **141**, 1697-1713.
148. S. Lin, S. Chen, Z. Chen, Q. Dai and C. Ke, X-ray-induced epithelial-mesenchymal transition in SW480 colorectal cancer cells and its potential mechanisms, *J buon*, 2017, **22**, 1457-1462.
149. S. Y. Lee, E. K. Jeong, M. K. Ju, H. M. Jeon, M. Y. Kim, C. H. Kim, H. G. Park, S. I. Han and H. S. Kang, Induction of metastasis, cancer stem cell phenotype, and oncogenic metabolism in cancer cells by ionizing radiation, *Mol Cancer*, 2017, **16**, 10.
150. J. Huang, J. Zhang, C. Shi, L. Liu and Y. Wei, Survival, recurrence and toxicity of HNSCC in comparison of a radiotherapy combination with cisplatin versus cetuximab: a meta-analysis, *BMC Cancer*, 2016, **16**, 689.
151. M. Bostan, G. G. Petrică-Matei, G. Ion, N. Radu, M. Mihăilă, R. Hainăroşie, L. I. Braşoveanu, V. Roman, C. Constantin and M. T. Neagu, Cisplatin effect on head and neck squamous cell carcinoma cells is modulated by ERK1/2 protein kinases, *Exp Ther Med*, 2019, **18**, 5041-5051.
152. W. Wang, M. K. Shanmugam, P. Xiang, T. Y. A. Yam, V. Kumar, W. S. Chew, J. K. Chang, M. Z. B. Ali, M. J. Y. Reolo, Y. X. Peh, S. Karim, A. Y. Y. Tan, T. Sanda, G. Sethi and D. R. Herr, Sphingosine 1-Phosphate Receptor 2 Induces Otoprotective Responses to Cisplatin Treatment, *Cancers (Basel)*, 2020, **12**.
153. L. Wang, F. Zhang, J. Y. Cui, L. Chen, Y. T. Chen and B. W. Liu, CAFs enhance paclitaxel resistance by inducing EMT through the IL-6/JAK2/STAT3 pathway, *Oncol Rep*, 2018, **39**, 2081-2090.
154. S. Lamouille, J. Xu and R. Derynck, Molecular mechanisms of epithelial-mesenchymal transition, *Nat Rev Mol Cell Biol*, 2014, **15**, 178-196.
155. N. Skrypek, S. Goossens, E. De Smedt, N. Vandamme and G. Berx, Epithelial-to-Mesenchymal Transition: Epigenetic Reprogramming Driving Cellular Plasticity, *Trends Genet*, 2017, **33**, 943-959.
156. S. Ding, W. Zhang, Z. Xu, C. Xing, H. Xie, H. Guo, K. Chen, P. Song, Y. Gu, F. Xiao, L. Zhou and S. Zheng, Induction of an EMT-like transformation and MET in vitro, *Journal of Translational Medicine*, 2013, **11**, 164.
157. S. W. Pyo, M. Hashimoto, Y. S. Kim, C. H. Kim, S. H. Lee, K. R. Johnson, M. J. Wheelock and J. U. Park, Expression of E-cadherin, P-cadherin and N-cadherin in oral squamous cell carcinoma: Correlation with the clinicopathologic features and patient outcome, *Journal of Cranio-Maxillofacial Surgery*, 2007, **35**, 1-9.
158. M. Diniz-Freitas, T. García-Caballero, J. Antúnez-López, J. M. Gándara-Rey and A. García-García, Reduced E-cadherin expression is an indicator of unfavourable prognosis in oral squamous cell carcinoma, *Oral Oncology*, 2006, **42**, 190-200.
159. P. Upadhaya, S. Giri, D. Barhoi and A. Bhattacharjee, Altered expression of junctional proteins as a potential biomarker in oral precancerous and cancerous patients, *Tissue Barriers*, 2022, **10**, 1973329.

160. J. Kang, E. Kim, W. Kim, K. M. Seong, H. Youn, J. W. Kim, J. Kim and B. Youn, Rhamnetin and cirsiliol induce radiosensitization and inhibition of epithelial-mesenchymal transition (EMT) by miR-34a-mediated suppression of Notch-1 expression in non-small cell lung cancer cell lines, *J Biol Chem*, 2013, **288**, 27343-27357.
161. X. Zhang, L. Zheng, Y. Sun, T. Wang and B. Wang, Tangeretin enhances radiosensitivity and inhibits the radiation-induced epithelial-mesenchymal transition of gastric cancer cells, *Oncol Rep*, 2015, **34**, 302-310.
162. J. H. Lee, J. W. Shim, Y. J. Choi, K. Heo and K. Yang, The combination of sorafenib and radiation preferentially inhibits breast cancer stem cells by suppressing HIF-1α expression, *Oncol Rep*, 2013, **29**, 917-924.
163. K. G. Lai, Y. H. Lin, C. T. Ho, C. Y. Chen, C. Y. Peng, T. Z. Liu and J. F. Chiou, Paclitaxel pretreatment overcomes hypoxia inducible factor-1α-induced radioresistance acquisition of human hepatoma and lung adenocarcinoma cells, *Life Sci*, 2015, **136**, 7-12.
164. H. Liu, W. Yang, H. Gao, T. Jiang, B. Gu, Q. Dong, W. Xu, S. Wu and X. Sun, Nimotuzumab abrogates acquired radioresistance of KYSE-150R esophageal cancer cells by inhibiting EGFR signaling and cellular DNA repair, *Onco Targets Ther*, 2015, **8**, 509-518.
165. W. Yin, Y. Liu, X. Liu, X. Ma, B. Sun and Z. Yu, Metformin inhibits epithelial-mesenchymal transition of oral squamous cell carcinoma via the mTOR/HIF-1α/PKM2/STAT3 pathway, *Oncology Letters*, 2020, **21**, 1-1.
166. J. Zavadil and E. P. Böttinger, TGF-beta and epithelial-to-mesenchymal transitions, *Oncogene*, 2005, **24**, 5764-5774.
167. A. Hollestelle, J. K. Peeters, M. Smid, M. Timmermans, L. C. Verhoog, P. J. Westenend, A. A. Heine, A. Chan, A. M. Sieuwerts, E. A. Wiemer, J. G. Klijn, P. J. van der Spek, J. A. Foekens, M. Schutte, M. A. den Bakker and J. W. Martens, Loss of E-cadherin is not a necessity for epithelial to mesenchymal transition in human breast cancer, *Breast Cancer Res Treat*, 2013, **138**, 47-57.
168. A. Chen, H. Beetham, M. A. Black, R. Priya, B. J. Telford, J. Guest, G. A. Wiggins, T. D. Godwin, A. S. Yap and P. J. Guilford, E-cadherin loss alters cytoskeletal organization and adhesion in non-malignant breast cells but is insufficient to induce an epithelial-mesenchymal transition, *BMC Cancer*, 2014, **14**, 552.
169. C. Y. Loh, J. Y. Chai, T. F. Tang, W. F. Wong, G. Sethi, M. K. Shanmugam, P. P. Chong and C. Y. Looi, The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges, *Cells*, 2019, **8**.

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