Department of Otorhinolaryngology – Head and Neck Surgery,
Helsinki University Central Hospital, and
Faculty of Medicine, University of Helsinki,
Finland

Transforming Growth Factor β1 Genotype, p16 Expression, and Treatment Outcome in Head and Neck Squamous Cell Carcinoma

Marie Lundberg

ACADEMIC DISSERTATION

To be presented by permission of the Medical Faculty of the University of Helsinki, in the Auditorium of the Department of Otorhinolaryngology - Head and Neck Surgery, Haartmaninkatu 4E, Helsinki on February 1st at 12 noon.

Helsinki 2013

Supervised by
Professor Antti Mäkitie, MD, PhD
Department of Otorhinolaryngology – Head and Neck Surgery
Helsinki University Central Hospital
University of Helsinki, Faculty of Medicine, Helsinki, Finland

Docent Petri Mattila, MD, PhD
Department of Otorhinolaryngology – Head and Neck Surgery
Helsinki University Central Hospital
University of Helsinki, Faculty of Medicine, Helsinki, Finland

Reviewed by
Professor Eva Munck-Wikland, MD, PhD
Department of Otorhinolaryngology – Head and Neck Surgery,
Karolinska Institutet, Stockholm, Sweden

Professor Timo Paavonen, MD, PhD
Department of Pathology, School of Medicine
University of Tampere, Tampere, Finland

Discussed with
Professor Johan Wennerberg, MD, PhD
Department of Otorhinolaryngology – Head and Neck Surgery,
Lund University, Lund, Sweden

ISBN 978-952-10-8437-9 (paperback)
ISBN 978-952-10-8438-6 (PDF)

http://ethesis.helsinki.fi
Unigrafia Oy, Helsinki 2013

To Nils, Mauritz, Edvin, and the baby

If evolution really works, how come mothers only have two hands?

Milton Berle

CONTENTS

ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to by their Roman numerals. The publishers kindly provided permission to reprint the articles.

I **Lundberg M, Pajusto M, Koskinen WJ, Mäkitie AA, Aaltonen LM, Mattila PS**
Association between transforming growth factor β1 genetic polymorphism and response to chemoradiotherapy in head and neck squamous cell cancer
Head & Neck 2009;31:664-72

II **Lundberg M, Saarilahti K, Mäkitie AA, Mattila PS**
TGFβ1 genetic polymorphism is associated with survival in head and neck squamous cell carcinoma independent of the severity of chemoradiotherapy induced mucositis
Oral Oncology 2010;46:369-72

III **Lundberg M, Leivo I, Saarilahti K, Mäkitie AA, Mattila PS**
Increased incidence of oropharyngeal cancer and p16 expression
Acta Oto-Laryngologica 2011;131:1008-11

IV **Lundberg M, Leivo I, Saarilahti K, Mäkitie AA, Mattila PS**
Transforming growth factor beta 1 genotype and p16 as prognostic factors in head and neck squamous cell carcinoma
Acta Oto-Laryngologica 2012;132:1006-12

ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous group of cancers originating from the aerodigestive epithelium of the upper respiratory tract. Although survival rates are improving, treatment outcome in certain patient subgroups is still disappointing. Molecular markers, such as p16, a surrogate marker for Human Papilloma Virus infection, and Epidermal Growth Factor Receptor (EGFR) have been suggested as relevant for prediction of treatment outcome. Another molecular marker under investigation is the single nucleotide polymorphism rs1800470 of the Transforming Growth Factor β1 gene (*TGFB1*) that affects levels of the pleiotrophic cytokine TGFβ1, an essential regulator of cell proliferation, immunomodulation, and cancer stem cell maintenance. The aim of this thesis was to investigate the value of these molecular markers in HNSCC, and their potential relation to treatment outcome. Furthermore, as the incidence of oropharyngeal cancer (OPSCC) is rapidly increasing in several western countries, we surveyed its relation to a possible increase in p16 overexpression in HNSCC in Finland.

Peripheral blood from a cohort of 175 consecutive patients was genotyped for rs1800470 of *TGFB1* by polymerase chain reaction (PCR), and expression of p16 and EGFR explored with immunohistochemistry. The results were correlated with clinical and histopathological variables. The Finnish Cancer Registry provided data on OPSCC incidence.

Age-standardized incidence in Finland of OPSCC was increasing and likewise the proportion of p16 overexpressing HNSCC. Overexpression of p16 was associated with improved survival in OPSCC, but with no association between EGFR expression and survival. The variant allele of *TGFB1* at rs1800470 was associated with improved outcome, especially in OPSCC, and in tumors treated with chemoradiotherapy. This result was not explainable by a decreased grade of chemoradiotherapy-induced acute mucositis or by altered treatment time. Improved survival was independent of p16 and EGFR expression, although carriers of the variant allele were more liable to have a p16-overexpressing tumor.

In HNSCC, the single nucleotide polymorphism rs1800470 of *TGFB1* is a potential independent prognostic marker that can, in combination with p16 expression, predict positive response to chemoradiotherapy.

SAMMANFATTNING

Cancer i huvud- och halsregionen (HNSCC) är en heterogen grupp cancertyper vilka utgår främst från skivepitelet i de övre luftvägarna. De viktigaste kända riskfakorerna för HNSCC är rökning, stort alkoholbruk och infektion med humant papillomvirus (HPV). Trots att man gjort framsteg inom behandlingen är prognosen för överlevnad fortfarande nedslående hos vissa patientgrupper. För att kunna optimera behandlingen på ett individuellt plan och förutspå överlevnaden har man föreslagit att molekylära markörer kunde vara användbara. Exempel på dessa är p16, en surrogatmarkör för infektion med HPV, och den epidermala tillväxtfaktorreceptorn EGFR. En annan intressant markör är den nukleära polymorfismen rs1800470 i genen för tillväxtfaktorn ”Transforming growth factor β1” (*TGFB1*). Denna polymorfism påverkar halten av TGFβ1, en cytokin som är avgörande för cellproliferation, immunomodulering och för upprätthållandet av cancerstamceller. Målet med denna avhandling var att undersöka dessa markörer hos patienter med HNSCC och om man med deras hjälp kan förutspå behandlingsförloppet. Eftersom incidensen för orofaryngealcancer (OPSCC) ökar i västvärlden undersökte vi om den ökar också i Finland, samt om denna eventuella ökning korrelerar med en förhöjd prevalens i p16-positiv HNSCC.

I studierna inkluderades 175 konsekutiva HNSCC-patienter vars blod genotypades för *TGFB1* rs1800470 med polymeras-kedjereaktion (PCR). Vi bestämde p16- och EGFR-expressionerna med immunohistokemi. Alla resultat korrelerades med kliniska och histopatologiska karakteristika. Det finländska cancerregistret tillhandahöll incidensuppgifter.

Den åldersstandardiserade OPSCC-incidensen ökade i Finland, liksom proportionen HNSCC som överexpresserade p16. OPSCC-patienter vars tumörer överexpresserade p16 hade en förbättrad överlevnad, men något samband mellan EGFR-expression och överlevnad kunde inte påvisas.

OPSCC-patienter som bar på variantallelen av *TGFB1* rs1800470 hade signifikant bättre överlevnad än patienter med normalallelen. Detsamma gällde för patienter som behandlats med chemoradioterapi. Fenomenen kunde inte förklaras med att dessa patienter skulle ha fått behandlingsorsakad akut infammation av munslemhinnan i lägre grad, eller av att deras behandlingstid skulle ha varit kortare. Den förbättrade överlevnaden var oavhängig p16 och EGFR expressioner, även om patienter som bar på *TGFB1*-variantallelen i aningen högre grad hade p16 överexpresserande tumörer.

Polymorfismen *TGFB1* rs1800470 är en potentiell självständig ny prognostisk markör som eventuellt, i kombination med p16-expression, kan användas för att förutspå en förbättrad respons vid behandling av HNSCC med chemoradioterapi.

ABBREVIATIONS

CRT	Chemoradiotherapy
CSC	Cancer stem cell
CT	Chemotherapy
DFS	Disease-free survival
DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
EMT	Epithelial-to-mesenchymal transition
Gy	Gray (unit of absorbed dose of ionizing radiation)
HNSCC	Head and neck squamous cell carcinoma
HPSCC	Hypopharyngeal squamous cell carcinoma
HPV	Human papilloma virus
HUCH	Helsinki University Central Hospital
IHC	Immunohistochemistry
IMRT	Intensity-modulated radiotherapy
ISH	In situ hybridization
LSCC	Laryngeal squamous cell carcinoma
NPC	Nasopharyngeal carcinoma
OPSCC	Oropharyngeal squamous cell carcinoma
OS	Overall survival
OSCC	Oral squamous cell carcinoma
PCR	Polymerase chain reaction
pRb	Retinoblastoma protein
RNA	Ribonucleic acid
RT	Radiotherapy
RT-PCR	Reverse transcriptase polymerase chain reaction
SNP	Single nucleotide polymorphism
TGFβR	Transforming growth factor beta receptor
TGFβ	Transforming growth factor beta
TGFB	Transforming growth factor beta gene
TNM	Tumor Node Metastasis

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the world's sixth most common cancer, accounting annually for 356 000 deaths (Globocan, 2008). Because of its nonspecific symptoms and the difficulty in examining epithelial linings in this area, diagnosis is often delayed, and patients at first presentation frequently show with locally disseminated disease. That the cure rates of high-stage, extra-capsular spread, and recurrent disease are poor (Wong et al. 1996; Thomas et al. 2005), and 5-year overall survival remains low (66%) (Pulte and Brenner 2010), makes it essential to diagnose HNSCC early and strive for permanent control with primary treatment.

Currently, treatment strategies rely on traditional clinical, histopathological, and radiological characteristics to determine disease stage according to a T (tumor), N (node), M (metastasis) classification system. Limitations to this approach exist, however, because the TNM classification neglects the tumor's biological characteristics such as human papilloma virus (HPV) as a causative agent. The HPV-induced subtype is associated with a favorable outcome, which is why HPV status, or possibly its surrogate marker p16, has become an important biomarker (Chaturvedi et al. 2011; O'Rorke et al. 2012).

Treatment has long been based on surgery and radiotherapy (RT) or a combination of these modalities. RT with concomitant chemotherapy (CT) has – because of its equal effectiveness yet fewer adverse effects, in addition to its use as an adjuvant, postoperative alternative – increasingly replaced surgery as primary treatment for some subsites (Argiris et al. 2008; Singh and Pfister 2008; Pignon et al. 2009). The ultimate goal of non-invasive treatment schemes is to eradicate the pluripotent, self-renewing, and refractile cancer stem cells (CSC) that can remain quiescent after treatment and cause relapse (Prince and Ailles 2008). In breast cancer, one of the controlling agents of CSCs is Transforming Growth Factor β (TGFβ) (Shipitsin et al. 2007). TGFβ plays an essential role in maintaining cell homeostasis, and in processes such as proliferation, immunosuppression, and apoptosis (Blobe et al. 2000; Akhurst 2004; Caja et al. 2012). The single nucleotide polymorphism rs1800470 of the TGFβ1 gene (*TGFB1*) elevates levels of the most abundant isoform of TGFβ; TGFβ1 (Awad et al. 1998; Yokota et al. 2000; Dunning et al. 2003), and has been associated with increased risk for breast cancer and nasopharyngeal carcinoma (NPC) (Dunning et al. 2003; Wei et al. 2007), and decreased survival in various tumors including breast- and esophageal cancer (Fukuchi et al. 2004; Shu et al. 2004). Its role as a prognostic marker in HNSCC seems to have undergone no prior investigation.

One prognostic marker currently receiving much attention is the epidermal growth factor receptor (EGFR), because its genetic mutations and expression profile serve as markers of treatment response to EGFR inhibitors in colon- and non-small-cell lung cancer. In HNSCC, the EGFR monoclonal antibody cetuximab serves primarily in the treatment of disseminated or relapsed disease in combination with RT or chemoradiotherapy (CRT), but since 90% of HNSCC cases overexpress EGFR, thus far no patient selection is possible based on its expression (Kalyankrishna and Grandis 2006; Moon et al. 2010; Langer 2012).

A search is ongoing for new biomarkers to guide the clinician towards an optimal individualized treatment strategy with a high survival rate and reasonable side-effects. This thesis focuses on the potential prognostic biomarkers p16, EGFR, and *TGFB1* rs1800470 to further improve treatment strategies in HNSCC.

REVIEW OF THE LITERATURE

Epidemiology and risk factors

Head and neck cancer is made up of a heterogeneous group of tumors. Their clinical patterns differ, although 90% arise from squamous epithelial cells of the upper aerodigestive tract. HNSCC is classified according to the anatomical site of origin. The most common location is the larynx, followed by the oral cavity and the oropharynx (Carvalho et al. 2005; Pulte and Brenner 2010). This thesis discusses HNSCC originating from the oral cavity, oropharynx, hypopharynx, larynx, and nasopharynx (Figure 1).

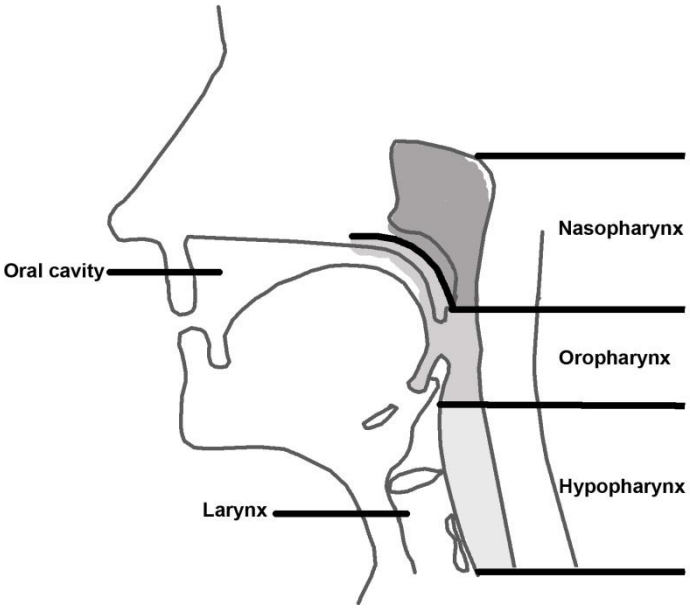

Figure 1 *Topography of head and neck squamous cell carcinoma subsites discussed here. Illustration by Marie Lundberg and Jenny Kuisma.*

In 2009, 724 new HNSCC cases were diagnosed in Finland, equaling an incidence of 11 per 100 000 person-years (Cancer Statistics, NORDCAN). In the USA the incidence is similar; 15 among men and 6 per 100 000 among women (Pulte and Brenner 2010). Average age at diagnosis is 60, although this age has, during the last three decades, decreased slightly (Argiris et al. 2008; Pulte and Brenner 2010). Overall incidence has also decreased in the western world, whereas in many developing countries it is on the rise (Warnakulasuriya 2009). This decrease occurs despite a growing number of cancers found in the oral tongue, base of the tongue, and the palatine tonsils, particularly among young people (Carvalho et al. 2005; Shiboski et al. 2005; Hammarstedt et al. 2007; Chaturvedi et al. 2008; Attner et al. 2010; Annertz et al. 2012). The decrease has been attributed to a lower prevalence of smoking, one of the major risk factors for HNSCC. The other major risk factor is alcohol, and together the two have a multiplicative effect (Pai and Westra 2009). Minor risk factors are poor dental hygiene, low socio-economic status, smokeless tobacco, betel-nut chewing, family history, certain occupational

factors, and infections such as Epstein-Barr virus infection (Rosenquist et al. 2005; Argiris et al. 2008; Pai and Westra 2009).

It has become evident that infection with HPV is a third major risk factor for HNSCC (Gillison et al. 2000; Syrjanen 2010). The incidence of HPV-associated cancer is highest in cancer of the tonsils and the base of the tongue (Kreimer et al. 2005; Syrjanen 2005; Hobbs et al. 2006; Koskinen et al. 2007; Pai and Westra 2009; Machado et al. 2010). HPV can possibly also cause oral squamous cell carcinoma (OSCC), as reported in a recent meta-analysis (Syrjanen et al. 2011), but for other types of HNSCC, causation remains unestablished (Torrente et al. 2011; Lewis et al. 2012; Wilson et al. 2012). Of oropharyngeal squamous cell carcinoma (OPSCC), 20 to 90% is estimated to be related to HPV infection (Hansson et al. 2005; Hammarstedt et al. 2006; Ragin and Taioli 2007; Attner et al. 2010), generally to HPV16 (>80%) and less generally to HPV18, 31, or 33 (Kreimer et al. 2005). These are the same oncogenic viruses that induce anogenital cancers (Kreimer et al. 2005; D'Souza et al. 2007; Syrjanen 2010). The evidence for a causal association is strengthened by the infrequency of oncogenic viruses in the normal population. Both in Sweden and the USA prevalence is around 1%, with peaks at ages 30 to 34 and 60 to 64 (Hansson et al. 2005; Gillison et al. 2012a). Patients with HPV-positive tumors are more likely to be non-smoking, non-drinking white men of high socioeconomic status (Gillison et al. 2000, 2008; D'Souza et al. 2007; Ernster et al. 2007; Chaturvedi et al. 2008; Ang et al. 2010; Guan et al. 2010; Liang et al. 2012). Incidence of HPV-positive OPSCC increased by as much as 225% from 1988 to 2004 in the USA (Chaturvedi et al. 2011), and in Sweden the proportion of HPV-positive OPSCC has doubled each decade since 1970, parallel to the rapid increase in incidence (Nasman et al. 2009). An analogous trend in incidence is occurring in other western countries (Charfi et al. 2008; Rietbergen et al. 2012), including Finland up to this millennium (Syrjanen 2004; Makitie et al. 2006).

Pathogenesis

The most common variant of HNSCC is recognized by keratin pearl formation with islands of squamous cells surrounded by stromal fibrosis. The less-common ones number five: spindle-cell carcinoma with non-cohesive islands of carcinoma resembling sarcoma; an exophytic but yet infiltrative non-metastazing verrucous type; papillary squamous-cell carcinoma recognizable by a prominent exophytic component; adenoid squamous-cell carcinoma; and a solid-lobuled basaloid type with an immature appearance, a high nuclear-to-cytoplasmic ratio, and only sparse or no keratinization. This last type is highly aggressive, with poor patient outcome (Pai and Westra 2009). The striking exception is the HPV-induced tumor with basaloid features but still an improved outcome (Adelstein et al. 2009; Pai and Westra 2009). HPV requires proliferating basal-layer cells for infection (zur Hausen 2002). Normally, a continuous layer of epithelial cells serves as a barrier against HPV infection, but in the pharyngeal lymphoid tissue the epithelium is disrupted and the basal membrane exposed, and therefore HPV can easily infect the basal stem cells that regenerate the epithelium, without any epithelial trauma (zur Hausen 2002; Pai and Westra 2009).

With the exception of drop-down carcinoma, which develops without epithelial dysplasia, HNSCC develops from mild dysplasia in the upper third of the epithelium, to moderate dysplasia, and finally to severe dysplasia or carcinoma in situ, involving the full thickness of the epithelium (Pai and Westra 2009). When carcinoma breaks through the basement membrane, it is classifiable as invasive. During this process, a number of genetic and epigenetic changes take place that accumulate in the tumor cell and its surroundings. These changes provide the tumor cell with its malignant potential, including infinite replicative potential, self-sufficiency in growth signals, insensitivity to anti-growth and apoptotic signals, angiogenetic potential, evasion of host immunity, and an ability to invade and metastasize (Hanahan and Weinberg 2011). An important step in enabling invasion and dissemination is the epithelial-to-mesenchymal transition (EMT), a process whereby epithelial cells undergo conversion to pluripotent mesenchymal cells, lose cell adherence and polarity, and acquire invasive potential, a process directed, amongst other factors, by TGFβ1 (Hanahan and Weinberg 2011).

Two crucial hallmarks in HNSCC carcinogenesis are the p53 and retinoblastoma protein (pRb) pathways, both regulators of cell-cycle progression. Tumor suppressor p53 is inactivated in a majority of HNSCC cases, either by somatic mutations (60%) or by oncoprotein E6 through infection with HPV (20%). Loss of p53 activity results in accumulation of DNA mutations that lead to increased genetic instability, and results in apoptotic resistance, uncontrolled proliferation, and cell immortalization (zur Hausen 2002).

In HPV-induced carcinogenesis, the pRb pathway is essential. Normally the binding of tumor suppressor p16, an INK4 cell-cycle inhibitor located on chromosome 9p21, to cyclinD1-cyclin-dependent-kinase complexes maintains pRb in its hypophosphorylated state, in which it binds the E2F transcription factor, thus preventing cell-cycle progression. When HPV integrates into the genome, HPV-oncoprotein E7 inactivates and degrades pRb, thereby releasing E2F and upregulating both cytoplasmic and nuclear p16. As p16 is a G1/S cell-cycle-phase checkpoint regulator, E7 forces the cell into the S phase, activating cell proliferation, malignant transformation, and immortalization (zur Hausen 2002; Leemans et al. 2011; El-Naggar and Westra 2012). Non-HPV-linked genetic alterations associated with HNSCC exist in the p16 gene (CDKN2A), leading to lost, diminished, or limited expression of p16 (Thomas and Primeaux 2012). These result in development of distant metastases (Namazie et al. 2002) and decreased survival (Ambrosch et al. 2001).

Diagnosis and treatment

Pre-treatment evaluation

HNSCC symptoms vary by anatomic location of the primary tumor. Common ones are hoarseness, pain, dysphagia, ulcers, and metastatic neck masses (Argiris et al. 2008). Based on findings from clinical examination, biopsies, radiologic imaging, and confirming histopathology, the tumor is staged according to its TNM classification (in

this thesis AJCC, sixth edition, 2002) where T (Tumor 1-4) represents the size and extent of the primary tumor, N (Node 0-3) the absence or presence and extent of regional metastatic disease, and M (Metastasis 0-1) the existence of distant metastasis. Based on its TNM classification, disease is categorized into Stages I to IV, where Stage IV cancers generally have invaded surrounding organs, have a regional metastasis greater than 3 cm in size, multiple metastases, or distant metastasis. The TMN classification is the most important tool for assessment of prognosis but does not include factors such as tumor differentiation (Grade), extra-capsular spread, or infiltration depth. Histological grading is based on squamous differentiation (i.e. keratinization), degree of cellular pleomorphism, and mitotic index (Thomas et al. 2005; Pai and Westra 2009).

Patients with HPV-positive OPSCC often have clinically aggressive disease; typically a small primary tumor with nodal metastasis at presentation, often of Stage III or IV (Hobbs et al. 2006; Hafkamp et al. 2008; Lewis et al. 2010; Machado et al. 2010). Surprisingly, these patients, especially if non-smokers, have a favorable prognosis (Ragin and Taioli 2007; Ang et al. 2010; Maxwell et al. 2010; Gillison et al. 2012b). Thus, HPV positivity could serve as a prognostic factor for improved survival (Ragin and Taioli 2007; Pai and Westra 2009; Ang et al. 2010; O'Rorke et al. 2012).

Therapeutic options

Traditionally the standard treatment for HNSCC is surgery including resection of the primary tumor and neck dissection in advanced disease. It allows for pathological staging, sometimes identifying micrometastasis or extra-capsular spread that will guide further treatment decisions. Surgery, however, has its anatomical and functional limitations, as surgeons strive for excision both with clear margins and with organ preservation. Trans-oral approaches and endoscopic and robotic techniques offer functional preservation combined with good oncological results, and microvascular flaps make even wider resections possible, although jeopardizing quality of life through resultant problems with speech, swallowing, breathing and lack of smell and taste (Argiris et al. 2008). To improve quality of life, treatment has moved towards a more conservative scheme with organ-sparing RT or CRT as first-line options for certain tumor sites.

For the 30 to 40% of HNSCC patients who present with early-stage disease (Stage I-II), either surgery, or RT as single modality are options, both resulting in high levels of tumor control (Argiris et al. 2008). Adjuvant RT or CRT are options in the case of positive or close margins, high T or N class (T3-4, N2-3), bone erosion, perineural invasion, or extra-capsular lymph-node infiltration. The typical RT scheme is daily fractions of 2.0 Gray (Gy) up to 50 Gy on the primary tumor and regional lymph nodes, followed by a booster of 20 Gy on the primary tumor and positive lymph nodes. The aim of fractionation is to increase the dose, while minimizing toxicity and risk for interruptions (Fu et al. 2000), as the loss of tumor control can reach 14% in only one week of interruption (Fowler and Lindstrom 1992). Intensity-modulated radiotherapy (IMRT) reduces toxic effects on healthy tissue, protecting it by three-dimensionally optimizing the irradiated tumor’s volume (Saarilahti et al. 2005; Argiris et al. 2008). IMRT can provide excellent locoregional tumor control (80-95%) and disease-free

survival (DFS) (46-95%) in all oral and pharyngeal subsites (Collan et al. 2011; Daly et al. 2011; Wang et al. 2012).

Concomitant CT further intensifies the effect of RT (Pignon et al. 2009; Bourhis et al. 2012). Overall survival (OS) is improved by adding CT to RT especially in OSCC and OPSCC (8%) but also in certain hypopharyngeal (HPSCC) and laryngeal cancers (LSCC, 5%) (Blanchard et al. 2011). Another meta-analysis demonstrated increased survival rates of 20% in locally advanced HNSCC (Cohen et al. 2004) and for these, concomitant CRT is often recommended in unresectable, in some nonsurgically treated resectable, and in postoperative high-risk patients (Bourhis et al. 2012; Nwizu et al. 2012). Toxicity increases substantially (Calais et al. 1999; Forastiere et al. 2003), but clear recommendations as to schedule, number of cycles, or useful combinations are nonexistent (Nwizu et al. 2012). Various agents have a therapeutic effect, but cisplatin has become the standard, being well tolerated and radiosensitizing (Cohen et al. 2004; Argiris et al. 2008). In Finland, cisplatin is usually administered weekly for 6 weeks at a dose of 40 mg/m². Common reasons for dose reduction are renal insufficiency, cytopenia, and neutropenic infections.

Novel agents for cancer treatment have come into clinical use through translational research. Cetuximab, a monoclonal antibody for EGFR, is the first molecular-targeted agent approved for treatment of locally advanced or metastatic HNSCC in combination with RT, or in combination with platinum-based CT for relapsed or metastasized disease (Astsaturov et al. 2006). Cetuximab combined with RT raises locoregional control and OS (49 vs. 29 months) in locally advanced tumors as compared with RT alone (Bonner et al. 2006). Cetuximab is well tolerated but is not considered superior to conventional therapy; it mostly serves as a secondary choice for selected cases with recurrent cancer (Bonner et al. 2006).

Complications of radiotherapy and chemoradiotherapy

Complications of RT and CRT are classified into acute toxicity and late sequelae. The late sequelae, including thyroid dysfunction, trismus, subcutaneous fibrosis, and osteoradionecrosis, can be minimized by reducing RT fraction dose. The most common late complication in HNSCC is xerostomia, at an incidence of 60 to 90% (Wijers et al. 2002). This results from salivary gland fibrosis and causes significant reduction in quality of life through problems with speech and swallowing, altered taste, and dental caries (Bhide et al. 2009). The large salivary glands can in part be spared with IMRT (Eisbruch 2005; Saarilahti et al. 2005; Saarilahti et al. 2006) but weight loss and prolonged use of percutaneous endoscopic gastrostomy as consequences of swallowing disorders are still common (Caudell et al. 2009).

Acute toxicity affects tissues that divide rapidly, such as the skin and the mucosa. Skin erythema is characterized by vasodilatation and increased permeability that lead to reduced perfusion and to vessel- and soft-tissue fibrosis. In HNSCC, skin erythema is seldom grave, whereas mucositis occurs in virtually all patients; in its severe form in 39% of irradiated patients, and in 79% after CRT (Herrstedt 2000). It is the most common dose-limiting complication in HNSCC treatment (Calais et al. 1999; Trotti et al. 2003). When RT and CT create DNA-strand breaks that, in combination with a

cytokine cascade, induce apoptosis of the basal epithelium, mucositis occurs. Fibroblasts in the mucosa are damaged, events which cause ulcerations then colonized by bacteria (Sonis 2004; Treister and Sonis 2007). These bacteria can easily penetrate vessels and, in neutropenic patients, cause septic infections.

Prevention of mucositis

Sucking ice cubes during CT is cheap, easy, and to some extent effective in prevention of mucositis. No radio-protective drugs have proven effective (Herrstedt 2000; Vissink et al. 2003), and some drugs suggested may even compromise survival (Ryu et al. 2007). Effort should be put into careful planning of treatment, into pain and inflammation relief, removal of mucosal-irritating factors, and dental hygiene (Herrstedt 2000; Vissink et al. 2003; Sonis 2004). TGFβ has been tested in a number of trials for its wound-healing effects (Blobe et al. 2000; Flanders and Burmester 2003). TGFβ3 reduces the incidence of oral mucositis by reducing epithelial cell proliferation (Spijkervet and Sonis 1998; Sonis 2004), and orally administered TGFβ2 and TGFβ3 have reduced CT-induced mucositis both in humans and in animals (Sonis et al. 1997; Wymenga et al. 1999; van't Land et al. 2002; Harsha et al. 2006). Thus, TGFβ might have the potential to reduce oral mucositis, but this remains to be verified in clinical work.

Survival

Several articles on HNSCC state that survival has remained static during the most recent decades, although all agree that diagnostic and treatment advances have improved quality of life (Forastiere et al. 2001; Ragin and Taioli 2007; White et al. 2010; Leemans et al. 2011). However, when Carvalho et al. (2005) analyzed the survival trend between 1973 and 1999, based on the Surveillance, Epidemiology, and End Results (SEER) database which covers about 10% of the US population, they observed an increase in the 5-year HNSCC survival rate. This finding was confirmed in 2010, with 5-year relative survival's being 55% in 1992-1996 compared to 66% in 2002-2006 (Pulte and Brenner 2010). Both studies concluded that a vast difference exists between anatomic sites with survival reaching only 34% for HPSCC compared to 67% for LSCC (Carvalho et al. 2005; Pulte and Brenner 2010). Survival improved the most in cancers of the tonsils (40 to 70%), the tongue (45 to 65%), and the oral cavity (54 to 63%), followed by NPC (Pulte and Brenner 2010). Survival for lip cancer remained unchanged, probably due to its primary high survival rate of 90% (Carvalho et al. 2005; Hakulinen et al. 2010; Pulte and Brenner 2010).

In the Nordic countries, the relative survival of HNSCC is among the highest in Europe and is still improving (Hakulinen et al. 2010). In Finland during 1995-1999, the 5-year OS for OPSCC was 45% and DFS was 67% (Makitie et al. 2006). In Sweden, survival for this patient group has improved, especially since the 1980s, for tonsil cancer from 37 to 62%, and for the base of the tongue from 32 to 51%, whereas age-standardized relative survival rates for tongue cancer have improved only insignificantly (42 to 44%) (Hammarstedt et al. 2011).

When comparing 1964-1968 with 1999-2003 in Finland, 5-year age-standardized relative survival of tongue cancer improved from 43 to 50% in men, oral cavity cancer 64 to 68% and cancer of the pharynx (including NPC, OPSCC, and HPSCC) 26 to 38%. In women, the improvement was 55 to 67%, 43 to 69%, and 21 to 51% (Hakulinen et al. 2010). Women thus have improved survival rates compared with those of men, and also a more rapid increase in survival over time. Relative survival tends to be highest among young patients, possibly due to a higher proportion of cancers caused by HPV, but as their survival has improved also for non-HPV-related cancers, factors such as comorbidity and fewer tobacco-smoking years also matter (Hakulinen et al. 2010; Pulte and Brenner 2010). Smoking status at diagnosis is a factor for poor prognosis associated with treatment response (Fountzilas et al. 1997), survival (Meyer et al. 2008; Duffy et al. 2009), and second primaries (Duffy et al. 2009). Although smoking is etiologically associated with HPV-negative OPSCC, HPV-positive patients are not exclusively non-smokers, and also in this prognostically favorable patient group, smoking alters survival rates (Hafkamp et al. 2008; Kumar et al. 2008; Gillison et al. 2012b), disease recurrence, and risk for second primaries (Maxwell et al. 2010; Gillison et al. 2012b).

Apart from tumor site, the most important predictor for outcome of HNSCC is stage (Shah and Lydiatt 1995; Carvalho et al. 2005; Thomas et al. 2005). Average survival of high-stage disease is 30 to 40% (Carvalho et al. 2005; Pulte and Brenner 2010). Other indicators of poor clinical outcome are positive margins after surgical excision, extra-capsular spread, and perineural invasion (Quon et al. 2001; Thomas et al. 2005). More accurate staging through advanced diagnostic tools, and cancer awareness leading to earlier diagnosis may be reasons for improved survival (Carvalho et al. 2005), but Pulte and Brenner (2010) found this unlikely, after demonstrating improved survival at all stages.

HPV-positive tumors have repeatedly been associated with improved survival (Weinberger et al. 2004; Ragin and Taioli 2007; Ang et al. 2010; Dayyani et al. 2010; Chaturvedi et al. 2011; O'Rorke et al. 2012). O'Rorke et al. (2012), assessing OS and DFS in HPV-positive HNSCC in a meta-analysis that included 52 studies, found both to be improved (Hazard ratio, HR, 0.46 for OS and 0.28 for DFS). Similar results are demonstrable: HPV-positive OPSCC patients were at 28% lower risk of death than were HPV-negative patients, and at a significantly lower risk (51%) for recurrence. In other anatomical locations, no difference in OS or DFS emerged (Ragin and Taioli 2007). The biological mechanism for this phenomenon remains unknown, but some speculate that HPV-positive tumors contain a functioning tumor-suppressor, p53, which renders the tumor susceptible to radiation-induced apoptosis, because HPV-positive tumors appear more sensitive to both RT and CRT (Dahlstrand et al. 2004; Kumar et al. 2007; Fakhry et al. 2008; Kumar et al. 2008; Worden et al. 2008; Ang et al. 2010; Lau et al. 2011). After treatment with primary surgery, improved survival has also resulted (Licitra et al. 2006).

During the last two decades, individualized regimes, combined therapy, and fast-track treatment have improved OS and organ preservation (Forastiere et al. 2001). Thus, we do have methods for improving survival in HNSCC patients, but we still lack the tools for grouping patients according to optimized individual treatment for improved survival,

and for risk evaluation of possible de-intensification of treatment protocols (Psyrri et al. 2012).

Prognostic markers

TNM classification has proven insufficient in predetermining a satisfactory treatment response in individual patients. The demand is for easily available molecular markers that distinguish good responders from bad responders (Thomas et al. 2005). Molecular markers could enable personalized treatment; individualized surgical methods, altered radiation fractionation schemes, novel combinations of RT, CT, and monoclonal antibodies, and de-intensified treatment schemes, all aiming for high survival rates with limited side-effects (Quon et al. 2001).

A good prognostic marker is versatile. It identifies patients at risk for aggressive disease, predicts therapy response, is noninvasive, and can prove clinically useful as a diagnostic tool, in patient counseling, in tailored treatment planning, and in follow-up (Quon et al. 2001; Thomas et al. 2005; Singh and Pfister 2008; Shah et al. 2009). Of the numerous prognostic markers analyzed, none have proven sufficient, and it is highly unlikely that any marker could singlehandedly provide the complete answer to treatment-strategy planning. With microarray technology, expression of thousands of genes can be investigated simultaneously. Several studies have identified gene profiles related to outcome, but none overlap sufficiently for clinical use (Thomas et al. 2005; Chung et al. 2006). The only prognostic marker that thus far provides information on survival is HPV status. It is therefore vital to combine information on other prognostic markers with patient HPV status. These studies evaluated three potential prognostic markers: p16 as a surrogate marker for HPV infection, EGFR, and the *TGFB1* polymorphism at rs1800470.

Several criteria for molecular markers are important regarding the care of HNSCC patients. The sample for marker determination should be easily, preferably noninvasively, accessible. The method should be sensitive and specific, standardized, widely available, and quick (Thomas et al. 2005), not lengthening the time-span from diagnosis to treatment that is so vital for survival (Fowler and Lindstrom 1992; Jensen et al. 2007). A new method needs to be verified in large, uniformly treated patient groups, preferably in prospective studies including anatomically and histopathologically homogenous tumors (Thomas et al. 2005).

Human papilloma virus and p16

The fact that HPV is a risk factor for OPSCC is recognized, but establishing the mere presence of HPV in OPSCC is insufficient for distinguishing causative transcriptionally active HPV from a latent infection. Numerous means to detect HPV in tumor cells exist, but to date no single method is unanimously accepted. Prevalence reports for high-risk HPV infection in HNSCC range from 0 to 100% (Kreimer et al. 2005; D'Souza et al. 2007), as a result of non-standardized detection methods, differences in type and quality of tissue material, and a varying prevalence among anatomic locations and populations.

Because HPV is an epithelial infection of stratified squamous epithelium, tissue biopsies are most useful for its detection. For early detection, applicable methods are direct swab, saliva collection, and oral rinsing, but although a large area can be sampled, cell collection from the tonsil crypts where HPV supposedly hibernates is uncertain (Kim et al. 2007; Venuti and Paolini 2012). As no blood-borne phase exists in HPV infection, all blood- and serum samples are by definition surrogate markers. Detection of the easily applicable serum antibodies E6, E7, or the structural late-capsid proteins L1 and L2 are useful in epidemiological studies, but because HPV can occur in other mucosal linings, these are not site specific, but rather represent cumulative exposure to HPV; they are therefore of limited utility (Mork et al. 2001; Adelstein et al. 2009). Recent findings suggest, however, that antibodies for E6 and E7 in sera could serve as prognostic markers for survival and follow-up, post treatment (Rubenstein et al. 2011; Liang et al. 2012).

The polymerase chain reaction (PCR) and reverse transcriptase PCR (RT-PCR) amplify a signal sequence of DNA or RNA, and are, as methods, vulnerable to segment loss during viral integration into host DNA. PCR and RT-PCR, executed with type-specific or broad-spectrum primer sets, can discriminate between HPV subtypes, but they are extremely sensitive and may not reflect a biologically active infection. They too easily amplify HPV from adjacent normal tissue, or from contamination, or amplify non-transcriptional HPV (Snijders et al. 2003; Adelstein et al. 2009). Quantification meets the same issue, although allowing for precise measurement of DNA or RNA, thereby distinguishing between integrated and episomal HPV (Venuti and Paolini 2012). The sensitivity for qRT-PCR is estimated at 92% and its specificity at 97% (Smeets et al. 2007). RT-PCR of E6 or E7 mRNA detects transcriptionally active HPV infection and is presently considered the gold standard (Venuti and Paolini 2012). It can be performed on formalin-fixed paraffin-embedded specimens, but fresh-frozen specimens are preferable. This method is time consuming and technically challenging, although it is convincingly associated with improved OS and DFS (Shi et al. 2009; Jung et al. 2010; Venuti and Paolini 2012).

In situ hybridization (ISH) allows for topographical detection and identification of integrated high-risk HPV DNA in tumor cell nuclei and is therefore correlated with biologically relevant infection. The method is standardized, technically validated, and interpretable with a light microscope, but it is generally type-specific and requires multiple probes (Adelstein et al. 2009; Lewis et al. 2010). It has a higher specificity than PCR-based methods have, but lacks sensitivity (83%) and may be regarded as technically too laborious for routine screening (Smeets et al. 2007; Schache et al. 2011; Venuti and Paolini 2012). HPV-DNA ISH has been found to be in high concordance with mRNA E6/E7 (Shi et al. 2009), and with a new E6/E7-mRNA ISH method (Ukpo et al. 2011), but clinically, ISH is regarded as insufficiently validated.

When HPV oncoprotein E7 degrades pRb, due to a lack of negative feedback, tumor suppressor p16 becomes overexpressed. Immunohistochemical (IHC) staining for p16 can therefore serve as a surrogate marker for transcriptionally active high-risk HPV infection. The advantages of p16 IHC over PCR or ISH analysis are its cost effectiveness, the commercially available and equally reliable monoclonal antibodies, and its simplicity of performance on formalin-fixed paraffin-embedded specimens

(Lewis 2012; Thomas and Primeaux 2012). In a p16-positive cell, the nucleus and cytoplasm are strongly and diffusely stained, with partial staining uncommon. A threshold of 70 to 75% is clinically relevant and has been associated with biologically active HPV (Ang et al. 2010; Lewis et al. 2010; Lewis 2012) and improved outcome (Weinberger et al. 2004; Reimers et al. 2007; Ang et al. 2010; Lewis et al. 2010). Sensitivity for HPV infection has been estimated at 100%, but p16 IHC lacks specificity (79%); p16 is expressed in a subset of tumors apparently lacking HPV DNA (Begum et al. 2007; Smeets et al. 2007; Ang et al. 2010; Lewis et al. 2010; Schache et al. 2011; Thomas and Primeaux 2012). The reason for this is unknown. Theories exist of HPV's being involved in tumorigenesis but later being concealed and p16 overexpressed through pRb deletion or suppression, or of p16 overexpression's being innate, or of p16 overexpression's being due to still-unidentified viruses (Lewis et al. 2010; El-Naggar and Westra 2012). The p16 false-positive samples can, of course, result from variabilities in technical practice and reporting, or can result from the fact that p16 is, biomechanically, a surrogate marker (El-Naggar and Westra 2012).

Epidermal growth factor receptor

The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor, a member of the ErbB/HER gene family. When one of 12 possible ligands binds to EGFR, the receptor is autophosphorylated through homo- or heterodimerization. Phosphorylation activates multiple signaling pathways that are potent oncogenic regulators and lead to cell-cycle progression, apoptosis, and enhanced tumor-cell motility, which alter tumor cell growth, invasion, angiogenesis, and metastasis (Thomas et al. 2005; Kalyankrishna and Grandis 2006; Moon et al. 2010).

Overexpression of EGFR in HNSCC is very common (80-92%) (Grandis and Tweardy 1993; Thomas et al. 2005). Variations exist between anatomical sites; overexpression is more common in pharyngeal and oral carcinoma than in LSCC (Takes et al. 1998). Expression increases with tumor progression; it is higher in carcinoma than in dysplasia, in high- stage disease than in low-stage disease, and in undifferentiated tumors, indicating that EGFR plays an important role in HNSCC pathogenesis (Shin et al. 1994; Reimers et al. 2007). Smoking can further raise levels of EGFR ligands, causing a positive feedback loop for tumor-cell growth (Pai et al. 2002; Du et al. 2005). Smoking is directly associated with high EGFR expression, possibly through hypoxia in the tumor tissue (Kalyankrishna and Grandis 2006; Kumar et al. 2007, 2008), and in smokers with EGFR-overexpressing OPSCC, prognosis is miserable (Kumar et al. 2008).

Several reports indicate that in HNSCC, EGFR overexpression is associated with clinically aggressive behavior, resistance to treatment, and worse outcome (Huang and Harari 2000; Chung et al. 2006; Kumar et al. 2007, 2008; Al-Swiahb et al. 2010; Hong et al. 2010). An inverse correlation has been documented between HPV infection and EGFR status in OPSCC (Kim et al. 2007; Kumar et al. 2008; Al-Swiahb et al. 2010; Hong et al. 2010), although not all studies could confirm this association (Reimers et al. 2007; Shi et al. 2009).

Because EGFR overexpression is common in HNSCC, it would be natural to believe that an activating mutation exists, causing the phenomenon. In both colon- and non-small-cell lung cancers, specific phenotypes responsive to EGFR-targeted treatment occur, and EGFR gene copy numbers correlate with RNA and protein expression (Lynch et al. 2004; Aiello et al. 2011). In HNSCC, EGFR-activating mutations are rare (1-7%) (Lee et al. 2005; Loeffler-Ragg et al. 2006), a connection between gene copy numbers and protein expression remains undemonstrated, and the relationship between gene copy numbers and outcome is uncertain (Chung et al. 2006; Moon et al. 2010; Langer 2012). The molecular mechanisms behind EGFR overexpression are poorly understood. However, some patients react to treatment with the RT-modulating monoclonal EGFR antibody cetuximab (Huang and Harari 2000; Moon et al. 2010). A clinically interesting question is how to determine which patients respond to this type of treatment.

Transforming growth factor β1

TGFβ is a pleiotropic polypeptide growth factor that virtually all cells both produce and have receptors for (Blobe et al. 2000). It regulates important processes that maintain homeostasis: proliferation, differentiation, angiogenesis, extra-cellular matrix formation, apoptosis, and immunosuppression (Blobe et al. 2000; Akhurst and Derynck 2001).

TGFβ is expressed in three isoforms (TGFβ1-3) with similar but context- and tissue-dependent effects. In tumor cells, the isoform most frequently upregulated is TGFβ1 (Derynck et al. 2001); it is secreted and stored in an inactive form in the extra-cellular matrix, and is activated only after being cleaved. TGFβ1 signals either through latent transcription factors called Smads (canonical signaling, Figure 2) or alternatively, when the Smad system is impaired, through non-canonical effector molecules. Both pathways are mediated through the cell-surface receptors TGFβRI, II, and III, of which the non-signaling type III is most abundant. Signaling begins with TGFβ1 binding to TGFβRII or TGFβRIII, which recruit, bind, and transphosphorylate TGFβRI. The receptors initiate intracellular signaling by phosphorylating, and thereby activating, Smad 2 or Smad 3, or both, which then form a complex with Smad 4. The Smad complex moves into the nucleus, where it interacts with numerous transcriptional factors, altering the fate of the cell (Figure 2) (Blobe et al. 2000; White et al. 2010; Tian et al. 2011).

The noncanonical pathway is stimulated through a growing number of effectors such as MAP kinases, growth- and survival kinases, GTP-binding proteins (Ras), and protein tyrosine kinases. How TGFβ1 activates these pathways remains unknown, but apparently the signaling can override normal growth inhibition (Akhurst and Derynck 2001; Zhang 2009; Tian et al. 2011).

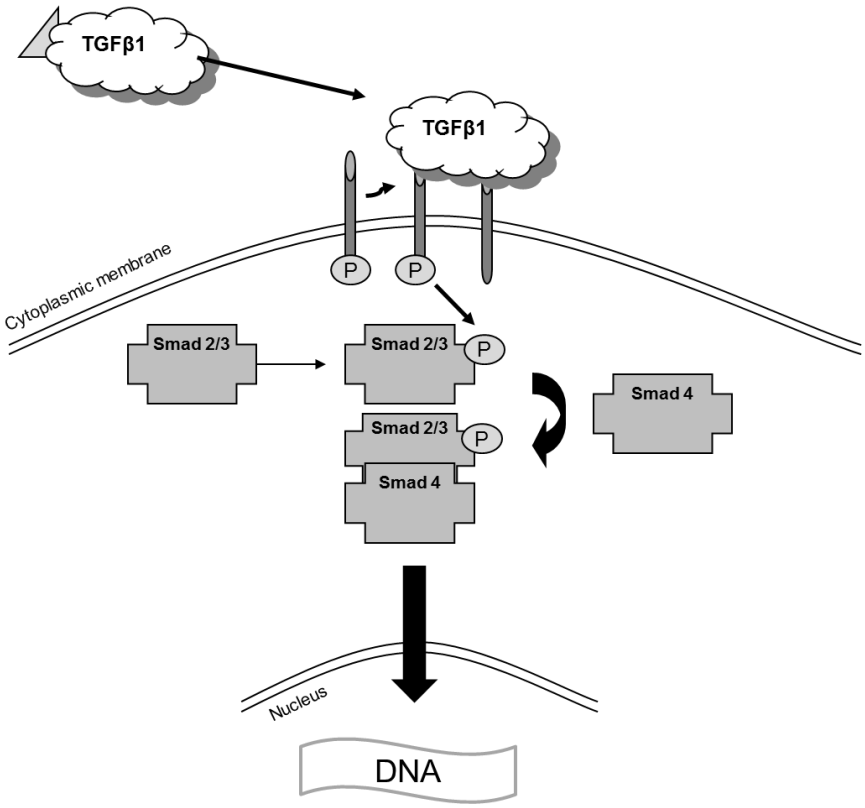

Figure 2 *Canonical signaling of TGFβ1.* *Illustration by Marie Lundberg.*

Transforming growth factor β1 in cancer

In a healthy cell, TGFβ1 is a potent growth inhibitor through its ability to induce cell cycle arrest in the G1 phase, when the cell allows for host-DNA repair or, alternatively, induces apoptosis. In early carcinogenesis, TGFβ1 inhibits epithelial cell growth, but later, the tumor cells develop resistance to cytostasis and apoptosis, even though the TGFβ1 signaling pathway remains intact (Akhurst and Derynck 2001), and they instead, when stimulated, proliferate (Tian et al. 2011). When TGFβ1 production is enhanced, surrounding tissues are affected: increased deposition of extra-cellular matrix occurs, plus altered adhesive properties, enhanced proteolytic activity, angiogenesis, cancer stem-cell maintenance, and activation of EMT. These changes influence the tumor's potential to invade and metastasize (Blobe et al. 2000; Akhurst and Derynck 2001; Shipitsin et al. 2007; Tian et al. 2011). TGFβ1 activates immortality processes (Tian et al. 2011) and affects immunity, both systemically and locally, helping tumor cells evade detection by host immunocytes (Blobe et al. 2000; Akhurst and Derynck 2001). Multiple molecular mechanisms and pathways are affected, overriding hallmarks of carcinogenesis, and in this, TGFβ1 plays an essential role (Tian et al. 2011). The dual role of TGFβ1 in carcinogenesis: acting both as a tumor suppressor and tumor stimulator, is difficult to understand, and many of its underlying molecular mechanisms still remain unrevealed.

Transforming growth factor β1 polymorphisms

The TGFβ1 gene (*TGFB1*) is located on chromosome 9q13, and polymorphism positions are defined relative to the first major transcription site (Figure 3). A number of single nucleotide polymorphisms (SNPs) have been identified, of which at least three are functional, modulating TGFβ1 plasma levels (Cambien et al. 1996; Awad et al. 1998; Kaklamani et al. 2005).

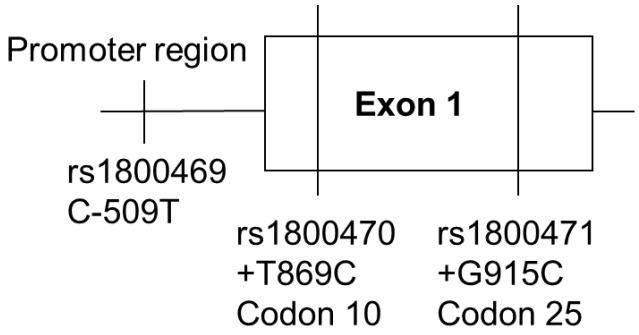

Figure 3 *Locations of the three major TGFB1 single nucleotide polymorphisms.* *Illustration by Marie Lundberg.*

Rs1800471 (+G915C) causes an exchange of the large, charged arginine to a small neutral proline next to the cleavage site of TGFβ1. The variant genotype is relatively rare, its frequency being 7 to 8% in one Czech population (Holla et al. 2002). It is associated with decreased production of TGFβ1 in vitro and with decreased lung fibrosis (Awad et al. 1998), and with improved survival in gastric cancer (Guan et al. 2009).

A thymidine (T) exchange to cytosine (C) at position 29 in the signaling sequence of *TGFB1* results in the substitution of the amino acid leucine (Leu) to proline (Pro). This SNP (rs1800470, formerly rs1982073, or +T869C) is localized to the region that directs TGFβ transport into the extracellular space. This SNP is very common and is documented in several human races and diseases; 16 to 39% of people are of wild-type Leu-Leu (TT) genotype, 45 to 61% are Leu-Pro (TC) heterozygotes, and 10 to 43% are variant homozygotes (Pro-Pro or CC) (Ziv et al. 2001; Dunning et al. 2003; Ziv et al. 2003; Ewart-Toland et al. 2004; Shu et al. 2004; Kaklamani et al. 2005; Wei et al. 2007). In Finland, percentages are reportedly a respective 53, 40, and 7 among breast-cancer patients, and among controls 51, 42, and 6 (Dunning et al. 2003).

The variant C-allele of rs1800470 has been associated with 2.8-fold higher TGFβ1 serum levels both in vivo and in vitro (Yokota et al. 2000; Dunning et al. 2003;). The polymorphism is in linkage disequilibrium with rs1800469 (C-509T), which is accordingly associated with higher TGFβ1 levels (Grainger et al. 1999). The effect may therefore result from the combination of these SNPs, but as rs1800469 is situated in a non-signaling promoter region, the increased secretion is mainly attributed to rs1800470 (Dunning et al. 2003; Guan et al. 2009).

The complicated dual role of TGFβ1, functioning both as a tumor suppressor and a promotor, is well illustrated in the rs1800470 discussion. Breast-cancer patients carrying the C-allele have had a reduced 5-year DFS (Shu et al. 2004). A large multicenter study

including 3987 patients and 3867 controls, 1000 of them from Finland, has shown an association with an 21% increased risk for the disease (Dunning et al. 2003). The researchers estimated that 3% of all breast cancers could be attributable to the variant CC phenotype (Dunning et al. 2003). A study from the Netherlands revealed a 1.4-fold increased risk for breast cancer for this variant genotype (Gonzalez-Zuloeta Ladd et al. 2007), but the inverse has also been reported in large case studies (Ziv et al. 2001; Le Marchand et al. 2004; Kaklamani et al. 2005). Hishida et al. (2003) found the variant allele protective in postmenopausal women and suggested that it is a susceptibility genotype confounded by other risk factors, such as hormonal status.

In patients with high TGFβ1-secreting esophageal tumors, overexpression of TGFβ1 in IHC staining and reduced expression of TGFβR both associated with high metastatic and progressive potential, and decreased survival rates (Fukai et al. 2003; Fukuchi et al. 2004). An American study failed to affirm any association between rs1800470 and survival in gastric cancer (Guan et al. 2009). In prostate cancer, patients with the CC genotype at rs1800470 exhibited no significant risk for cancer, but the homozygotes for the linkage disequilibrium T-allele of rs1800469 showed a 2.4-fold higher risk for more advanced stage cancer (Ewart-Toland et al. 2004), and in a Swedish study, elevated production of TGFβ1 was associated with poor clinical outcome (Wikstrom et al. 1998).

Transforming growth factor β1 polymorphisms in head and neck squamous cell carcinoma

In HNSCC the TGFβ pathway is often disrupted (Garrigue-Antar et al. 1995; Bennett et al. 2009; White et al. 2010). Downregulation of TGFβRII (Lu et al. 2006), loss of TGFβ1 expression (Logullo et al. 2003), and altered Smad expression (Baez et al. 2005; Mangone et al. 2010) are all associated with HNSCC. Surprisingly, few studies address *TGFB1* polymorphisms in HNSCC.

Two studies explore the polymorphisms rs1800469 and rs1800470 and risk for NPC in Chinese populations (Wei et al. 2007; Hu et al. 2012). In the first report, including 108 NPC patients and 120 matched controls, both variant alleles (rs1800469>T and rs1800470>C) were individually associated with an increased risk for NPC, and their combination aggravated it (Odds ratio, OR, 1.68) (Wei et al. 2007). The second study, four times as large, presented the opposite results concerning rs1800469, and found no association between risk and rs1800470 (Hu et al. 2012). Furthermore, the variant allele homozygote frequency did vary substantially: 41 and 40% compared to 15 and 24% (Wei et al. 2007; Hu et al. 2012).

The C-allele of rs1800470, but not the other two major polymorphisms individually, has been associated with HPV16-positive OPSCC in 200 American patients (OR 1.97). When all three variant genotypes were combined, this association was even higher (OR 2.28), indicating that *TGFB1* polymorphisms might even form tumor cells susceptible to HPV16 (Guan et al. 2010).

AIMS OF THE STUDIES

The general objective of this thesis was to contribute to the search for prognostic markers for treatment response in HNSCC. The specific aims of the studies were:

- To detect a possible correlation between the *TGFB1* polymorphism at rs1800470 and prognosis in HNSCC.

- To learn whether, in HNSCC patients treated with CRT, rs1800470 of *TGFB1* affects the grade of mucositis and affects treatment time.

- To assess changes in OPSCC incidence in Finland, and to evaluate the correlation of p16 overexpression with this incidence.

- To discover the inter-relationships of the biomarkers p16, EGFR, and rs1800470 of *TGFB1*, and their association with HNSCC patient survival.

PATIENTS AND METHODS

Patient series (I, II, III, IV)

Patients included in Studies I to IV were diagnosed and treated for HNSCC at the Helsinki University Central Hospital (HUCH) between 1990 and 2007. All studies were retrospective. HUCH's patient records provided data on patient and tumor characteristics, treatment, and survival. At the Department of Otorhinolaryngology, HNSCC patients who provide written consent donate peripheral blood for future cancer research. Those who made up the basis of our studies, the 175 patients in our first study, were consecutive donors to this tumor bank.

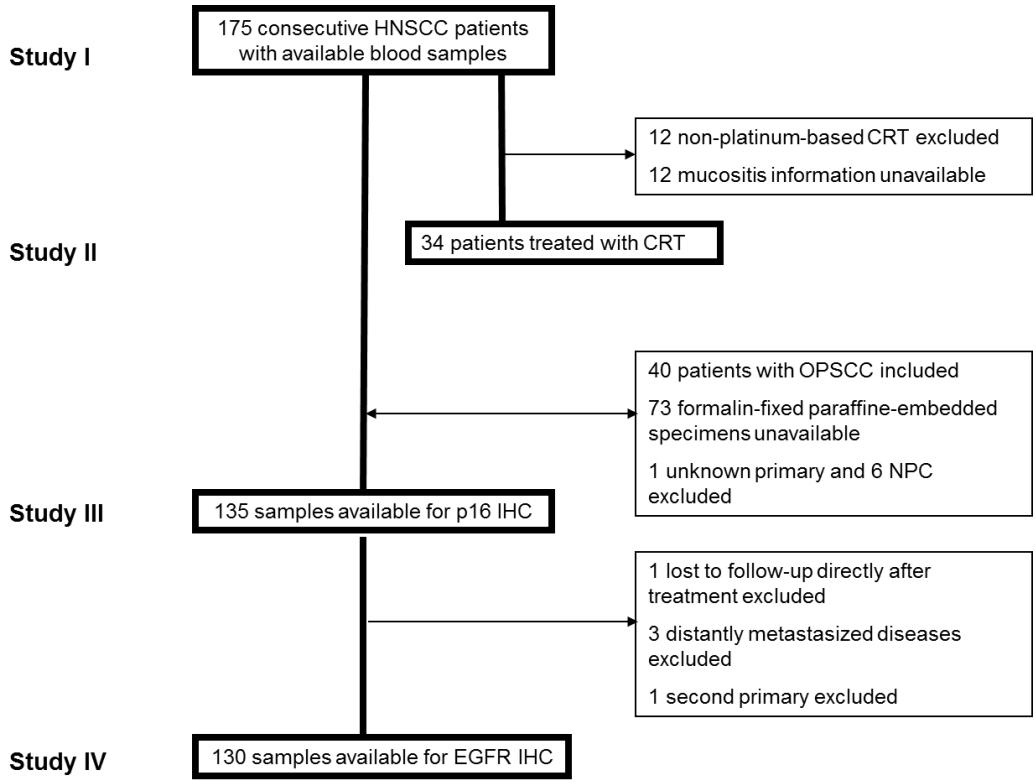

Figure 4 *Patient selection in Studies I to IV.*

Since the four studies were based on the same patient series, characteristics were similar. The majority of the patients were men (74-78%) in all four studies. Mean age ranged from 56 (II) to 60 years (I). Tumor site and stage proportions varied to some extent among the studies (Table 1). In Study II, only those patients treated with CRT were enrolled, which explains the absence there of OSCC and Stage I disease. From Studies III and IV we excluded NPC and unknown carcinomas because of the small number of samples available. The distribution of more high-staged tumors in Studies III and IV compared to Study I is a result of the 40 new OPSCC patients (Figure 4), a cohort including all patients at the Department of Oncology treated with IMRT and concomitant CT in 2001-2007, with a follow-up of a minimum 2 years (Collan et al. 2011).

		Study I N=175 (%)	Study II N =34 (%)	Study III N=135 (%)	Study IV N=130 (%)
Tumor site	**Oral**	45 (26)	-	18 (13)	18 (14)
	Oropharynx	33 (18)	7 (21)	60 (44)	59 (45)
	Hypopharynx	30 (18)	12 (35)	22 (16)	20 (15)
	Larynx	58 (33)	8 (23)	35 (26)	33 (25)
	Nasopharynx	7 (4)	6 (18)	-	-
	Unknown	2 (1)	1 (3)	-	-
Stage	**I**	47 (27)	-	13 (10)	13 (10)
	II	26 (15)	1 (3)	15 (11)	15 (12)
	III	36 (20)	11 (32)	32 (24)	30 (23)
	IV	66 (38)	22 (65)	75 (55)	72 (55)

Table 1 *Tumor sites and stages in Studies I-IV.*

Patient data on smoking, excessive alcohol use, and body mass index were assessed in Study I (unpublished data). According to patient records, 115 patients (66%) smoked or had smoked, and 36 (21%) used excessive amounts of alcohol. Information on height and weight were accessible for 119 patients. Average body mass index was 20.2, ranging from 15.0 to 38.2. The data were not further analyzed because of the uncertainty and unavailability of facts, due to the studies' retrospective nature.

Polymerase chain reaction (I, IV)

From washed leukocytes, DNA for *TGFB1* genotyping was retrieved from the peripheral whole blood samples of the departmental HNSCC tumor bank. For real-time PCR sequencing of DNA, we used TaqMan chemistry with the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Detection primers and probes for genotyping of *TGFB1* at rs1800470 were purchased from Applied Biosystems, according to Dunning et al. (2003), and are listed in Table 2. The reaction was performed in a 20µl mixture containing 2x TaqMan Universal Master Mix (Applied Biosystems) and 200 nM of primers and probes. The Reporter dye FAM was covalently attached to the 5′ end of the E2 probe, and VIC reporter dye to the 5′ end of the E6 probe. TAMRA quencher dye served for both probes. Each PCR plate contained two DNA negative controls.

Oligo name	Sequences 5'->3'
Forward primer	TCTCCCTGAGGACCTCAGCTT
Reverse primer	GCAGCTTGGACAGGATCTGG
C-allele probe	VIC-CTGCTGC**C**GCTGCTGCTACC-TAMRA
T-allele probe	FAM-CTGCTGC**T**GCTGCTGCTACCG-TAMRA

Table 2 *Primers and probes for rs1800470 of TGFB1.*

Immunohistochemistry (III, IV)

We attempted to retrieve formalin-fixed paraffin-embedded specimens from all 215 tumors, but only 142 cases were available, of which 1 unknown primary and 6 NPC were excluded to form adequately sized groups (Figure 4). The samples were reviewed before IHC at the Department of Pathology, HUCH. IHC staining was performed in a LabVision Immunostainer (Labvision, Fremont, CA, USA) at HUSLAB on 5µm tissue slices. For epitope retrieval of p16 and EGFR, we used the following antibodies: p16ink clone E6H4 (ready-to use, CINtec Histology Kit 9511, MTM Laboratories, Heidelberg, Germany) and EGFR (1:25 mouse-clone 31G7, 28-0005, Zymed Laboratories Inc., San Fransisco, CA, USA). Diaminobenzidine functioned as the chromogenic reporter in the polymer-based detection system (Envision, K5007, DakoCytomation).

Scoring of immunohistochemical stainings (III, IV)

All IHC stainings were scored by an experienced pathologist (Prof. I. Leivo) and the author (M.L.). Staining of p16 in the tumor cell nucleus and cytoplasm was scored into three categories (Figure 5). The staining was positive if more than 70% of the cells were stained, intermediate for 30 to 69% staining, and negative if less than 30% were stained. Only four tumors (3%) stained intermediately (30-40%) and were therefore combined with the negative group.

EGFR staining of tumor cells was similarly scored into three categories: no staining, low staining (≤50%), and high staining (>50%, Figure 5). Intensity of staining was not regarded in the classification, and cytoplasmic staining without membrane staining was classified as negative. In the analysis, only the high-expressing category (>50%) was considered positive according to Reimers et al. (2007). In both IHC experiments, non-neoplastic epithelial cells – when found – served as internal negative controls.

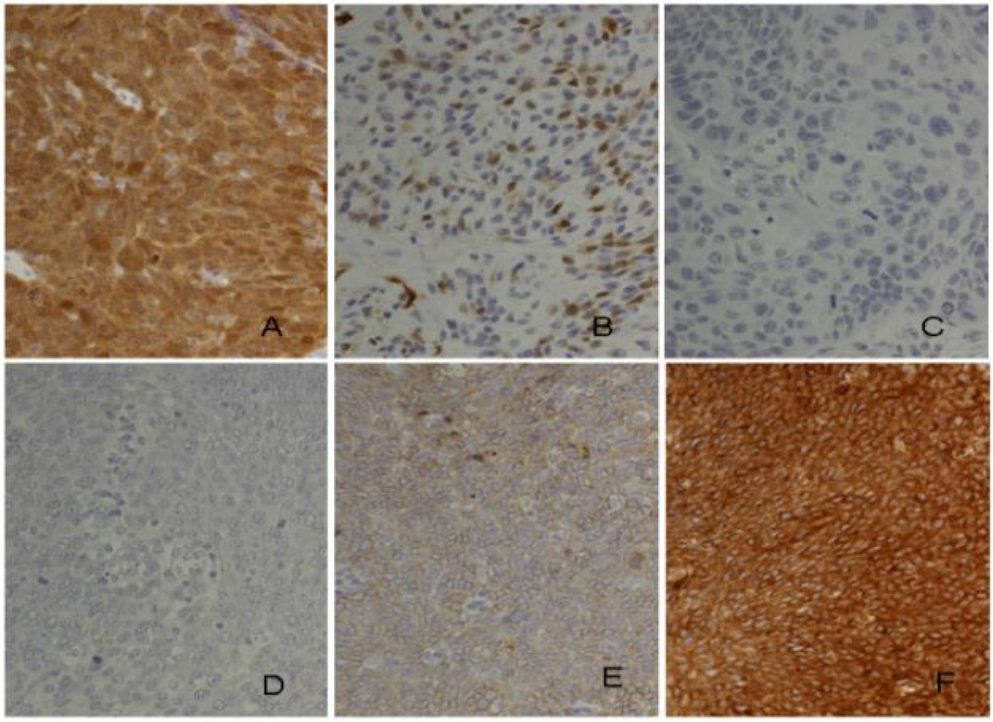

Figure 5 *Scoring of p16 and EGFR. A) p16-positive (≥70%), B) p16-negative (30-69%), C) p16-negative, D) EGFR-negative, E) EGFR-negative (≤50%), EGFR-positive (>50%).* *Photograph by Docent Jaana Hagström.*

Grading of mucositis and dermatitis (II)

Information on dose of chemotherapeutic agent and RT, grade and treatment of mucositis and dermatitis, treatment time, and interruptions in treatment schedule and their reasons came from patient records of the Department of Oncology, HUCH. If many evaluations on mucositis and dermatitis were available, the highest grade was registered. Mucositis and dermatitis were scored according to the Acute Radiation Morbidity Criteria by the Radiation Therapy Oncology Group (RTOG), which includes the clinical view and need for analgesics (Table 3) (Cox et al. 1995). Grade 0 is the normal baseline and Grade 5 radiation effects leading to death. Scoring can begin from commencement of treatment and continues through day 90 when the Criteria of Late Effects should be utilized.

Grade	1	2	3	4
Oral mucosa	Injection; may experience mild pain not requiring analgesics	Patchy mucositis which may produce an inflammatory serosanguinitis discharge; may experience moderate pain requiring analgesia	Confluent fibrinous mucositis; may include severe pain requiring opioid analgesics	Ulceration, hemorrhage, or necrosis
Skin	Follicular, faint, or dull erythema/ epilation/dry/ desquamation/ decreased sweating	Tender or bright erythema, patchy moist desquamation/ moderate edema	Confluent, moist desquamation other than skin folds, pitting edema	Ulceration, hemorrhage, necrosis

Table 3 *RTOG acute radiation morbidity criteria (Cox et al. 1995).*

Incidence and survival (I, II, III, IV)

Survival data were calculated from date of diagnosis (I, IV), or from the end of treatment (II), because the diagnosis date was unavailable at the Department of Oncology. OS endpoint was date of death for any reason, or, if alive, the last date of follow-up. DFS was defined as the day of tumor recurrence, second primary diagnosis, death for any reason, or last date of follow-up (Chua et al. 2005). Survival data were updated between studies so that the shortest follow-up time was 2 years (I), extending to more than 2.5 years (IV). Mean follow-up time ranged from 3.5 to 4.75 years (I, IV), median from 2.9 to 4.75 (II, IV).

Data on absolute and age-standardized incidence of OPSCC in Finland were provided by the Finnish Cancer Registry (Finnish Cancer Registry special tabulation, May 2010). For sufficient follow-up time, the last two available decades corresponding to our patient material were included: 1989-2008 for absolute and 1987-2006 for age-standardized incidence.

Ethical considerations (I, II, III, IV)

The Research Ethics Board at HUCH approved all study protocols. Because of the retrospective nature of these studies, we obtained no informed consent. However, all patients had given their voluntary written consent before donating blood samples to the tumor bank of the Department of Otorhinolaryngology – Head and Neck Surgery, at HUCH. These samples can legally serve in future cancer research. The Research Ethics Board approved this proceeding.

Statistical aspects (I,II,III,IV)

Statistical analyses were performed with StatView software (SAS Institute Inc., Cary, NC, USA) in Studies I and II, and PASW 18.0 software (SPSS Inc., Chicago, IL, USA) in Studies III and IV. Cross-tabulations with chi-square test, or Fisher's exact test when samples were small, were chosen for analyses of contingency variables. With Student's *t*-test, means across categorical variables were compared. All *p*-values were two-sided. With the Kaplan-Meier method, the OS and DFS across various variables were evaluated, with log-rank score determining their statistical significance. The hazard ratio (HR) of confounding factors was monitored with the Cox regression model. A *p*-value ≤ 0.05 was considered statistically significant.

RESULTS

Transforming growth factor β1 (I, IV)

In the patient material of Study I, the C-allele frequency of rs1800470 was 29%. Only seven patients were CC homozygotes (4%). The CT/CC genotype was most common among OPSCC (70%) and LSCC (62%), followed by NPC and HPSCC. The calculated C-allele frequency was 39% in OPSCC patients, whereas other anatomic locations reached frequencies between 19% (OSCC) and 32% (LSCC, unpublished data). The unknown primaries were too few to be rated. The frequencies were similar in Study IV, where only 95 patients of the cohort were genotyped, but in Study II, which did not include OSCC, the C-allele frequency was somewhat higher (34%). The multivariate analysis revealed no correlation between the *TGFB1* genotype and gender, age, or stage.

Mucositis (II)

The majority (59%) of patients treated with platinum-based CRT suffered from severe, Grade 3-4 mucositis, but none had dose-limiting dermatitis. Of the 12 patients (35%) who were hospitalized because of CRT-induced infections, only one, suffering from varicella zoster infection, had low-grade mucositis. The hospitalization lasted on average for 5.5 days (range 3-19), and interfered with treatment schedule in half the cases (two each with mucositis or, neutropenic infections, one each with varicella zoster or, worsened general condition). The average treatment gap was 8 days (N=8, range 2-16), and average treatment time was 53 days (range 42-66), only insignificantly shorter among patients with low-grade mucositis than in high-grade (52.8 vs. 54.4 days, p=0.80).

In this cohort, neither age, gender, cisplatin dose (mg/m²), RT dose (Gy), nor treatment mode (three-dimensional RT vs. IMRT) affected grade of mucositis in regression analysis, although patients treated with three-dimensional RT tended to suffer from higher-grade mucositis (OR 1.90, 95% Confidence Interval, CI, 0.27-13.43, p=0.52), as also did carriers of the C-allele (OR 2.65, 95% CI 0.50-13.89, p=0.25).

p16 (III, IV)

The p16 expression was assessed in 135 HNSCC tumor specimens, of which 48 (36%) were positive. Of 60 OPSCC cases, 41 (68%) overexpressed p16. The frequency of p16-positive specimens was correlated with decade of diagnosis (1990-1999 vs. 2000-2007), with a significant increase from 22 to 41% observed (OR 2.50, 95% CI 1.04-6.03, p=0.05, Table 2, Study III). Although the majority of the positive specimens represented OPSCC, only five samples were from the former decade, with no significant difference. Comparing 2000-2003 and 2004-2007 for OPSCC, the proportion of positive samples

increased from 61 to 73%. The percentage of p16 positive samples in other types of HNSCC was low: in OSCC and LSCC 6%, and in HPSCC 18%. Those patients with p16-positive tumors were on average younger on diagnosis; 55 vs. 60 years (p<0.01). This was true also for OPSCC (p=0.01). No significant difference in gender distribution existed, but the p16-positive tumors tended to be of higher stage (p=0.09) with a smaller primary tumor (T1-2 vs. 3-4, p=0.04), and more regional metastases on diagnosis (N0-1 vs. N2-3, p<0.001).

Incidence of oropharyngeal carcinoma (III)

The age-standardized incidence of OPSCC increased in Finland from 1987 to 2006 for both sexes; in total from 0.43 (1987-1991) to 0.85 (2002-2006) per 100 000 person-years. The corresponding figures for men were 0.68 to 1.2, and for women 0.17 to 0.5 (Figure 6). During a similar period (1989-2008), the absolute incidence increased from 0.66 per 100 000 person-years in the first 5 years to 1.36 per 100 000 during the last 5-year period (p<0.01 Figure 1, Study III).

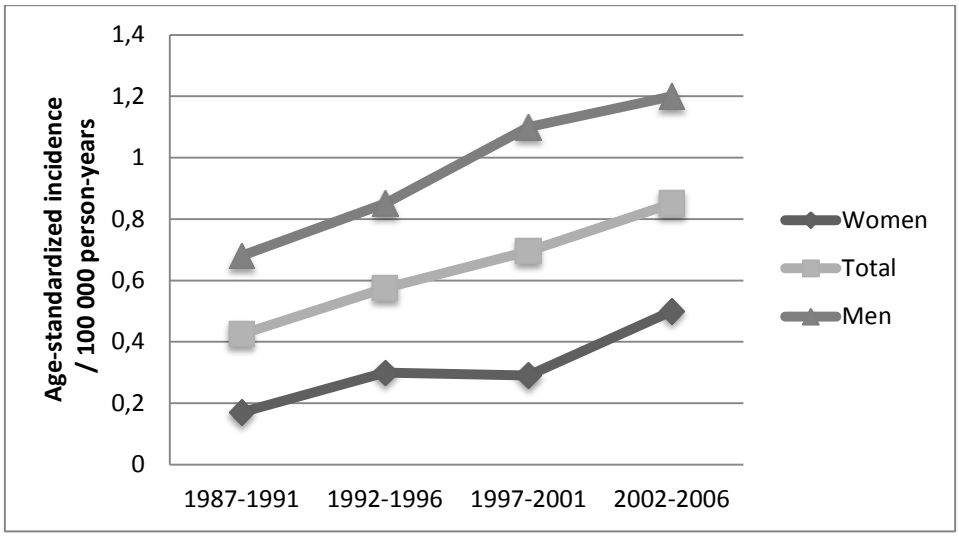

Figure 6 *Changes in the age-standardized incidence (per 100 000 person-years) of OPSCC in Finland during 1987-2006.*

Epidermal growth factor receptor (IV)

EGFR expression was negative in only 5 tumors (4%), whereas 40 tumors (31%) had intermediate staining and 85 (65%) strong staining. The two former categories were combined in analysis (Reimers et al. 2007). EGFR was overexpressed in 65 to 80% of all cases in all other locations except LSCC that were positive in 42% of cases (p=0.11, unpublished data). The co-factors age, gender, stage, and TNM classification were unassociated with EGFR expression. The mean age among the EGFR-positive patients was slightly younger (57) than in the EGFR-negative population (59 years, p=0.42).

Relations between markers (IV)

The whole cohort (N=130) was assessed for associations between p16 and EGFR without revealing any that were significant (OR 0.85, p=0.70). The tendency was towards an inverse correlation, especially in OPSCC where EGFR was overexpressed in 65% of the p16-positive tumors compared to 79% in the p16-negative group (OR 0.50, p=0.37). In the whole cohort (N=130), the corresponding percentages were 63 and 67.

The variant C-allele of *TGFB1* at rs1800470 was more common in the p16-positive group, in which 15 of the 20 patients (75%) were carriers, compared to 57% of the p16-negative patients (Table 4). This association was, however, nonsignificant (OR 2.21, p=0.20). No association existed between EGFR and the *TGFB1* genotype (OR 0.86, p=0.83).

	p16-positive **N= 20**	**p16-negative** **N= 75**	**EGFR-positive** **N= 57**	**EGFR-negative** **N= 38**
***TGFB1* wild type (TT) N=37 (%)**	5 (14)	32 (86)	23 (62)	14 (37)
***TGFB1* variant (CC/CT) N=58 (%)**	15 (26)	43 (74)	34 (59)	24 (41)

Table 4 *The distribution of associations between p16, EGFR, and rs1800470 of TGFB1 (N=95).*

Among OPSCC patients, of 16 p16-positive tumors, 14 (88%) were carriers of the C-allele, whereas allele frequencies were equally distributed among p16-negative tumors. Although the association between p16 overexpression and the C-allele was strong within the p16-positive cohort, when this was combined with the p16-negative group, the result remained nonsignificant (OR 6.00, 95% CI 0.86-41.90, p=0.09). EGFR was not associated with the *TGFB1* genotype in OPSCC (OR 0.844, p=0.83).

Survival outcome (I, II, IV)

The mean OS ranged from 42 months (I) to 57 months (IV). During the longer follow-up in Study IV, 61% of the patients remained recurrence-free. The recurrences were equally distributed across primary sites, with 14, regional nodes with 15, and distant metastases also with 15. Seven patients (5%) developed a second primary tumor.

In HNSCC patients, the variant C-allele of *TGFB1* at rs1800470 was significantly associated with OS (p=0.02) and DFS (Figure 1 Study I, p<0.05). The results were confined to OPSCC (p=0.02) and HPSCC (p=0.04), although LSCC and OSCC also demonstrated the same trend (Figure 2, Study I). As a single marker, p16 expression was, associated with improved OS (p=0.01) and DFS (p<0.01), independent of age,

gender, or stage (HR 0.27, 85% CI 0.11-0.67, $p<0.01$). This was true in all subtypes of cancer, but most convincingly in OPSCC (OS $p=0.016$, DFS $p=0.018$, Figure 7).

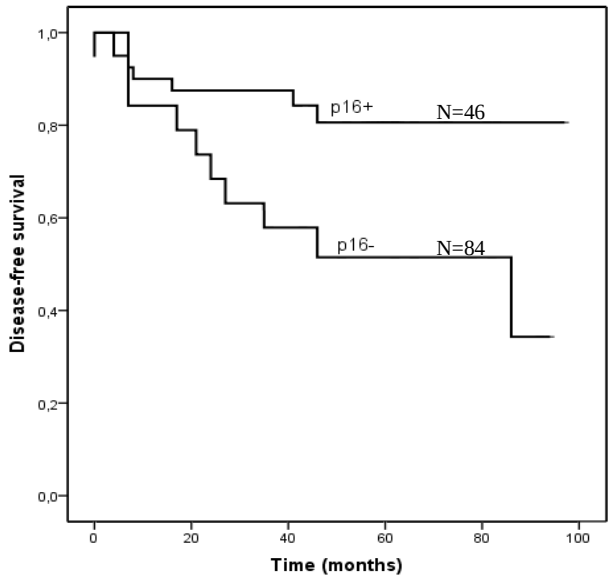

Figure 7 *Disease-free survival based on the p16 marker in OPSCC.*

EGFR status did not alter the results. It was not associated with survival (OS $p=0.56$ and DFS $p=0.70$), or with anatomical site. In multivariate analysis for OS (N=130), EGFR status remained unassociated with survival when adjusted for patient age, gender, stage, p16 status (N=130, HR 0.97, $p=0.85$), and *TGFB1* genotype (N=95, HR 1.15, $p=0.69$). Tumors overexpressing a combination of p16 and EGFR had the best odds for survival (OS $p=0.079$, DFS $p=0.056$, Figure 8).

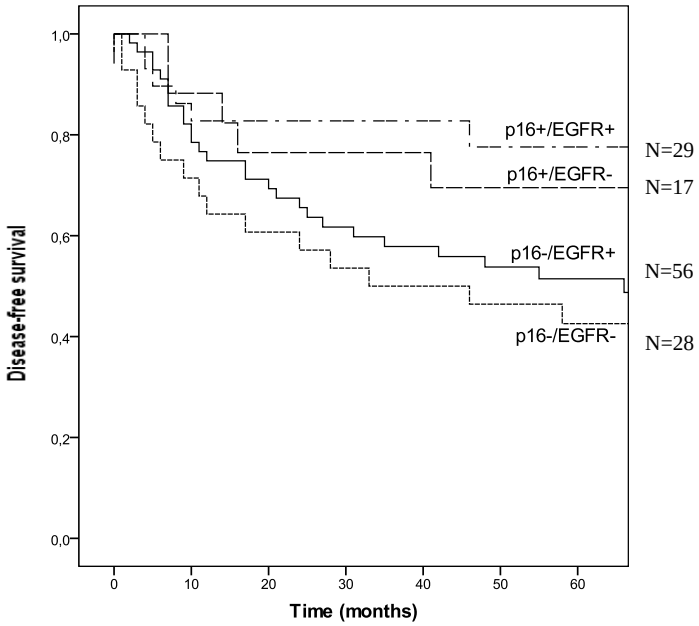

Figure 8 *Disease-free survival based on the various combinations of markers p16 and EGFR combined (N=130).*

As survival was most improved in patients with the variant C-allele of *TGFB1* at rs1800470 treated with CRT (HR 3.42, p=0.03, Figure 4 and Table 2, Study I) these 37 patients were analyzed for OS and DFS in combination with p16 status. Kaplan-Meier analysis suggested that the combination of the variant C-allele and p16 overexpression results in an improved OS and DFS, but that the *TGFB1* genotype correlates more strongly with survival, because survival was improved among carriers of the variant allele independent of p16 status. This finding was verified in multivariate analysis, where the carriers of the rs1800470 C-allele had an improved DFS (HR 0.44, p=0.06) and OS (HR 0.31, p=0.02) compared to that of those with the TT genotype.

DISCUSSION

In this thesis we assessed the correlation between the *TGFB1* genetic polymorphism at rs1800470 and treatment outcome, and the genotype's possible relations with prognostic markers p16 and EGFR. In patients with various malignant tumors, such as esophageal, gastric, lung, and breast cancers, an increased production of TGFβ1 has been associated with poor prognosis (Ghellal et al. 2000; Saito et al. 2000; Fukai et al. 2003; Fukuchi et al. 2004; Shu et al. 2004). Contrary to what was expected, HNSCC carriers of the TGFβ1 high-producing variant C-allele showed improved survival. When stratifying for anatomical sites, improved survival was confined to OPSCC and HPSCC, although other sites demonstrated the same trend. In further analysis, the TGFβ1-linked effect on survival was pronounced in patients treated with CRT; carriers of the variant allele had a significantly better outcome (HR 3.42) than the outcome of patients with the wild-type genotype. After assessment for overexpression of p16 and EGFR by IHC, analysis suggested that CRT-treated carriers of the *TGFB1* variant allele whose tumors overexpressed p16 showed the best prognosis, but the *TGFB1* genotype remained an independent prognostic marker for survival. These results did not seem to result from aberrant *TGFB1* allele frequencies, because similar distributions have emerged among healthy individuals in Finland (Dunning et al. 2003), indicating that the SNP is unrelated to HNSCC risk.

The *TGFB1* polymorphism at rs1800470 has strengths as a prognostic marker: it is accessible from a blood sample and various forms of genetic testing are widely available, as are the well-documented oligonucleotides. Genotyping can be executed quickly, and problems with storage and possible degradation of DNA can be overcome. Results are easy to interpret and independent of interpersonal scoring. The test could be standardized and the prospective results could guide the clinician in the treatment strategy.

All our studies had a long follow-up, and the patient series of 175 is, size-wise, fairly reasonable for an HNSCC study. The major weakness of this thesis is the highly heterogeneous patient material in regard to the anatomic site of the primary tumor, its histopathology, and thus the treatment. We inevitably restricted patient numbers by use of stratified categories, and therefore the results need confirmation in larger trials. Other weaknesses are the retrospective nature of the studies, and the absence of data on smoking status, a variable possibly affecting survival endpoints. The lengthy patient-entry time makes treatment protocols difficult to compare, since treatment regimens have changed over time, moving from three-dimensional RT to IMRT, and from combined treatment protocols towards more organ-sparing regimens. Although rs1800470 is important in TGFβ1 regulation, investigating only one SNP and not confirming its consequences for TGFβ1 levels in HNSCC are acknowledged weaknesses, as is confirmation of HPV infection by p16 IHC alone.

Transforming growth factor β1 genotype and survival

No other studies, to our knowledge, address the impact of the *TGFB1* rs1800470 genotype on survival in HNSCC. The SNP is most thoroughly investigated in breast cancer, where results are inconsistent. The variant allele has been associated with both decreased (Shu et al. 2004) and increased survival (Gonzalez-Zuloeta Ladd et al. 2007). One report states that survival is stage-dependent; in early-stage disease the T-allele was associated with higher risk of death but in late-stage disease with lower risk for recurrence (Mu et al. 2008). The C-allele has also been associated with risk for late-stage disease (Shin et al. 2005). We found the beneficial survival figures to be confined to Stage IV disease, but when further analyzed, the result was more likely to be a function of TGFβ1-induced CRT reactivity, because CRT is usually administered in the management of high-stage disease.

A plausible explanation for our results could be that the TGFβ1 high-producers have enhanced tolerance to CRT. We therefore investigated the most significant acute side-effect in HNSCC treatment, the grade of mucositis, which can cause interruptions in treatment, altering outcome. This explanation is not farfetched, because TGFβ1 participates in wound healing and plays a central role in the mitigation of post-irradiation injury, its production being activated within one hour after RT (Peterson 1992). TGFβ has also been topically administered as a CRT-protective agent in multiple trials, some of them with positive results (Sonis et al. 1997; Wymenga et al. 1999; van't Land et al. 2002; Harsha et al. 2006). This explanation was, however, incorrect in this cohort, as the grade of mucositis was similar in both the rs1800470 variant and wild-type genotype groups. No variations occurred in treatment interruptions or treatment time. Apparently, here, TGFβ1 level in sera had no effect on grade of mucositis.

Possible mechanisms behind response to chemoradiotherapy

The mechanism behind the enhanced survival of the C-allele carriers treated with CRT remains unknown. Although a highly speculative theory, this effect may have been mediated through cancer stem cells (CSC). These cells are the main targets of CRT, as the quiescent remaining ones presumably cause tumor regrowth and relapse (Eyler and Rich 2008; Chen et al. 2009). CSCs are pluripotent cells with properties that include the potential to differentiate into heterogeneous tumors, the capability of indefinite self-renewing, metastasizing, and invading (Jordan et al. 2006). The latter two traits are dependent upon the CSC's inappropriate activation through EMT, a key step in embryogenesis in which the cell loses polarity, cell-to-cell, and cell-to-extra-cellular matrix contact (Jordan et al. 2006; Eyler and Rich 2008).

TGFβ interacts with CSCs, maintaining them in breast cancer (Shipitsin et al. 2007). It initiates and stimulates EMT in various cancers, including HNSCC cell lines (Mani et al. 2008; Yu et al. 2011). In keratinocytes, TGFβ affects CSC differentiation (Schober and Fuchs 2011), and in glioblastoma CSCs, its selective inhibition enhances radiation response (Zhang et al. 2011). In HNSCC, TGFβ is suggested to crosstalk with the CSC transcriptional repressor BMI-1 in vitro, which, when suppressed, enhances survival after CRT (Chen et al. 2010; Kim et al. 2010). It is possible that TGFβ1 activates

quiescent CSCs, rendering them susceptible to CRT, thereby explaining the improved survival.

Another theoretical possibility is that CRT reactivity is conveyed through TGFβ influence on the CSC microenvironment. CSCs are environment-dependent to remain undifferentiated and pluripotent (Prince and Ailles 2008). As a large part of HNSCC consists of non-parenchymal cells where TGFβ exerts its impact on the extra-cellular matrix, on angiogenesis, and on immunosuppression, the CSCs may be sensitized to CRT through changes in their environment.

Transforming growth factor β1 and p16

Numerous reports have demonstrated improved survival among patients with HPV-positive tumors (Ragin and Taioli 2007; Kumar et al. 2008; Lassen et al. 2009; Nichols et al. 2009; Ang et al. 2010; Dayyani et al. 2010; Chaturvedi et al. 2011; O'Rorke et al. 2012). Indications also exist of a connection between improved survival and an enhanced reactivity to CRT and RT (Kumar et al. 2007; Fakhry et al. 2008; Worden et al. 2008; Nichols et al. 2009; Sedaghat et al. 2009; Lassen 2010; Syrjanen 2010). As the carriers of the variant C-allele at rs1800470 showed improved survival when treated with CRT, we investigated the possible association between the HPV surrogate marker p16 and the SNP.

The result suggested that p16 is an independent prognostic factor in OPSCC, and possibly in HNSCC. Furthermore, the p16 overexpression showed no correlation with the *TGFB1* SNP at rs1800470, and the combination of the two molecular markers seemed to predict improved survival. The analysis included only 37 patients, however, so the result must be considered preliminary.

In 2010 Guan et al. published a study in which OPSCC patients with the variant C-allele of rs1800470 experienced a doubled risk for developing a HPV16-positive tumor. Our series suggested a similar trend, although the association did not reach statistical significance. As our patient series was smaller and less homogenous, it is possible that the *TGFB1* genotype is only a susceptibility marker for HPV infection, and that the improved survival of the variant allele reflects the enhanced survival in HPV-induced tumors. This would explain why survival was prolonged especially in OPSCC, which had the highest frequency of the C-allele and is more likely to harbor HPV. The improved survival of the variant allele was, on the other hand, closely associated with primary CRT treatment without surgical resection, whereas the increased survival of HPV-positive OPSCC seems to be independent of treatment mode. This discrepancy argues for rs1800470 as being an independent molecular marker (Licitra et al. 2006; Lindquist et al. 2007; Fakhry et al. 2008; Kumar et al. 2008).

Suggestions are that TGFβ1 and HPV are connected through immunosuppression. An HPV infection can be transient or be self-limited. In susceptible cells, infection can become permanent and progress into malignancies (zur Hausen 2002). As TGFβ1 is involved in suppressing T-helper cells, it may induce an escape from host immune surveillance, making the cell susceptible to persistent HPV infection (zur Hausen 2002).

In cervical cancer, HPV infection has been suggested to elevate TGFβ1 levels as carcinogenesis progresses, altering T-helper cell cytokine expression (Alcocer-Gonzalez et al. 2006), which further induces immunosuppression. TGFβ1 levels are additionally altered through oncogenes E6 and E7, because they can transactivate TGFβ1 transcription (Peralta-Zaragoza et al. 2006), and because E7 can block TGFβ1 tumor-suppressor function (Lee et al. 2002). Apparently TGFβ1 and HPV interact, but whether the patient's genome is associated with a predisposition to HPV infection, with its carcinogenic development, or with increased risk for malignant transformation is unknown, as is the mechanism behind the enhanced survival after CRT.

p16 as a prognostic marker

In this thesis, each tumor's HPV association was assessed by IHC for p16. Of the OPSCC patients, 68% overexpressed p16, which in line with the literature (Charfi et al. 2008; Ang et al. 2010; Lewis et al. 2010; Lau et al. 2011; Ukpo et al. 2011). Patient characteristics and survival accordingly agreed with the literature, as patients with p16-positive tumors were on average younger with small primary tumors and early nodal metastasis, and yet had enhanced survival compared to those with p16-negative tumors (Hobbs et al. 2006; Fakhry et al. 2008; Ang et al. 2010; Syrjanen 2010).

The causative role of HPV in OSCC is controversial, and risk-estimates for HPV association range from 0.32 to 363 (Syrjanen et al. 2011). In two systematic reviews, HPV prevalence ranged from 24 to 34% (Kreimer et al. 2005; Syrjanen et al. 2011). Our results did not confirm a correlation between OSCC and HPV, demonstrating a significantly lower p16 overexpression of 5.6%. The p16 positivity of 18% in HPSCC is in line with other's findings (Wilson et al. 2012), whereas 6% in LPSCC is lower than high-risk HPV infection-rates reported in one meta-analysis (Kreimer et al. 2005). The low detection rates in non-OPSCC cases can be explained by p16's being validated only in OPSCC (Lewis et al. 2012), and by the small sample sizes. The methodological variances and the HPV prevalence in non-OPSCC are highly variable in the literature, with a lack of case-control studies confirming HPV causativity for non-OPSCC (Kreimer et al. 2005; Hobbs et al. 2006; Syrjanen et al. 2011; Torrente et al. 2011; Lewis 2012; Lewis et al. 2012; Wilson et al. 2012).

All single HPV-detection methods show limitations, and the preferable one is disputed. For full maximal sensitivity and specificity, algorithms suggested have had p16 IHC followed by HPV16 ISH or PCR for p16-positive cases (Smeets et al. 2007; Adelstein et al. 2009; Singhi and Westra 2010; Schache et al. 2011; Rietbergen et al. 2012). The latter combination correlates 98% with E6 mRNA RT-PCR analysis. Arguments also exist of p16's being a sufficient marker for transcriptionally active HPV infection, because HPV-specific tests and p16 IHC are highly correlated; reported discrepancies range between 1 and 7% (Weinberger et al. 2004; Ang et al. 2010; Lewis et al. 2010; Singhi and Westra 2010; Schache et al. 2011; Thavaraj et al. 2011; Ukpo et al. 2011; Lewis 2012).

Although p16 IHC still lacks standardized criteria for technical performance, scoring, and interpretation, it is extensively used, reproducible, cost-effective, easily performed

and interpretable. Patients with p16-positive OPSCC have distinct characteristics, and show a clinical cancer course divergent from that of p16-negative patients. Most importantly, their survival is improved, independent of HPV involvement, by ISH or PCR (Weinberger et al. 2004; Begum et al. 2005; Ragin and Taioli 2007; Reimers et al. 2007; Gillison et al. 2008; Nichols et al. 2009; Ang et al. 2010; Dayyani et al. 2010; Lewis et al. 2010; Lewis 2012; Thomas and Primeaux 2012). Therefore, one emerging view is that p16 IHC serves as a reliable surrogate marker for high-risk HPV infection, and it has been suggested as a relevant marker for determining a patient's tumor course as clinically favorable (Ang et al. 2010; Hoffmann et al. 2010; Lewis et al. 2010; Lewis 2012), at least for patients with strong p16 expression in basaloid non- or partially keratinizing suspected or diagnosed OPSCC (El-Naggar and Westra 2012). But p16 is by default a surrogate marker; although providing important prognostic information, it cannot be the sole base for randomized treatment studies (Pannone et al. 2007; Smeets et al. 2007; Hoffmann et al. 2012; Holzinger et al. 2012; Liang et al. 2012), since false-positive results can have devastating consequences for patients in clinical de-escalation programs.

In the present studies, detection methods were not compared, and p16-positive samples remained unconfirmed by more specific methods. Thus, up to 21% of the positive samples may have proven negative in further testing. In this cohort, the p16-positive tumors offered a significantly lower risk of death, and risk for recurrence, when compared to p16-negative ones, in accordance with findings in the literature. Our studies support the evidence of p16 IHC's being a strong independent molecular marker for survival in OPSCC, even though it lacks specificity.

Epidermal growth factor receptor expression

The majority of HNSCC cells overexpress EGFR. This was also true in our studies, where 96% of the tumor cells expressed EGFR, 65% in more than half of the tumor cells. As demonstrated, expression was lower in LSCC than at other sites (Takes et al. 1998), indicating a differing tumor biology. This overexpression was not associated with survival in our analysis. We demonstrated no significant association between EGFR and rs1800470 of *TGFB1*. Although low EGFR expression (<50%) tended to be linked to p16 overexpression, the prognostic value of EGFR IHC remained negative. Documentation of EGFR expression as being inversely correlated with HPV status is not unanimous. Some suggest that the inversely correlated pair could serve as prognostic markers for improved OS and DFS (Reimers et al. 2007; Kumar et al. 2008; Kong et al. 2009; Hong et al. 2010) whereas others, including our group, cannot confirm this (Ulanovski et al. 2004; Kim et al. 2007).

The differences in techniques, antibody clones, and scoring contribute to the disagreement between results. Scoring of EGFR is difficult, with many matters unsettled. The threshold for positive samples ranges from 10 to 50% (Reimers et al. 2007; Al-Swiahb et al. 2010; Hong et al. 2010). For classification, some studies include staining intensity, some have formulas for concurrent scoring of intensity and frequency, and some use continuous scales (Shin et al. 1994; Chung et al. 2006; Kumar et al. 2008). In our studies, the threshold for staining positivity was relatively high

(Reimers et al. 2007), enabling a distinction between low and high expression. It is possible that a different threshold would have altered the results, but ultimately more validation of EGFR IHC is needed before it can serve as a relevant prognostic marker. Our heterogeneous population and small subgroups can also explain our lack of prognostic validity for EGFR. The association between EGFR and survival is not as strong as the association for p16, and therefore in our small patient population we found no correlation.

One interesting fact is that TGFβ is able to transactivate the EGFR pathway (Caja et al. 2007). In in vivo experiments on HNSCC, cells expressing high levels of TGFβ were more resistant to treatment with cetuximab (Bedi et al. 2012), indicating that the *TGFB1* genotype could possibly distinguish among patients who react to treatment with monoclonal EGFR antibodies. This needs further study, however.

Incidence of oropharyngeal cancer in Finland

OPSCC incidence in the western world is increasing (Tachezy et al. 2005; Hammarstedt et al. 2007; Adelstein et al. 2009; Nasman et al. 2009; Warnakulasuriya 2009; Attner et al. 2010; Blomberg et al. 2011; Chaturvedi et al. 2011), as in Finland until 2000 (Syrjanen 2004; Makitie et al. 2006). We have demonstrated that this increase is still ongoing, and the rate has become almost three-fold since the 1950's (Syrjanen 2004). The result is obvious both in terms of absolute incidence and age-standardized incidence, and for both genders. The result is strengthened by the highly accurate nationwide data of the Finnish Cancer Registry, and by the fact that the rates included cancers of the base of the tongue, which has not always been the case in OPSCC reports. The timespan was indeed only 20 years, but combined with previous Finnish reports, follow-up extends to over 50 years.

Simultaneously with the increase in OPSCC incidence, the proportion of p16-positive HNSCC is increasing. Because of a shortage of OPSCC samples from the 1990's, a significant increase in p16-overexpressing OPSCC over time was impossible to document, but with a larger population, including not only southern Finland, and a longer timespan, it is plausible that this phenomenon could be true also in OPSCC. An increase in HPV-related tumors has occurred parallel to OPSCC's rising incidence, with evidence strengthened by diminishing non-HPV-related OPSCC in regions with reduced tobacco consumption (Tachezy et al. 2005; Hammarstedt et al. 2006; Nasman et al. 2009; Attner et al. 2010; Marur et al. 2010; Chaturvedi et al. 2011). HPV-related HNSCC may be sexually acquired, because of associations with higher number of partners, younger age at sexual debut, and the increasing practice of oral sex (Schwartz et al. 1998; Gillison et al. 2000; D'Souza et al. 2007; Heck et al. 2010). HPV prevalence is correspondingly increasing in the cervix, although most countries have experienced large reductions in cervical cancer incidence after the introduction of effective screening programs (Nieminen et al. 1999; Bray et al. 2005). For HNSCC there exists no efficient screening. Thus far, non-invasive sampling is uneffective in the oropharynx (Venuti and Paolini 2012), and no premalignancies analogous to those found in cervical cancer have been defined in OPSCC.

Future perspectives

At the moment, eradication of HNSCC is still out of sight. OPSCC incidence is growing, and environmental influences causing chronic inflammation and genetic alterations show no signs of diminishing. The HPV vaccines developed against cervical cancer could change this situation, as they prevent the same oncogenic HPV types causing OPSCC and therefore theoretically could prevent a majority of OPSCC cases. In cervical pre-malignancies, vaccination can even cause spontaneous regression, thus inhibiting cancer (zur Hausen 2002). It is possible that vaccination for OPSCC would have a similar effect, although the existence of potentially malignant HPV-associated disorders has been suggested only in OSCC (Syrjanen et al. 2011). In the USA, Chaturvedi et al. (2011) estimate that by 2020 we will see more HPV-positive OPSCC annually than cervical cancers. This naturally evokes discussion on whether or not to start OPSCC vaccine trials, and why not include young men in the HPV-vaccination programs? If vaccination proves effective, it will provide the final proof for HPV's being an etiological agent in OPSCC (Haverkos 2004).

For HNSCC patients, wide heterogeneity exists in clinical outcome. Clinical investigation of intensified versus de-intensified treatment schemes combined with research into amelioration of toxic effects is warranted, as is research into development of new agents for treatment, and identification of biomarkers that can guide treatment decisions. The treatment could be even further individually optimized, combining surgery, CT, RT, antibodies, and possible new agents and methods with special attention to minimizing toxicity and simultaneously eliminating both the tumor and CSCs (Psyrri et al. 2012). Hopefully, after assessment in larger trials, the *TGFB1* rs1800470 genotype will, in combination with p16, serve as a prognostic marker for an enhanced response to CRT treatment.

CONCLUSIONS

Based on the results presented here, the following conclusions can be drawn:

- Among HNSCC patients treated with CRT, carriers of the variant C-allele of *TGFB1* at rs1800470 show improved survival.
- In HNSCC, the TGFβ1 genotype seems to show no association with grade of mucositis after treatment with CRT, or with CRT treatment time.
- The incidence of OPSCC is rising, and the frequency of p16-overexpressing HNSCC tumors in Finland is increasing.
- Genetic polymorphism at rs1800470 of *TGFB1*, independent of p16 overexpression, seems to be a predictive marker for HNSCC treated with CRT.

ACKNOWLEDGEMENTS

This study was conducted at the Department of Otorhinolaryngology - Head and Neck Surgery, Helsinki University Central Hospital, 2006-2013. I am deeply grateful to everyone who guided and aided me during all these years. My warmest thanks for giving me the opportunity to do research alongside with clinical work go to the Chief of the Clinic Group, Docent Hans Ramsay, to the Head of the Department, Docent Heikki Rihkanen, and to our always so-inspiring Professor Anne Pitkäranta. My research has been financially supported by the Research Fund of Helsinki University Central Hospital (EVO), by Finska Läkaresällskapet, and by Stiftelsen Dorothea Olivia, Karl Walter och Jarl Walter Perkléns minne. They are all gratefully acknowledged.

My sincere gratitude is owed my supervisors for all the time you have invested in this thesis. Professor Antti Mäkitie receives thanks for making me interested in head and neck cancers, for all the encouragement and trust, and for always having my back. Docent Petri Mattila, you have introduced me to the world of science by sharing all your great (and sometimes frighteningly high-aiming) ideas, and for patiently teaching me all I know about statistics and scientific writing. Thank you.

I thank the official reviewers of this thesis: Professors Eva Munck-Wikland and Timo Paavonen, for their positivity and constructive criticism, which greatly improved this manuscript. Dr. Carol Norris is thanked for the excellent language editing of this text.

A special thanks to all my co-authors: to Professor Ilmo Leivo for your expertise and for all those long hours over the microscope, not shortened by numerous interesting, non-scientific discussions, and for teaching me basic pathology (also not connected to my thesis), to Docent Kauko Saarilahti for your prompt answers, help with gathering oncologic data, and for sharing your oncologic expertise, and to Mervi Pajusto, PhD, Docent Leena-Maija Aaltonen, and Walter Koskinen, PhD, for getting me started with the first study. Eija Nenye deserves a special mention for technical assistance while I ran off to Pakistan with Doctors without Borders, as does Mia Kero for performing IHCs, and Docent Jaana Hagström for photographing the IHC expression patterns, and for many good hints on child care.

I thank my friends and colleagues at the Department of Otorhinolaryngology for providing a warm, ambitious, and inspirational atmosphere; especially Riitta Saarinen, Katri Aro, and Laura Mäkinen, for sharing practical advice, laughter, and agony in the process of writing a thesis. I hope I will soon be back!

I want to thank my family: my parents Barbara and Uffe Cederqvist for teaching me that nothing is impossible through always believing in me, for compensating when I constantly ran out of time with practicalities such as keeping our house standing, our renovation going on, and plants alive, as well as sharing the big baby-sitting burden with my ever-so-helpful parents-in-law Susanne and Karl Erik Lundberg.

I am happy that our children have received the same gift I did from my parents: siblings. Thank you Simon, Christoffer, and Jenny for your love, friendship, and support, especially, during this work, when it comes to crashed computers, lay-out, and picture editing. Thanks also to my sister-in-law, Jenny Sylvin, for the Swedish language

editing, and to Stefan Lönnberg for sharing your knowledge about cervical cancer and for showing interest in this thesis.

Finally, my deepest thanks to my beloved husband Nils for your encouraging, everlastingly positive attitude towards life, and for sometimes making me sit down to consider what is really important. You and our boys Mauritz and Edvin, who fill my days with joy, happiness, and surprises, and the (hopefully still unborn) baby – you mean the world to me.

Grankulla, November 2012

Marie Lundberg

REFERENCES

Adelstein DJ, Ridge JA, Gillison ML, Chaturvedi AK, D'Souza G, Gravitt PE, Westra W, Psyrri A, Kast WM, Koutsky LA, Giuliano A, Krosnick S, Trotti A, Schuller DE, Forastiere A, Ullmann CD. Head and neck squamous cell cancer and the human papillomavirus: Summary of a national cancer institute state of the science meeting, November 9-10, 2008, Washington, D.C. Head Neck 2009;31:1393-422.

Aiello M, Vella N, Cannavo C, Scalisi A, Spandidos DA, Toffoli G, Buonadonna A, Libra M, Stivala F. Role of genetic polymorphisms and mutations in colorectal cancer therapy. Mol Med Report 2011;4:203-8.

Akhurst RJ. TGF beta signaling in health and disease. Nat Genet 2004;36:790-2.

Akhurst RJ and Derynck R. TGF-beta signaling in cancer-a double-edged sword. Trends Cell Biol 2001;11:S44-51.

Alcocer-Gonzalez JM, Berumen J, Tamez-Guerra R, Bermudez-Morales V, Peralta-Zaragoza O, Hernandez-Pando R, Moreno J, Gariglio P, Madrid-Marina V. In vivo expression of immunosuppressive cytokines in human papillomavirus-transformed cervical cancer cells. Viral Immunol 2006;19:481-91.

Al-Swiahb JN, Huang CC, Fang FM, Chuang HC, Huang HY, Luo SD, Chen CH, Chen CM, Chien CY. Prognostic impact of p16, p53, epidermal growth factor receptor, and human papillomavirus in oropharyngeal cancer in a betel nut-chewing area. Arch Otolaryngol Head Neck Surg 2010;136:502-8.

Ambrosch P, Schlott T, Hilmes D, Ruschenburg I. p16 alterations and retinoblastoma protein expression in squamous cell carcinoma and neighboring dysplasia from the upper aerodigestive tract. Virchows Arch 2001;438:343-9.

Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24-35.

Annertz K, Anderson H, Palmer K, Wennerberg J. The increase in incidence of cancer of the tongue in the Nordic countries continues into the twenty-first century. Acta Otolaryngol 2012;132:552-7.

Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet 2008;371:1695-709.

Astsaturov I, Cohen RB, Harari P. Targeting epidermal growth factor receptor signaling in the treatment of head and neck cancer. Expert Rev Anticancer Ther 2006;6:1179-93.

Attner P, Du J, Nasman A, Hammarstedt L, Ramqvist T, Lindholm J, Marklund L, Dalianis T, Munck-Wikland E. The role of human papillomavirus in the increased incidence of base of tongue cancer. Int J Cancer 2010;126:2879-84.

Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: Association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation 1998;66:1014-20.

Baez A, Cantor A, Fonseca S, Marcos-Martinez M, Mathews LA, Muro-Cacho CA, Munoz-Antonia T. Differences in Smad4 expression in human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck squamous cell carcinoma. Clin Cancer Res 2005;11:3191-7.

Bedi A, Chang X, Noonan K, Pham V, Bedi R, Fertig EJ, Considine M, Califano JA, Borello I, Chung CH, Sidransky D, Ravi R. Inhibiton of transforming growth factor beta enhances the in vivo antitumor efficacy of epidermal growth factor receptor-targeted therapy. Mol Cancer Ther 2012;11:2429-39.

Begum S, Gillison ML, Nicol TL, Westra WH. Detection of human papillomavirus-16 in fine-needle aspirates to determine tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. Clin Cancer Res 2007;13:1186-91.

Begum S, Cao D, Gillison M, Zahurak M, Westra WH. Tissue distribution of human papillomavirus 16 DNA integration in patients with tonsillar carcinoma. Clin Cancer Res 2005;11:5694-9.

Bennett KL, Romigh T, Eng C. Disruption of transforming growth factor-beta signaling by five frequently methylated genes leads to head and neck squamous cell carcinoma pathogenesis. Cancer Res 2009;69:9301-5.

Bhide SA, Miah AB, Harrington KJ, Newbold KL, Nutting CM. Radiation-induced xerostomia: Pathophysiology, prevention and treatment. Clin Oncol (R Coll Radiol) 2009;21:737-44.

Blanchard P, Baujat B, Holostenco V, Bourredjem A, Baey C, Bourhis J, Pignon JP, MACH-CH Collaborative g. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): A comprehensive analysis by tumour site. Radiother Oncol 2011;100:33-40.

Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. N Engl J Med 2000;342:1350-8.

Blomberg M, Nielsen A, Munk C, Kjaer SK. Trends in head and neck cancer incidence in Denmark, 1978-2007: Focus on human papillomavirus associated sites. Int J Cancer 2011;129:733-41.

Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, Ove R, Kies MS, Baselga J, Youssoufian H, Amellal N, Rowinsky EK, Ang KK. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567-78.

Bourhis J, Sire C, Graff P, Gregoire V, Maingon P, Calais G, Gery B, Martin L, Alfonsi M, Desprez P, Pignon T, Bardet E, Rives M, Geoffrois L, Daly-Schveitzer N, Sen S, Tuchais C, Dupuis O, Guerif S, Lapeyre M, Favrel V, Hamoir M, Lusinchi A, Temam S, Pinna A, Tao YG, Blanchard P, Auperin A. Concomitant chemoradiotherapy versus acceleration of radiotherapy with or without concomitant chemotherapy in locally advanced head and neck carcinoma (GORTEC 99-02): An open-label phase 3 randomised trial. Lancet Oncol 2012;13:145-53.

Bray F, Loos AH, McCarron P, Weiderpass E, Arbyn M, Moller H, Hakama M, Parkin DM. Trends in cervical squamous cell carcinoma incidence in 13 european countries: Changing risk and the effects of screening. Cancer Epidemiol Biomarkers Prev 2005;14:677-86.

Caja L, Kahata K, Moustakas A. Context-dependent action of transforming growth factor beta family members on normal and cancer stem cells. Curr Pharm Des 2012;18:4072-86

Caja L, Ortiz C, Bertran E, Murillo MM, Miro-Obradors MJ, Palacios E, Fabregat I. Differential intracellular signalling induced by TGF-beta in rat adult hepatocytes and hepatoma cells: Implications in liver carcinogenesis. Cell Signal 2007;19:683-94.

Calais G, Alfonsi M, Bardet E, Sire C, Germain T, Bergerot P, Rhein B, Tortochaux J, Oudinot P, Bertrand P. Randomized trial of radiation therapy versus concomitant

chemotherapy and radiation therapy for advanced-stage oropharynx carcinoma. J Natl Cancer Inst 1999;91:2081-6.
Cambien F, Ricard S, Troesch A, Mallet C, Generenaz L, Evans A, Arveiler D, Luc G, Ruidavets JB, Poirier O. Polymorphisms of the transforming growth factor-beta 1 gene in relation to myocardial infarction and blood pressure. The etude cas-temoin de l'infarctus du myocarde (ECTIM) study. Hypertension 1996;28:881-7.
Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP. Trends in incidence and prognosis for head and neck cancer in the United States: A site-specific analysis of the SEER database. Int J Cancer 2005;114:806-16.
Caudell JJ, Schaner PE, Meredith RF, Locher JL, Nabell LM, Carroll WR, Magnuson JS, Spencer SA, Bonner JA. Factors associated with long-term dysphagia after definitive radiotherapy for locally advanced head-and-neck cancer. Int J Radiat Oncol Biol Phys 2009;73:410-5.
Charfi L, Jouffroy T, de Cremoux P, Le Peltier N, Thioux M, Freneaux P, Point D, Girod A, Rodriguez J, Sastre-Garau X. Two types of squamous cell carcinoma of the palatine tonsil characterized by distinct etiology, molecular features and outcome. Cancer Lett 2008;260:72-8.
Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncology 2008;26:612-9.
Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011;29:4294-301.
Chen YC, Chang CJ, Hsu HS, Chen YW, Tai LK, Tseng LM, Chiou GY, Chang SC, Kao SY, Chiou SH, Lo WL. Inhibition of tumorigenicity and enhancement of radiochemosensitivity in head and neck squamous cell cancer-derived ALDH1-positive cells by knockdown of bmi-1. Oral Oncol 2010;46:158-65.
Chen YC, Chen YW, Hsu HS, Tseng LM, Huang PI, Lu KH, Chen DT, Tai LK, Yung MC, Chang SC, Ku HH, Chiou SH, Lo WL. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophys Res Commun 2009;385:307-13.
Chua YJ, Sargent D, Cunningham D. Definition of disease-free survival: This is my truth-show me yours. Ann Oncol 2005;16:1719-21.
Chung CH, Ely K, McGavran L, Varella-Garcia M, Parker J, Parker N, Jarrett C, Carter J, Murphy BA, Netterville J, Burkey BB, Sinard R, Cmelak A, Levy S, Yarbrough WG, Slebos RJ, Hirsch FR. Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. J Clin Oncol 2006;24:4170-6.
Cohen EE, Lingen MW, Vokes EE. The expanding role of systemic therapy in head and neck cancer. J Clin Oncol 2004;22:1743-52.
Collan J, Lundberg M, Vaalavirta L, Back L, Kajanti M, Makitie A, Tenhunen M, Saarilahti K. Patterns of relapse following surgery and postoperative intensity modulated radiotherapy for oral and oropharyngeal cancer. Acta Oncol 2011;50:1119-25.
Cox JD, Stetz J, Pajak TF. Toxicity criteria of the radiation therapy oncology group (RTOG) and the European organization for research and treatment of cancer (EORTC). Int J Radiat Oncol Biol Phys 1995;31:1341-6.

Dahlstrand H, Dahlgren L, Lindquist D, Munck-Wikland E, Dalianis T. Presence of human papillomavirus in tonsillar cancer is a favourable prognostic factor for clinical outcome. Anticancer Res 2004;24:1829-35.

Daly ME, Le QT, Jain AK, Maxim PG, Hsu A, Loo BW,Jr, Kaplan MJ, Fischbein NJ, Colevas AD, Pinto H, Chang DT. Intensity-modulated radiotherapy for locally advanced cancers of the larynx and hypopharynx. Head Neck 2011;33:103-11.

Dayyani F, Etzel CJ, Liu M, Ho CH, Lippman SM, Tsao AS. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). Head Neck Oncol 2010;2:15.

Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. Nat Genet 2001;29:117-29.

D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356:1944-56.

Du B, Altorki NK, Kopelovich L, Subbaramaiah K, Dannenberg AJ. Tobacco smoke stimulates the transcription of amphiregulin in human oral epithelial cells: Evidence of a cyclic AMP-responsive element binding protein-dependent mechanism. Cancer Res 2005;65:5982-8.

Duffy SA, Ronis DL, McLean S, Fowler KE, Gruber SB, Wolf GT, Terrell JE. Pretreatment health behaviors predict survival among patients with head and neck squamous cell carcinoma. J Clin Oncol 2009;27:1969-75.

Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V, Pharoah PD, Easton DF, Ponder BA, Metcalfe JC. A transforming growth factorbeta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer Res 2003;63:2610-5.

Eisbruch A. Intensity-modulated radiation therapy in the treatment of head and neck cancer. Nat Clin Pract Oncol 2005;2:34-9.

El-Naggar AK and Westra WH. p16 expression as a surrogate marker for HPV-related oropharyngeal carcinoma: A guide for interpretative relevance and consistency. Head Neck 2012;34:459-61.

Ernster JA, Sciotto CG, O'Brien MM, Finch JL, Robinson LJ, Willson T, Mathews M. Rising incidence of oropharyngeal cancer and the role of oncogenic human papilloma virus. Laryngoscope 2007;117:2115-28.

Ewart-Toland A, Chan JM, Yuan J, Balmain A, Ma J. A gain of function TGFB1 polymorphism may be associated with late stage prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13:759-64.

Eyler CE and Rich JN. Survival of the fittest: Cancer stem cells in therapeutic resistance and angiogenesis. J Clin Oncol 2008;26:2839-45.

Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008;100:261-9.

Flanders KC and Burmester JK. Medical applications of transforming growth factor-beta. Clin Med Res 2003;1:13-20.

Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. N Engl J Med 2001;345:1890-900.

Forastiere AA, Goepfert H, Maor M, Pajak TF, Weber R, Morrison W, Glisson B, Trotti A, Ridge JA, Chao C, Peters G, Lee DJ, Leaf A, Ensley J, Cooper J. Concurrent

chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. N Engl J Med 2003;349:2091-8.

Fountzilas G, Kosmidis P, Avramidis V, Nikolaou A, Kalogera-Fountzila A, Makrantonakis P, Bacoyiannis C, Samantas E, Skarlos D, Daniilidis J. Long-term survival data and prognostic factors of a complete response to chemotherapy in patients with head and neck cancer treated with platinum-based induction chemotherapy: A Hellenic co-operative oncology group study. Med Pediatr Oncol 1997;28:401-10.

Fowler JF and Lindstrom MJ. Loss of local control with prolongation in radiotherapy. Int J Radiat Oncol Biol Phys 1992;23:457-67.

Fu KK, Pajak TF, Trotti A, Jones CU, Spencer SA, Phillips TL, Garden AS, Ridge JA, Cooper JS, Ang KK. A radiation therapy oncology group (RTOG) phase III randomized study to compare hyperfractionation and two variants of accelerated fractionation to standard fractionation radiotherapy for head and neck squamous cell carcinomas: First report of RTOG 9003. Int J Radiat Oncol Biol Phys 2000;48:7-16.

Fukai Y, Fukuchi M, Masuda N, Osawa H, Kato H, Nakajima T, Kuwano H. Reduced expression of transforming growth factor-beta receptors is an unfavorable prognostic factor in human esophageal squamous cell carcinoma. Int J Cancer 2003;104:161-6.

Fukuchi M, Miyazaki T, Fukai Y, Nakajima M, Sohda M, Masuda N, Manda R, Tsukada K, Kato H, Kuwano H. Plasma level of transforming growth factor beta1 measured from the azygos vein predicts prognosis in patients with esophageal cancer. Clin Cancer Res 2004;10:2738-41.

Garrigue-Antar L, Munoz-Antonia T, Antonia SJ, Gesmonde J, Vellucci VF, Reiss M. Missense mutations of the transforming growth factor beta type II receptor in human head and neck squamous carcinoma cells. Cancer Res 1995;55:3982-7.

Ghellal A, Li C, Hayes M, Byrne G, Bundred N, Kumar S. Prognostic significance of TGF beta 1 and TGF beta 3 in human breast carcinoma. Anticancer Res 2000;20:4413-8.

Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008;100:407-20.

Gillison ML, Broutian T, Pickard RK, Tong ZY, Xiao W, Kahle L, Graubard BI, Chaturvedi AK. Prevalence of oral HPV infection in the United States, 2009-2010. JAMA 2012a;307:693-703.

Gillison ML, Zhang Q, Jordan R, Xiao W, Westra WH, Trotti A, Spencer S, Harris J, Chung CH, Ang KK. Tobacco smoking and increased risk of death and progression for patients with p16-positive and p16-negative oropharyngeal cancer. J Clin Oncol 2012b;30:2102-11.

Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709-20.

Gonzalez-Zuloeta Ladd AM, Arias-Vasquez A, Siemes C, Coebergh JW, Hofman A, Witteman J, Uitterlinden A, Stricker BH, van Duijn CM. Transforming-growth factor beta1 Leu10Pro polymorphism and breast cancer morbidity. Eur J Cancer 2007;43:371-4.

Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93-7.

Grandis JR and Tweardy DJ. Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res 1993;53:3579-84.

Guan X, Zhao H, Niu J, Tan D, Ajani JA, Wei Q. Polymorphisms of TGFB1 and VEGF genes and survival of patients with gastric cancer. J Exp Clin Cancer Res 2009;28:94.

Guan X, Sturgis EM, Lei D, Liu Z, Dahlstrom KR, Wei Q, Li G. Association of TGF-beta1 genetic variants with HPV16-positive oropharyngeal cancer. Clinical Cancer Research 2010;16:1416-22.

Hafkamp HC, Manni JJ, Haesevoets A, Voogd AC, Schepers M, Bot FJ, Hopman AH, Ramaekers FC, Speel EJ. Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. Int J Cancer 2008;122:2656-64.

Hakulinen T, Tryggvadottir L, Gislum M, Storm HH, Bray F, Klint A, Engholm G. Trends in the survival of patients diagnosed with cancers of the lip, oral cavity, and pharynx in the Nordic countries 1964-2003 followed up to the end of 2006. Acta Oncol 2010;49:561-77.

Hammarstedt L, Lu Y, Marklund L, Dalianis T, Munck-Wikland E, Ye W. Differential survival trends for patients with tonsillar, base of tongue and tongue cancer in Sweden. Oral Oncol 2011;47:636-41.

Hammarstedt L, Dahlstrand H, Lindquist D, Onelov L, Ryott M, Luo J, Dalianis T, Ye W, Munck-Wikland E. The incidence of tonsillar cancer in Sweden is increasing. Acta Otolaryngol 2007;127:988-92.

Hammarstedt L, Lindquist D, Dahlstrand H, Romanitan M, Dahlgren LO, Joneberg J, Creson N, Lindholm J, Ye W, Dalianis T, Munck-Wikland E. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. Int J Cancer 2006;119:2620-3.

Hanahan D and Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011;144:646-74.

Hansson BG, Rosenquist K, Antonsson A, Wennerberg J, Schildt EB, Bladstrom A, Andersson G. Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: A population-based case-control study in southern Sweden. Acta Otolaryngol 2005;125:1337-44.

Harsha WTF, Kalandarova E, McNutt P, Irwin R, Noel J. Nutritional supplementation with transforming growth factor-beta, glutamine, and short chain fatty acids minimizes methotrexate-induced injury. J Pediatr Gastr Nutr 2006;42:53-8.

Haverkos HW. Viruses, chemicals and co-carcinogenesis. Oncogene 2004;23:6492-9.

Heck JE, Berthiller J, Vaccarella S, Winn DM, Smith EM, Shan'gina O, Schwartz SM, Purdue MP, Pilarska A, Eluf-Neto J, Menezes A, McClean MD, Matos E, Koifman S, Kelsey KT, Herrero R, Hayes RB, Franceschi S, Wunsch-Filho V, Fernandez L, Daudt AW, Curado MP, Chen C, Castellsague X, Ferro G, Brennan P, Boffetta P, Hashibe M. Sexual behaviours and the risk of head and neck cancers: A pooled analysis in the international head and neck cancer epidemiology (INHANCE) consortium. Int J Epidemiol 2010;39:166-81.

Herrstedt J. Prevention and management of mucositis in patients with cancer. Int J Antimicrob Agents 2000;16:161-3.

Hishida A, Iwata H, Hamajima N, Matsuo K, Mizutani M, Iwase T, Miura S, Emi N, Hirose K, Tajima K. Transforming growth factor B1 T29C polymorphism and breast cancer risk in Japanese women. Breast Cancer 2003;10:63-9

Hobbs CG, Sterne JA, Bailey M, Heyderman RS, Birchall MA, Thomas SJ. Human papillomavirus and head and neck cancer: A systematic review and meta-analysis. Clin Otolaryngol 2006;31:259-66.

Hoffmann M, Ihloff AS, Gorogh T, Weise JB, Fazel A, Krams M, Rittgen W, Schwarz E, Kahn T. p16(INK4a) overexpression predicts translational active human papillomavirus infection in tonsillar cancer. Int J Cancer 2010;127:1595-602.

Hoffmann M, Tribius S, Quabius ES, Henry H, Pfannenschmidt S, Burkhardt C, Gorogh T, Halec G, Hoffmann AS, Kahn T, Rocken C, Haag J, Waterboer T, Schmitt M. HPV DNA, E6*I-mRNA expression and p16INK4A immunohistochemistry in head and neck cancer - how valid is p16INK4A as surrogate marker? Cancer Lett 2012;323:88-96.

Holla LI, Fassmann A, Benes P, Halabala T, Znojil V. 5 polymorphisms in the transforming growth factor-beta 1 gene (TGF-beta 1) in adult periodontitis. J Clin Periodontol 2002;29:336-41.

Holzinger D, Schmitt M, Dyckhoff G, Benner A, Pawlita M, Bosch FX. Viral RNA patterns and high viral load reliably define oropharynx carcinomas with active HPV16 involvement. Cancer Res 2012;72:4993-5003.

Hong A, Dobbins T, Lee CS, Jones D, Jackson E, Clark J, Armstrong B, Harnett G, Milross C, O'Brien C, Rose B. Relationships between epidermal growth factor receptor expression and human papillomavirus status as markers of prognosis in oropharyngeal cancer. Eur J Cancer 2010;46:2088-96.

Hu S, Zhou G, Zhang L, Jiang H, Xiao M. The effects of functional polymorphisms in the TGFbeta1 gene on nasopharyngeal carcinoma susceptibility. Otolaryngol Head Neck Surg 2012;146:579-84.

Huang SM and Harari PM. Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: Inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. Clin Cancer Res 2000;6:2166-74.

Jensen AR, Nellemann HM, Overgaard J. Tumor progression in waiting time for radiotherapy in head and neck cancer. Radiother Oncol 2007;84:5-10.

Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med 2006;355:1253-61.

Jung AC, Briolat J, Millon R, de Reynies A, Rickman D, Thomas E, Abecassis J, Clavel C, Wasylyk B. Biological and clinical relevance of transcriptionally active human papillomavirus (HPV) infection in oropharynx squamous cell carcinoma. Int J Cancer 2010;126:1882-94.

Kaklamani VG, Baddi L, Liu J, Rosman D, Phukan S, Bradley C, Hegarty C, McDaniel B, Rademaker A, Oddoux C, Ostrer H, Michel LS, Huang H, Chen Y, Ahsan H, Offit K, Pasche B. Combined genetic assessment of transforming growth factor-beta signaling pathway variants may predict breast cancer risk. Cancer Res 2005;65:3454-61.

Kalyankrishna S and Grandis JR. Epidermal growth factor receptor biology in head and neck cancer. J Clin Oncol 2006;24:2666-72.

Kim RH, Lieberman MB, Lee R, Shin KH, Mehrazarin S, Oh JE, Park NH, Kang MK. Bmi-1 extends the life span of normal human oral keratinocytes by inhibiting the TGF-beta signaling. Exp Cell Res 2010;316:2600-8.

Kim SH, Koo BS, Kang S, Park K, Kim H, Lee KR, Lee MJ, Kim JM, Choi EC, Cho NH. HPV integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR and c-myc during tumor formation. Int J Cancer 2007;120:1418-25.

Kong CS, Narasimhan B, Cao H, Kwok S, Erickson JP, Koong A, Pourmand N, Le QT. The relationship between human papillomavirus status and other molecular prognostic markers in head and neck squamous cell carcinomas. Int J Radiat Oncol Biol Phys 2009;74:553-61.

Koskinen WJ, Brondbo K, Mellin Dahlstrand H, Luostarinen T, Hakulinen T, Leivo I, Molijn A, Quint WG, Roysland T, Munck-Wikland E, Makitie AA, Pyykko I, Dillner J, Vaheri A, Aaltonen LM. Alcohol, smoking and human papillomavirus in laryngeal carcinoma: A Nordic prospective multicenter study. J Cancer Res Clin Oncol 2007;133:673-8.

Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: A systematic review. Cancer Epidemiol Biomarkers Prev 2005;14:467-75.

Kumar B, Cordell KG, Lee JS, Prince ME, Tran HH, Wolf GT, Urba SG, Worden FP, Chepeha DB, Teknos TN, Eisbruch A, Tsien CI, Taylor JM, D'Silva NJ, Yang K, Kurnit DM, Bradford CR, Carey TE. Response to therapy and outcomes in oropharyngeal cancer are associated with biomarkers including human papillomavirus, epidermal growth factor receptor, gender, and smoking. Int J Radiat Oncol Biol Phys 2007;69:S109-11.

Kumar B, Cordell KG, Lee JS, Worden FP, Prince ME, Tran HH, Wolf GT, Urba SG, Chepeha DB, Teknos TN, Eisbruch A, Tsien CI, Taylor JM, D'Silva NJ, Yang K, Kurnit DM, Bauer JA, Bradford CR, Carey TE. EGFR, p16, HPV titer, bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. J Clin Oncol 2008;26:3128-37.

Langer CJ. Exploring biomarkers in head and neck cancer. Cancer 2012;118:3882-92.

Lassen P. The role of human papillomavirus in head and neck cancer and the impact on radiotherapy outcome. Radiother Oncol 2010;95:371-80.

Lassen P, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. J Clin Oncol 2009;27:1992-8.

Lau HY, Brar S, Klimowicz AC, Petrillo SK, Hao D, Brockton NT, Kong CS, Lees-Miller SP, Magliocco AM. Prognostic significance of p16 in locally advanced squamous cell carcinoma of the head and neck treated with concurrent cisplatin and radiotherapy. Head Neck 2011;33:251-6.

Le Marchand L, Haiman CA, van den Berg D, Wilkens LR, Kolonel LN, Henderson BE. T29C polymorphism in the transforming growth factor beta1 gene and postmenopausal breast cancer risk: The multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2004;13:412-5.

Lee DK, Kim BC, Kim IY, Cho EA, Satterwhite DJ, Kim SJ. The human papilloma virus E7 oncoprotein inhibits transforming growth factor-beta signaling by blocking binding of the smad complex to its target sequence. J Biol Chem 2002;277:38557-64.

Lee JW, Soung YH, Kim SY, Nam HK, Park WS, Nam SW, Kim MS, Sun DI, Lee YS, Jang JJ, Lee JY, Yoo NJ, Lee SH. Somatic mutations of EGFR gene in squamous cell carcinoma of the head and neck. Clin Cancer Res 2005;11:2879-82.

Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011;11:9-22.

Lewis JS,Jr. p16 immunohistochemistry as a standalone test for risk stratification in oropharyngeal squamous cell carcinoma. Head and Neck Pathol 2012;6:S75-82.
Lewis JS,Jr, Ukpo OC, Ma XJ, Flanagan JJ, Luo Y, Thorstad WL, Chernock RD. Transcriptionally-active high-risk human papillomavirus is rare in oral cavity and laryngeal/hypopharyngeal squamous cell carcinomas--a tissue microarray study utilizing E6/E7 mRNA in situ hybridization. Histopathology 2012;60:982-91.
Lewis JS,Jr, Thorstad WL, Chernock RD, Haughey BH, Yip JH, Zhang Q, El-Mofty SK. p16 positive oropharyngeal squamous cell carcinoma:An entity with a favorable prognosis regardless of tumor HPV status. Am J Surg Pathol 2010;34:1088-96.
Liang C, Marsit CJ, McClean MD, Nelson HH, Christensen BC, Haddad RI, Clark JR, Wein RO, Grillone GA, Houseman EA, Halec G, Waterboer T, Pawlita M, Krane JF, Kelsey KT. Biomerkers of HPV in head and neck squamous cell carcinoma. Cancer Res 2012;72:5000-13.
Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R, Oggionni M, Rossini C, Cantu G, Squadrelli M, Quattrone P, Locati LD, Bergamini C, Olmi P, Pierotti MA, Pilotti S. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol 2006;24:5630-6.
Lindquist D, Romanitan M, Hammarstedt L, Nasman A, Dahlstrand H, Lindholm J, Onelov L, Ramqvist T, Ye W, Munck-Wikland E, Dalianis T. Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. Mol Oncol 2007;1:350-5.
Loeffler-Ragg J, Witsch-Baumgartner M, Tzankov A, Hilbe W, Schwentner I, Sprinzl GM, Utermann G, Zwierzina H. Low incidence of mutations in EGFR kinase domain in caucasian patients with head and neck squamous cell carcinoma. Eur J Cancer 2006;42:109-11.
Logullo AF, Nonogaki S, Miguel RE, Kowalski LP, Nishimoto IN, Pasini FS, Federico MH, Brentani RR, Brentani MM. Transforming growth factor beta1 (TGFbeta1) expression in head and neck squamous cell carcinoma patients as related to prognosis. J Oral Pathol Med 2003;32:139-45.
Lu SL, Herrington H, Reh D, Weber S, Bornstein S, Wang D, Li AG, Tang CF, Siddiqui Y, Nord J, Andersen P, Corless CL, Wang XJ. Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. Genes Dev 2006;20:1331-42.
Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129-39.
Machado J, Reis PP, Zhang T, Simpson C, Xu W, Perez-Ordonez B, Goldstein DP, Brown DH, Gilbert RW, Gullane PJ, Irish JC, Kamel-Reid S. Low prevalence of human papillomavirus in oral cavity carcinomas. Head Neck Oncol 2010;2:6.
Makitie AA, Pukkila M, Laranne J, Pulkkinen J, Vuola J, Back L, Koivunen P, Grenman R. Oropharyngeal carcinoma and its treatment in finland between 1995-1999: A nationwide study. Eur Arch Otorhinolaryngol 2006;263:139-43.
Mangone FR, Walder F, Maistro S, Pasini FS, Lehn CN, Carvalho MB, Brentani MM, Snitcovsky I, Federico MH. Smad2 and Smad6 as predictors of overall survival in oral squamous cell carcinoma patients. Mol Cancer 2010;9:106.

Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704-15.

Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: A virus-related cancer epidemic. Lancet Oncol 2010;11:781-9.

Maxwell JH, Kumar B, Feng FY, Worden FP, Lee JS, Eisbruch A, Wolf GT, Prince ME, Moyer JS, Teknos TN, Chepeha DB, McHugh JB, Urba SG, Stoerker J, Walline HM, Kurnit DM, Cordell KG, Davis SJ, Ward PD, Bradford CR, Carey TE. Tobacco use in human papillomavirus-positive advanced oropharynx cancer patients related to increased risk of distant metastases and tumor recurrence. Clin Cancer Res 2010;16:1226-35.

Meyer F, Bairati I, Fortin A, Gelinas M, Nabid A, Brochet F, Tetu B. Interaction between antioxidant vitamin supplementation and cigarette smoking during radiation therapy in relation to long-term effects on recurrence and mortality: A randomized trial among head and neck cancer patients. Int J Cancer 2008;122:1679-83.

Moon C, Chae YK, Lee J. Targeting epidermal growth factor receptor in head and neck cancer: Lessons learned from cetuximab Exp Biol Med 2010;235:907-20.

Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, Moller B, Pukkala E, Schiller JT, Youngman L, Lehtinen M, Dillner J. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001;344:1125-31.

Mu L, Katsaros D, Lu L, Preti M, Durando A, Arisio R, Yu H. TGF-beta1 genotype and phenotype in breast cancer and their associations with IGFs and patient survival. Br J Cancer 2008;99:1357-63.

Namazie A, Alavi S, Olopade OI, Pauletti G, Aghamohammadi N, Aghamohammadi M, Gornbein JA, Calcaterra TC, Slamon DJ, Wang MB, Srivatsan ES. Cyclin D1 amplification and p16 (MTS1/CDK4I) deletion correlate with poor prognosis in head and neck tumors. Laryngoscope 2002;112:472-81.

Nasman A, Attner P, Hammarstedt L, Du J, Eriksson M, Giraud G, Ahrlund-Richter S, Marklund L, Romanitan M, Lindquist D, Ramqvist T, Lindholm J, Sparen P, Ye W, Dahlstrand H, Munck-Wikland E, Dalianis T. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: An epidemic of viral-induced carcinoma? Int J Cancer 2009;125:362-6.

Nichols AC, Faquin WC, Westra WH, Mroz EA, Begum S, Clark JR, Rocco JW. HPV-16 infection predicts treatment outcome in oropharyngeal squamous cell carcinoma. Otolaryngol Head Neck Surg 2009;140:228-34.

Nieminen P, Kallio M, Anttila A, Hakama M. Organised vs. spontaneous pap-smear screening for cervical cancer: A case-control study. Int J Cancer 1999;83:55-8.

Nwizu T, Ghi MG, Cohen EE, Paccagnella A. The role of chemotherapy in locally advanced head and neck squamous cell carcinoma. Semin Radiat Oncol 2012;22:198-206.

O'Rorke M, Ellison M, Murray L, Moran M, James J, Anderson L. Human papillomavirus related head and neck cancer survival: A systematic review and meta-analysis. Oral Oncol 2012;48:1191-201

Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: A novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med 2002;8:289-93.

Pai SI and Westra WH. Molecular pathology of head and neck cancer: Implications for diagnosis, prognosis, and treatment. Annu Rev Pathol 2009;4:49-70.

Pannone G, Sanguedolce F, De Maria S, Farina E, Lo Muzio L, Serpico R, Emanuelli M, Rubini C, De Rosa G, Staibano S, Macchia L, Bufo P. Cyclooxygenase isozymes in oral squamous cell carcinoma:A real-time RT-PCR study with clinic pathological correlations. Int J Immunopathol Pharmacol 2007;20:317-24.

Peralta-Zaragoza O, Bermudez-Morales V, Gutierrez-Xicotencatl L, Alcocer-Gonzalez J, Recillas-Targa F, Madrid-Marina V. E6 and E7 oncoproteins from human papillomavirus type 16 induce activation of human transforming growth factor beta1 promoter throughout Sp1 recognition sequence. Viral Immunol 2006;19:468-80.

Peterson DE. Oral toxicity of chemotherapeutic agents. Semin Oncol 1992;19:478-91.

Pignon JP, le Maitre A, Maillard E, Bourhis J, MACH-NC Collaborative G. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 93 randomised trials and 17,346 patients. Radiother Oncol 2009;92:4-14.

Prince ME and Ailles LE. Cancer stem cells in head and neck squamous cell cancer. J Clin Oncol 2008;26:2871-5.

Psyrri A, Sasaki C, Vassilakopoulou M, Dimitriadis G, Rampias T. Future directions in research, treatment and prevention of HPV-related squamous cell carcinoma of the head and neck. Head Neck Pathol 2012;6:S121-8.

Pulte D and Brenner H. Changes in survival in head and neck cancers in the late 20th and early 21st century: A period analysis. Oncologist 2010;15:994-1001.

Quon H, Liu FF, Cummings BJ. Potential molecular prognostic markers in head and neck squamous cell carcinomas. Head Neck 2001;23:147-59.

Ragin CC and Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: Review and meta-analysis. Int J Cancer 2007;121:1813-20.

Reimers N, Kasper HU, Weissenborn SJ, Stutzer H, Preuss SF, Hoffmann TK, Speel EJ, Dienes HP, Pfister HJ, Guntinas-Lichius O, Klussmann JP. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. Int J Cancer 2007;120:1731-8.

Rietbergen M, Leemans C, Bloemena E, Heideman D, Braakhuis B, Hesselink A, Witte B, de Jong R, Meijer C, Snijders P, Brakenhoff R. Increasing prevalence rates of HPV attributable oropharyngeal squamous cell carcinomas in the Netherlands as assessed by a validated test algorithm. Int J Cancer 2012;Sep 5 (Epub ahead of print).

Rosenquist K, Wennerberg J, Schildt EB, Bladstrom A, Goran Hansson B, Andersson G. Oral status, oral infections and some lifestyle factors as risk factors for oral and oropharyngeal squamous cell carcinoma. A population-based case-control study in southern Sweden. Acta Otolaryngol 2005;125:1327-36.

Rubenstein LM, Smith EM, Pawlita M, Haugen TH, Hamšíková E, Turek LP. Human papillomavirus serologic follow-up response and relationship to survival in head and neck cancer: A case-comparison study. Infect Agent Cancer 2011;6:9.

Ryu JK, Swann S, LeVeque F, Scarantino CW, Johnson D, Chen A, Fortin A, Pollock J, Kim H, Ang KK. The impact of concurrent granulocyte macrophage-colony stimulating factor on radiation-induced mucositis in head and neck cancer patients: A double-blind placebo-controlled prospective phase III study by radiation therapy oncology group 9901. Int J Radiat Oncol Biol Phys 2007;67:643-50.

Saarilahti K, Kouri M, Collan J, Kangasmaki A, Atula T, Joensuu H, Tenhunen M. Sparing of the submandibular glands by intensity modulated radiotherapy in the treatment of head and neck cancer. Radiother Oncol 2006;78:270-5.

Saarilahti K, Kouri M, Collan J, Hamalainen T, Atula T, Joensuu H, Tenhunen M. Intensity modulated radiotherapy for head and neck cancer: Evidence for preserved salivary gland function. Radiother Oncol 2005;74:251-8.

Saito H, Tsujitani S, Oka S, Kondo A, Ikeguchi M, Maeta M, Kaibara N. An elevated serum level of transforming growth factor-beta 1 (TGF-beta 1) significantly correlated with lymph node metastasis and poor prognosis in patients with gastric carcinoma. Anticancer Res 2000;20:4489-93.

Schache AG, Liloglou T, Risk JM, Filia A, Jones TM, Sheard J, Woolgar JA, Helliwell TR, Triantafyllou A, Robinson M, Sloan P, Harvey-Woodworth C, Sisson D, Shaw RJ. Evaluation of human papilloma virus diagnostic testing in oropharyngeal squamous cell carcinoma: Sensitivity, specificity, and prognostic discrimination. Clin Cancer Res 2011;17:6262-71.

Schober M and Fuchs E. Tumor-initiating stem cells of squamous cell carcinomas and their control by TGF-beta and integrin/focal adhesion kinase (FAK) signaling. Proc Natl Acad Sci U S A 2011;108:10544-9.

Schwartz SM, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, Mao EJ, Fitzgibbons ED, Huang S, Beckmann AM, McDougall JK, Galloway DA. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst 1998;90:1626-36.

Sedaghat AR, Zhang Z, Begum S, Palermo R, Best S, Ulmer KM, Levine M, Zinreich E, Messing BP, Gold D, Wu AA, Niparko KJ, Kowalski J, Hirata RM, Saunders JR, Westra WH, Pai SI. Prognostic significance of human papillomavirus in oropharyngeal squamous cell carcinomas. Laryngoscope 2009;119:1542-9.

Shah JP and Lydiatt W. Treatment of cancer of the head and neck. CA Cancer J Clin 1995;45:352-68.

Shah NG, Trivedi TI, Tankshali RA, Goswami JV, Jetly DH, Shukla SN, Shah PM, Verma RJ. Prognostic significance of molecular markers in oral squamous cell carcinoma: A multivariate analysis. Head Neck 2009;31:1544-56.

Shi W, Kato H, Perez-Ordonez B, Pintilie M, Huang S, Hui A, O'Sullivan B, Waldron J, Cummings B, Kim J, Ringash J, Dawson LA, Gullane P, Siu L, Gillison M, Liu FF. Comparative prognostic value of HPV16 E6 mRNA compared with in situ hybridization for human oropharyngeal squamous carcinoma. J Clin Oncol 2009;27:6213-21.

Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: Increasing trends in the U.S. population ages 20-44 years. Cancer 2005;103:1843-9.

Shin A, Shu XO, Cai Q, Gao YT, Zheng W. Genetic polymorphisms of the transforming growth factor-beta1 gene and breast cancer risk: A possible dual role at different cancer stages. Cancer Epidemiol Biomarkers Prev 2005;14:1567-70.

Shin DM, Ro JY, Hong WK, Hittelman WN. Dysregulation of epidermal growth factor receptor expression in premalignant lesions during head and neck tumorigenesis. Cancer Res 1994;54:3153-9.

Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K. Molecular definition of breast tumor heterogeneity. Cancer Cell 2007;11:259-73.

Shu XO, Gao YT, Cai Q, Pierce L, Cai H, Ruan ZX, Yang G, Jin F, Zheng W. Genetic polymorphisms in the TGF-beta 1 gene and breast cancer survival: A report from the shanghai breast cancer study. Cancer Res 2004;64:836-9.

Singh B and Pfister DG. Individualized treatment selection in patients with head and neck cancer: Do molecular markers meet the challenge? J Clin Oncol 2008;26:3114-6.

Singhi AD and Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. Cancer 2010;116:2166-73.

Smeets SJ, Hesselink AT, Speel EJ, Haesevoets A, Snijders PJ, Pawlita M, Meijer CJ, Braakhuis BJ, Leemans CR, Brakenhoff RH. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer 2007;121:2465-72.

Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: Relationship between analytical and clinical sensitivity. J Pathol 2003;201:1-6.

Sonis ST. The pathobiology of mucositis. Nat Rev Cancer 2004;4:277-84.

Sonis ST, Van Vugt AG, Brien JP, Muska AD, Bruskin AM, Rose A, Haley JD. Transforming growth factor-beta 3 mediated modulation of cell cycling and attenuation of 5-fluorouracil induced oral mucositis. Oral Oncol 1997;33:47-54.

Spijkervet FK and Sonis ST. New frontiers in the management of chemotherapy-induced mucositis. Curr Opin Oncol 1998;10:S23-7.

Syrjanen S. The role of human papillomavirus infection in head and neck cancers. Ann Oncol 2010;21:243-5.

Syrjanen S. Human papillomavirus (HPV) in head and neck cancer. J Clin Virol 2005;32:S59-66.

Syrjanen S. HPV infections and tonsillar carcinoma. J Clin Pathol 2004;57:449-55.

Syrjanen S, Lodi G, von Bultzingslowen I, Aliko A, Arduino P, Campisi G, Challacombe S, Ficarra G, Flaitz C, Zhou HM, Maeda H, Miller C, Jontell M. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: A systematic review. Oral Dis 2011;17:58-72.

Tachezy R, Klozar J, Salakova M, Smith E, Turek L, Betka J, Kodet R, Hamsikova E. HPV and other risk factors of oral cavity/oropharyngeal cancer in the Czech republic. Oral Dis 2005;11:181-5.

Takes RP, Baatenburg de Jong RJ, Schuuring E, Litvinov SV, Hermans J, Van Krieken JH. Differences in expression of oncogenes and tumor suppressor genes in different sites of head and neck squamous cell. Anticancer Res 1998;18:4793-800.

Thavaraj S, Stokes A, Guerra E, Bible J, Halligan E, Long A, Okpokam A, Sloan P, Odell E, Robinson M. Evaluation of human papillomavirus testing for squamous cell carcinoma of the tonsil in clinical practice. J Clin Pathol 2011;64:308-12.

Thomas GR, Nadiminti H, Regalado J. Molecular predictors of clinical outcome in patients with head and neck squamous cell carcinoma. Int J Exp Pathol 2005;86:347-63.

Thomas J and Primeaux T. Is p16 immunohistochemistry a more cost-effective method for identification of human papilloma virus-associated head and neck squamous cell carcinoma? Ann Diagn Pathol 2012;16:91-9.

Tian M, Neil JR, Schiemann WP. Transforming growth factor-beta and the hallmarks of cancer. Cell Signal 2011;23:951-62.

Torrente MC, Rodrigo JP, Haigentz M,Jr, Dikkers FG, Rinaldo A, Takes RP, Olofsson J, Ferlito A. Human papillomavirus infections in laryngeal cancer. Head Neck 2011;33:581-6.

Treister N and Sonis S. Mucositis: Biology and management. Curr Opin Otolaryngol Head Neck Surg 2007;15:123-9.

Trotti A, Bellm LA, Epstein JB, Frame D, Fuchs HJ, Gwede CK, Komaroff E, Nalysnyk L, Zilberberg MD. Mucositis incidence, severity and associated outcomes in patients with head and neck cancer receiving radiotherapy with or without chemotherapy: A systematic literature review. Radiother Oncol 2003;66:253-62.

Ukpo OC, Flanagan JJ, Ma XJ, Luo Y, Thorstad WL, Lewis JS,Jr. High-risk human papillomavirus E6/E7 mRNA detection by a novel in situ hybridization assay strongly correlates with p16 expression and patient outcomes in oropharyngeal squamous cell carcinoma. Am J Surg Pathol 2011;35:1343-50.

Ulanovski D, Stern Y, Roizman P, Shpitzer T, Popovtzer A, Feinmesser R. Expression of EGFR and cerb-B2 as prognostic factors in cancer of the tongue. Oral Oncol 2004;40:532-7.

van't Land B, Meijer HP, Frerichs J, Koetsier M, Jager D, Smeets RL, M'Rabet L, Hoijer M. Transforming growth factor-beta2 protects the small intestine during methotrexate treatment in rats possibly by reducing stem cell cycling. Br J Cancer 2002;87:113-8.

Venuti A and Paolini F. HPV detection methods in head and neck cancer. Head and Neck Pathol 2012;6:S63-74.

Vesco KK, Whitlock EP, Eder M, Burda BU, Senger CA, Lutz K. Risk factors and other epidemiologic considerations for cervical cancer screening: A narrative review for the U.S. preventive services task force. Ann Intern Med 2011;155:698-705.

Vissink A, Jansma J, Spijkervet FK, Burlage FR, Coppes RP. Oral sequelae of head and neck radiotherapy. Crit Rev Oral Biol Med 2003;14:199-212.

Wang J, Shi M, Hsia Y, Luo S, Zhao L, Xu M, Xiao F, Fu X, Li J, Zhou B, Long X. Failure patterns and survival in patients with nasopharyngeal carcinoma treated with intensity modulated radiation in northwest China: A pilot study. Radiat Oncol 2012;7:2.

Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009;45:309-16.

Wei YS, Zhu YH, Du B, Yang ZH, Liang WB, Lv ML, Kuang XH, Tai SH, Zhao Y, Zhang L. Association of transforming growth factor-beta1 gene polymorphisms with genetic susceptibility to nasopharyngeal carcinoma. Clin Chim Acta 2007;380:165-9.

Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Sasaki C, Rimm DL, Psyrri A. Prognostic significance of p16 protein levels in oropharyngeal squamous cell cancer. Clin Cancer Res 2004;10:5684-91.

White RA, Malkoski SP, Wang XJ. TGFbeta signaling in head and neck squamous cell carcinoma. Oncogene 2010;29:5437-46.

Wijers OB, Levendag PC, Braaksma MM, Boonzaaijer M, Visch LL, Schmitz PI. Patients with head and neck cancer cured by radiation therapy: A survey of the dry mouth syndrome in long-term survivors. Head Neck 2002;24:737-47.

Wikstrom P, Stattin P, Franck-Lissbrant I, Damber JE, Bergh A. Transforming growth factor beta1 is associated with angiogenesis, metastasis, and poor clinical outcome in prostate cancer. Prostate 1998;37:19-29.

Wilson DD, Rahimi AS, Saylor DK, Stelow EB, Jameson MJ, Shonka DC, Reibel JF, Levine PA, Read PW. p16 not a prognostic marker for hypopharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2012;138:556-61.

Wong DT, Todd R, Tsuji T, Donoff RB. Molecular biology of human oral cancer. Crit Rev Oral Biol Med 1996;7:319-28.

Worden FP, Kumar B, Lee JS, Wolf GT, Cordell KG, Taylor JM, Urba SG, Eisbruch A, Teknos TN, Chepeha DB, Prince ME, Tsien CI, D'Silva NJ, Yang K, Kurnit DM, Mason HL, Miller TH, Wallace NE, Bradford CR, Carey TE. Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: Response and survival positively associated with HPV16 copy number. J Clin Oncol 2008;26:3138-46.

Wymenga AN, van der Graaf WT, Hofstra LS, Spijkervet FK, Timens W, Timmer-Bosscha H, Sluiter WJ, van Buuren AH, Mulder NH, de Vries EG. Phase I study of transforming growth factor-beta3 mouthwashes for prevention of chemotherapy-induced mucositis. Clin Cancer Res 1999;5:1363-8.

Yokota M, Ichihara S, Lin TL, Nakashima N, Yamada Y. Association of a T29-->C polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to myocardial infarction in Japanese. Circulation 2000;101:2783-7.

Yu C, Liu Y, Huang D, Dai Y, Cai G, Sun J, Xu T, Tian Y, Zhang X. TGF-beta1 mediates epithelial to mesenchymal transition via the TGF-beta/Smad pathway in squamous cell carcinoma of the head and neck. Oncol Rep 2011;25:1581-7.

Zhang M, Kleber S, Rohrich M, Timke C, Han N, Tuettenberg J, Martin-Villalba A, Debus J, Peschke P, Wirkner U, Lahn M, Huber PE. Blockade of TGF-beta signaling by the TGFbetaR-I kinase inhibitor LY2109761 enhances radiation response and prolongs survival in glioblastoma. Cancer Res 2011;71:7155-67.

Zhang YE. Non-smad pathways in TGF-beta signaling. Cell Res 2009;19:128-39.

Ziv E, Cauley J, Morin PA, Saiz R, Browner WS. Association between the T29-->C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: The study of osteoporotic fractures. JAMA 2001;285:2859-63.

Ziv E, Kahn A, Cauley J, Morin P, Saiz R, Browner W. No association between the TGF-beta 1 Leu10Pro polymorphism and osteoporosis among white women in the United States. Am J Med 2003;114:227-31.

zur Hausen H. Papillomaviruses and cancer: From basic studies to clinical application. Nat Rev Cancer 2002;2:342-50.

ORIGINAL PUBLICATIONS