CLINICAL INDICATORS OF THE DEVELOPMENT OF A SECOND ORAL MALIGNANCY AT A PREVIOUSLY TREATED CANCER SITE: EARLY RESULTS OF A LONGITUDINAL STUDY

By

Denise Marie Laronde

B.A., The University of British Columbia, 1985
Dip. D. H., Vancouver Community College, 1989

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In

THE FACULTY OF GRADUATE STUDIES

Dentistry

THE UNIVERSITY OF BRITISH COLUMBIA

2005

© Denise Marie Laronde, 2005

ABSTRACT

Oral squamous cell carcinoma (SCC) has a poor 5-year survival rate of just over 50%, largely due to a high rate of second oral malignancies (SOM) including both recurrences and second primary tumours. Current clinicopathological indicators for oral premalignant lesions (OPLs) at high-risk of progressing into cancer are based on primary OPLs. Little is known whether these risk indicators apply to OPLs at previously treated cancer sites, which are particularly difficult to differentiate from reactive changes resulting from aggressive treatment of the tumours.

Objective: to discover which clinicopathological indicators, if any, could significantly predict a SOM at the previously treated cancer site at around 1 year (8 – 16 months) after treatment of the cancer.

Method: 84 patients with oral cancer (treated with intent to cure) being followed prospectively in the Oral Oncology/Oral Dysplasia Clinic were used in this thesis. Three categories of data were collected: (1) demographic and habit information (age, gender, ethnicity and tobacco habits) (2) primary tumour information (stage, site, histology and treatment of the tumour) and (3) clinicopathological features of post-treatment cancer site during follow up (the presence of an OPL, size, appearance, toluidine blue (TB) staining, histopathology and treatment of the OPLs).

Results: 18 patients (21%) have developed a SOM at the treated cancer site (SOM group) within an average of 26 (± 14) months. Follow-up time for 66 patients who did not develop SOM (non-SOM group) was 28 (± 15) months. Demographics, smoking habit and features of the primary oral cancer did not predict SOM.

Of the clinicopathological features of post-treatment cancer site during follow up, appearance, histopathology and treatment of OPLs did not predict SOM. There was a trend in increasing size of OPLs in the SOM group (14 ± 16 mm in diameter vs. 6 ± 5 for non-SOM group, $P = 0.07$). However, 2 significant predictors were found. The presence of leukoplakia at the prior cancer site was significantly associated with SOM both at one-year post tumour treatment (72% vs. 15% in non-SOM group, $P < 0.001$) and ever during follow up (83% vs. 36%, $P = 0.001$). Uptake of TB stain was also significantly associated with SOM both at one-year post tumour treatment (50% vs. 11% in non-SOM group, $P = 0.001$) and during the entire follow-up (67% vs. 25%, $P = 0.002$).

Conclusion: The results showed that presence of an OPL at the previous tumour site (regardless of its appearance and size) and TB positivity were significant risk predictors for SOM.

DEDICATION

For my Dad, who loved to learn and inspired all my education.

I love you and miss you.

This is what I learned in 'the little red school house' today.

Bless you.

ACKNOWLEDGEMENTS

I would like to thank Drs. Lewei Zhang and Miriam Rosin for the wonderful opportunity they presented me by allowing me to join their research group. Their trust in my ability has been most encouraging and greatly appreciated. A very special thank you to Professor Bonnie Craig, my dental hygiene mentor, for taking me on in the first place. I would also like to thank Dr Allan Hovan, who talked me into the whole thing and Dr Robert Priddy for his all his support from day 1. Thanks to all the members of the Oral Health Study and my friends and co-workers at the BCCA Oral Oncology/Oral Dysplasia clinic.

A very special and heartfelt thank-you to my mother Ruby, and my siblings, Michéle and David, for all their love and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

BCCA	British Columbia Cancer Agency
CIS	carcinoma-in-situ
HNCA	head and neck cancer
LOH	loss of heterozygosity
LP	lichen planus
OCLP	Oral Cancer Prevention Longitudinal study
OPL	oral premalignant lesion
OR	Odds Ratio
PPT	second primary tumour, same site
SCC	squamous cell carcinoma
SD	standard deviation
SFT	second field tumour
SOM	second oral malignancy
SPT	second primary tumour
TSG	tumour suppressor gene
UADT	upper aerodigestive tract
VC	verrucous carcinoma
WHO	World Health Organization

I. INTRODUCTION

I.1. Overview and Statistics

In Canada, 1 in 4.3 women and 1 in 3.6 men will die of cancer (NCIC, 2003).

Oral cancer is the sixth most common cancer in the western world (Johnson, 1998; Shah *et al.,* 2003; Warnakulasuriya, 2002) and the most life threatening disease of the oral mucosa (Burkhardt, 1985). Almost 96% of all head and neck cancers are carcinomas (Silverman, 2003); almost 90% of these originate in the epithelium of the oral cavity and are known as oral squamous cell carcinomas (SCC) (Hoffman *et al.,* 1998; Das and Nagpal, 2002). Oral SCC is one of the most challenging cancers to manage (Antoniades *et al.,* 2003). Despite new technology to aid in the diagnosis and treatment, the five-year survival rate for oral cancer has not improved significantly in the last twenty years (Day *et al.,* 1994a; Vokes *et al.,* 1993). The frequent development of second malignant tumours both at the previously treated cancer site and at second primary sites has had the greatest effect on preventing any improvement in this statistic. It is the goal of this study to find clinicopathological data that would aid clinicians in their ability to determine the risk of a second oral malignancy (SOM) at a former tumour site approximately one year (8 - 16 months) following treatment to cure the target tumour.

I.1.1. World Oral Cancer Statistics

It is estimated that approximately 250,000 new cases of oral cancer will occur each year, accounting for approximately 6% of all cancers worldwide (IARC-WHO, 2002). The prevalence of oral cancer varies worldwide. In the United States approximately 30,000 new cases of oral and oropharyngeal cancer will occur in the next year, with almost twice as many cases in males than in females, particularly males over the age of 50 (American Cancer Society, 2004; Jemal *et al.,* 2002). The expected yearly death rate is estimated to be more than 7,000 deaths with a similar male: female ratio of 2:1 (American Cancer Society, 2004). In the US, cancer of the oral cavity accounts for 3% of the estimated new cases of cancer in males for 2004. The estimated 5-year survival rate is 57%, slightly higher than that of ten years previous, with rates much lower if regional or distant metastasis is involved. The National Cancer Database (NCDB) in the US (Hoffman *et al.,* 1998) claims that the second highest percentage of tumours in the head and neck are in the oral cavity.

La Vecchia *et al.* (2004), analyzed the WHO mortality database to gain information regarding trends in oral cancer mortality in Europe from 1980 - 1999. The authors found in central and Eastern Europe the rate of oral cancer mortality is still increasing, especially in Hungary, Slovakia, Slovenia and Russia. However,

in western European countries oral cancer mortality in men is starting to decline with the exception of Belgium, Denmark, Greece, Portugal and Scotland. Although the oral cancer mortality rate in women is comparatively low, most countries, especially Hungary, have seen a rise in the rate. The authors view this as a reflection of the increased alcohol and tobacco use by women in these countries. In fact, the changing patterns of alcohol and tobacco use may be associated with all the changes. As a result of the very high oral cancer mortality rates in some central and eastern European countries the authors call for urgent tobacco and alcohol control in this region. The American Cancer society (2004) lists Hungary (10.6 per 100,000), Slovakia (9.5 per 100,000) and Croatia (7.2 per 100,000) with the highest death rates in men and Cuba (1.6 per 100,000), Hungary (1.6 per 100,000) and Denmark (1.3 per 100,000) with the highest oral cancer death rates in women. In an earlier paper by La Vecchia *et al.* (1997) the truncated rate of oral cancer for Hungarian men and women respectively, aged 35 – 64 years, was 39.5 per 100,000 and 4.3 per 100,000. Franceschi, Bidoli *et al.* (2000) also studied the incidence rates of oral cancer in different countries. In their study the highest rate of oral cancer was at Bas Rhin in northern France, where the male incidence rate was 20.4 per 100,000. Other populations with a high incidence of oral cancer for men were regions of India (6.3 – 13.2 per 100,000), Slovakia (10.5 per 100,000) and Afro-American men (10.0 per 100,000). The highest incidence rate for women was in the Asian countries of India and the Philippines (Franceschi, Bidoli *et al.,* 2000).

The rate of oral cancer in India is particularly high. Silverman (2003) claims the rate varies from 15 - 65% of total cancers, dependent on the region of the country, with the highest incidence rate in the south. In the Asian country of Taiwan, the mortality rate from oral cancer increased from 3.6 to 6.4 per 100,000 between the years of 1971 and 1994 (Shiu *et al.,* 2000).

I.1.2. Canadian Statistics

The National Cancer Institute of Canada (NCIC) (2003) estimated that nearly 140,000 new cases of cancer and more than 68,000 deaths due to cancer would occur in 2003. Thirty-one hundred of these cases were expected to be oral cancer (includes cancer of the pharynx) with a ratio of 2:1 males to females. Deaths due to oral cancer were estimated at 1100 with similar male to female ratio. These numbers rank oral cancer as the 7th most common cancer in men and the 15th most common cancer in woman in Canada (NCIC, 2003). On a list of 45 countries, the American Cancer Society (2004) estimates that Canada has the 25th highest death rate for oral cavity cancer in men (2.3 per 100,000) and the 19th highest death rate for women (0.8 per 100,000).

I.1.3. British Columbian Statistics

The British Columbia Cancer Agency (BCCA) states that the head and neck cancer incidence rate for BC is 22.9 per 100,000 males and 14.0 per 100,000 females (BCCA, 2003). The NCIC estimated the 2003 oral cancer incidence rates for British Columbia to be 11 per 100,000 and 6 per 100,000 in males and females, respectively. The mortality rate in BC for oral cancer is 4 per 100,000 and 2 per 100,000 for males and females respectively, while the actual deaths were estimated to be 90 males and 40 females. Actual data (NCIC, 2003) had 230 and 130 new cases of oral cancer diagnosed in BC in 1999 for men and women respectively. For the same year, 85 men and 40 women died from oral cancer.

I.2. Histology of the oral mucosa

The oral mucosa consists of all soft tissue anterior to the pharyngeal tonsillar pillars and soft palate and bound anteriorly by the vermilion border of the lip. Oral mucosa consists of stratified squamous epithelium overlying the connective tissue (lamina propria) (WHO, 1978). Stratified squamous epithelium is made up of three cell types. The only cells that divide and are therefore the target of carcinogens are the basal cells, which line the basement membrane. The next layers of squamous cell epithelium are the prickle cells also known as the spinous

or intermediate layer. The top layer, the stratum corneum, is composed of varying degrees of keratin. There are variations in the type and thickness of the epithelium throughout the mouth, dependent on the function of that area of tissue. Keratin acts as a protective barrier in the oral mucosa by helping the mucosa withstand normal wear and tear — the more work an area is subjected to the greater the keratinization. The epithelium can be orthokeratinized (stratum granulosum and nonnucleated keratin), parakeratinized (pyknotic nuclei in the keratin) or nonkeratinized. Areas such as the hard palate, the attached gingiva and the dorsal surface of the tongue, which are subjected to a significant amount of abrasion, are highly keratinized. The buccal mucosa has thick parakeratinized tissue and may have a line, parallel to the occlusal surface of the teeth, that is keratinized, known as linea alba. The soft palate has a thin parakeratinized layer and there is no keratinized tissue on the floor of the mouth, alveolar mucosa, and the lateral and ventral surface of the tongue (WHO, 1978). Rete pegs are projections of epithelial tissue, which extend into the underlying connective tissue, increasing the surface area between the epithelium and connective tissue. In nonkeratinized tissue, the number and depth of the rete pegs is related to the function of the tissue in the area. If the area suffers minimal trauma, such as the floor of the mouth, the rete pegs are shallow and few in number while areas that are subjected to more friction have deeper rete pegs (WHO, 1978).

The connective tissue is separated from the epithelium by the basement membrane, and is composed of blood vessels, nerves, salivary glands, adipose tissue, and fibrous tissue including collagen (WHO, 1978).

The thickness of the epithelium, the amount of pigmentation and the underlying vascularity of the connective tissue influence the colour of the tissue. The nonkeratinized tissue should be a pink or pale red colour while the keratinized tissue will be a paler shade of pink. The amount of melanin present may influence the colour of the tissue and is usually associated with the level of skin pigmentation. (WHO, 1978)

I.3. Etiology of Oral Cancer

The process of oral carcinogenesis is very complex and dependent upon each patient's unique response to purported known and unknown carcinogens.

The exact etiologies of OPLs and SCC are not entirely known. Tobacco is considered to be the primary etiological factor for SCC. Alcohol is another purported cause of oral cancer both alone and synergistically with tobacco. The heavy use of both tobacco and alcohol puts patients at the greatest risk of developing oral cancer. Shah *et al.* (2003) claim that more than 90% of oral cancer is a result of tobacco, heavy alcohol use, and poor diet. Other risk factors

include betel quid chewing, Human Papilloma virus subtypes 16 and 18 (HPV-16, HPV-18) (Bouquot and Whitaker, 1994), ultra-violet light (lips), immunosuppression (due to disease or medications) and a genetic predisposition. Controversy remains whether the presence of other oral pathology such as candidiasis, oral lichen planus, dental trauma from ill-fitting dentures or poor oral hygiene affects the malignant transformation rate. *Candida* has been found to generate nitrosamines, a carcinogen, although the connection is not yet apparent (Silverman and Sugerman, 2000).

I.3.1. Tobacco and Oral Cancer

Tobacco, as mentioned, is the strongest risk factor for oral cancer. According to the NCIC (2003) the most significant cause of all cancer is tobacco. It is estimated that 4 million people die worldwide every year as a result of tobacco use (Silverman, 2003). Tobacco is available in many forms and can be smoked, chewed or snuffed. Due to differences in the preparation of tobacco products worldwide and variation in the way it is used there is some geographic variation in the reported oral cancer risk associated with its use. In the western world tobacco is most associated with cancer of the floor of mouth while in India the buccal mucosa is the most common site of tobacco related oral cancer (Silverman, 2003).

Smoked tobacco products in developed countries include the cigarette, cigar and pipe. Bidi is a form of cigarette smoked in India made up of tobacco powder rolled up in a dried piece of temburni leaf and has been found, particularly in Indian males, to be associated with an increased prevalence of leukoplakia (Gupta, 1984). Not surprisingly, the odds of developing oral cancer increase with the amount of tobacco smoked (Reichart, 2001). The number of compounds reportedly identified in tobacco smoke range from 3,050 (Reichart, 2001) to 4000 (Silverman, 2003) approximately 300 of them are toxic, tumourigenic and carcinogenic (Das and Nagpal, 2002; Reichart, 2001). The tars are thought to be the most carcinogenic, and nicotine the most addictive (Silverman, 2003).

Rodriguez *et al.* (2004) studied the risk factors in young (<46 years old) oral and pharyngeal cancer patients and discovered that tobacco use was associated with 77% of the cancers in this study population and found as both the duration of habit and amount smoked increased so did the risk of oral cancer. This response also holds true for dysplasia. Jaber *et al.* (1999) found that patients who smoked more than 20 cigarettes a day, particularly unfiltered, were at a much higher risk of developing dysplasia than nonsmoking individuals. La Vecchia *et al.* (1999) concluded that the risk of oral cancer decreased for former smokers as the time since they last smoked increased. For former smokers who had quit for 10 years or more the risk of oral cancer was found to be on par with never smokers.

Tobacco use has also been found to correlate with risk of a second oral cancer. Silverman (2003) stated that oral cancer patients who do not change their habits are at a much greater risk of developing a second oral malignancy (SOM). Day *et al.* (1994a) compared the smoking and alcohol habits of 80 patients who developed a second cancer of the upper aerodigestive tract. The risk of second cancers increased with the duration and amount smoked. There was no decrease in risk of a second cancer in patients who quit at or after the first tumour was diagnosed but there was a significantly decreased risk of a second cancer in patients who had quit smoking more than one year prior to the initial diagnosis, in fact the risk for these patients was similar to lifelong nonsmokers. Individuals who had quit 5 years or more before the initial diagnosis had an even greater reduction in risk of SOM. The authors concluded that the length of time since the patient had quit smoking was inversely proportional to the risk of a second malignancy. Khuri *et al.* (2001) also found patients who were still smoking had a greater risk of a SOM than never smokers.

Smokeless tobacco can be used in the form of snuff or chewing tobacco. Tobacco-specific N-nitrosamines are believed to be the main carcinogen found in smokeless tobacco (Scully, 1995). Snuff is available dry, moist or in sachets. Dry snuff, inhaled through the nose, is more common in Europe than in North America. India has many varieties of smokeless tobacco. Some are marketed and sold as a dentifrice under the misguided belief that tobacco is good for the teeth (Gupta, 1992). In the United Stated there is a rise in the use of smokeless

tobacco, moist snuff and loose leaf chewing tobacco, in young adults and children, predominantly male (Glover and Glover, 1992; Poulson, Lindenmuth and Greer, 1984) and this increase in use has led to an increase of oral premalignant lesions (OPL) and malignant lesions in young Americans (Lippman and Hong, 1989). In a study by Poulson, Lindenmuth and Greer (1984) of 56 teenage subjects who admitted to using smokeless tobacco, 33 of whom had an oral lesion, and 4 had more than one lesion. All the lesions were found in the area where the subjects placed the tobacco. The investigators also found a dose-response relationship between exposure and risk of a lesion. More lesions were associated with the use of snuff versus chewing tobacco. The most common description for a smokeless tobacco lesion is white, wrinkled and thickened mucosa (Squier, 1984).

In a study of major league baseball players by Greene *et al.* (1992), the prevalence of oral lesions was associated with the frequency and amount of smokeless tobacco used. Snuff, again was associated with a higher percentage of lesions than chewing tobacco. Snuff was found to be more likely to cause a lesion at the site of tobacco placement than chewing tobacco (Kaugers *et al.*, 1992). In this study, 13% of almost 350 smokeless tobacco users who had been using the product for more than 6 months had a lesion (hyperkeratosis or dysplasia).

I.3.2. Betel Quid and Oral Cancer Risk

Betel quid is a popular product chewed in many Asian countries. Typically, betel quid is made up of a betel leaf, pieces of areca nut, tobacco, a few drops of lime (calcium hydroxide) and flavouring agents. There are many variations of betel quid and it can be chewed with or without tobacco but it is by far more common to have tobacco included (Gupta, 1992). The carcinogen effects of betel quid with tobacco have been shown in many studies by demonstrating increased frequency of OPLs and oral SCC in people using betel quid (Gupta, 1984; Shui et al., 2000; Jacob et al., 2004). Betel quid without a tobacco component is also carcinogenic and has recently been categorized as a Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer (Jacob et al., 2004).

I.3.3. Alcohol and Oral Cancer Risk

The effect of alcohol on its own in the etiology of oral cancer has been difficult to study as results are hampered by the low numbers of heavy drinkers who do not use tobacco and hence there is a limited amount of research. Results are also skewed by a wide variation between countries and regions regarding types of alcohol consumed and alcohol measurements. The processing of alcohol also varies greatly by country and some types of alcohol may have more carcinogenic

impurities than others (Wight and Ogden, 1998). The accuracy of self-reported alcohol use is also questionable as there may be a reluctance or inability on the part of the study participant to give accurate data. The results of a study done by Fioretti *et al.* (1999) found an association between alcohol use and oral cancer in a nonsmoking population. A trend was also found between risk of oral cancer and duration of alcohol use. Franceschi, Levi *et al.,* 2000, found that the risk of oral cancer by very heavy alcohol drinkers (≥ 91 drinks a week) persisted even after they quit drinking.

As mentioned earlier there is a reported synergistic effect on oral carcinogenesis when alcohol and tobacco are used jointly. It has been speculated that alcohol acts as a co-carcinogen by making the oral mucosa more susceptible to the carcinogenic effects of the alcohol itself as well as other carcinogens such as tobacco (Silverman, 2003). In a large study by Blot *et al.* (1988) an increased risk for oral and oropharyngeal cancer was found for both tobacco and alcohol use separately as well as a multiplicative effect when used together. Heavy smokers and drinkers were found to have a 38-fold increased risk of oral and pharyngeal cancer in males and more than a 100-fold risk in females. The authors also discovered that risk of oral cancer decreased as the time interval since last tobacco use increased. Former smokers who had not smoked in 10 years or more had an Odds Ratio (OR) for oral cancer risk equal to never smokers. This study also found an increased risk for cigar and pipe smokers and

smokeless tobacco users. Overall, the authors concluded that tobacco and alcohol contributed to almost 75% of oral and pharyngeal cancer in the US.

In both the aforementioned studies by Rodriguez *et al.* (2004) and Jaber *et al.* (1999) the heavy use of tobacco and alcohol was found to have the highest risk for developing an oral cancer in the former and dysplasia in the latter. In fact the Odds ratio for young heavy smokers and drinkers in the Rodriguez study was > 48. Although the risk was highest for subjects who smoked and drank in Jaber's study, nondrinking smokers were found to have a higher risk of developing dysplasia than nonsmoking drinkers.

Alcohol use among both smokers and nonsmokers increased risk with the highest risk found in current smokers and drinkers. Talamini *et al.* (1998) studied the risk of oral and pharyngeal cancer in nonsmoking heavy drinkers and nondrinking heavy smokers and found both groups had an increased risk of disease. Nonsmoking drinkers who consumed 35 - 55 drinks per week had an OR of 5.0 while those who drank more than 56 drinks per week had an OR of 5.3. The OR for never drinking heavy smokers (> 25 cigarettes per day) was 7.2.

Alcohol use has also been implicated in the risk of second primary cancers of the aerodigestive tract. In a study by Day *et al.* (1994a), patients who drank 15 or more beer per week were at an increased risk of a second cancer.

I.3.4. Human Papilloma Virus and Oral Cancer

The human papilloma viruses (HPV) are a large group of viruses that are responsible for a variety of oral and skin pathology including warts, condylomas, papillomas and cancers in a number of organs including uterine cervix, anogenital area and nasopharynx (Silverman, 2003). The two subtypes purported to be associated with an increased risk of oral cancer are HPV-16 and HPV-18. HPV-16 is the most common HPV type in head and neck SCC (HNSCC). The frequency of high-risk HPV in oral SCC is generally believed to be low, although there is a high frequency of high-risk HPVs associated with SCC in the oropharynx; particularly Waldeyer's tonsillar ring (Hoffman *et al.,* 2004).

HPV-16 activates oncogenes E6 and E7 by deleting E2 and E1 inhibitory genes (Ha and Califano, 2004). E6 and E7 proteins alter the function of the tumour suppressor genes p53 and Rb, cell cycle regulators (Hoffman *et al.,* 2004), leading to cell proliferation (Ha and Califano, 2004).

Smith *et al.* (2004) examined oral exfoliated cells collected via a mouth rinse for HPV and concluded that the presence of high-risk HPV subtypes (including HPV-16 and HPV-18) in exfoliated cells was a risk factor for head and neck cancer (HNCA). A synergistic effect was found in heavy drinking (>21 drinks/week) patients who also presented with high-risk HPV subtypes.

I.3.5. Host factors and Oral Cancer

All diseases are the result of the interactions of external factors and host factors. Inherited genetic factors such as decreased ability in clearing carcinogens and in repairing DNA damage could place a person at a greater risk of developing cancer, which could explain cancers in young people with no apparent etiologies. Cusumano and Persky (1988) found that young (< 35 years of age) females with oral cancer lacked the usual etiological factors of oral cancer, namely alcohol and tobacco, making this group of patients a distinctive entity within oral cancer. The authors discovered that these women presented at a more advanced stage and had a worse survival rate when compared to all oral cancer patients in their research. The advanced stage of presentation was thought to be due to diagnostic delays associated with their age – cancer wasn't initially suspected in patients that young. This group of patients may be a result of altered immunity. Mork, Møller and Glattre (1999) looked for an increased risk in upper aerodigestive tract (UADT) cancer in the families of patients with head and neck cancer diagnosed before the age of 45. Interestingly, the authors found a significantly higher risk of UADT cancers in first-degree relatives of female head and neck cancer patients but not on relatives of male head and neck cancer patients. However, Jin et al. (1999) found that the genetic alterations in young oral cancer patients were similar to those seen in older cancer patients.

Other factors that decrease the host abilities to resist cancer such as poor diet and immunosuppression could make an individual prone to cancer development. Poor nutrition was considered to be the third most important factor in oral cancer according to Rodriguez *et al.* (2004) and that it along with tobacco and alcohol account for 85% of oral cancers. Risk of oral cancer is associated with increased fat intake and inversely associated with the intake of fruits and vegetables (Silverman, 2003). One of the protective elements currently under study is lycopene, the chemical that makes tomatoes red. In a study by De Stefani *et al.* (2000) a protective effect from tomatoes, tomato based foods and oral lycopene was found. It has been speculated that lycopene helps re-establish communication between oral cells (Lund, 1998).

The association between oral cancer and immunosuppression has been shown in a number of studies. Patients who are immunocompromised due to immunosuppressive medications, such as patients who have had a bone marrow transplant, are also at risk for OPLs and SCC. Zhang *et al.* (2002) concluded that posttransplant patients with graft versus host disease (GVHD) should be monitored very closely. The authors also found that HPV was associated with posttransplant oral SCCs.

I.3.6. Other Etiologies and Oral Cancer

There is some debate whether oral lichen planus can be considered a premalignant lesion although it is considered one by the WHO (1978). Oral lichen planus, with a prevalence rate as high as 1% of the general population (as reviewed in Zhang *et al.,* 2000), is a chronic inflammatory disease of the immune system of unknown etiology, more common in females than males, and has various presentations, including reticular, erosive, and plaque-like. Biopsy is required for diagnosis especially at high-risk sites such as the lateral and ventral tongue and sites with an erosive or red component to the lesion (Silverman, 2003). Oral lichen planus (LP) lesions of the tongue, an area not often associated with lichenoid type reactions, should be monitored closely (Larsson and Warfvinge, 2003). Two theories proposed by Zhang *et al. (*1997) state that either oral lichen planus has a very small malignant potential or that oral lichen planus should only be considered premalignant when accompanied by dysplasia. The presence of any degree of dysplasia should not be diminished by the signs of oral lichen planus but considered a proper dysplasia (Zhang *et al.,* 2000). The separation of oral lichen planus without dysplasia from lichenoid dysplasia (dysplastic lesions with lichenoid features) may prove to significantly reduce the reported malignant potential of oral lichen planus. A third category is lichenoid reactions, a lesion presenting clinically as oral lichen planus but with a known etiology. In a study by Zhang *et al.* (1997) histological samples of oral lichen

planus were examined for loss of heterozygosity. A smaller percentage of oral lichen planus exhibited loss of heterozygosity (LOH) than that of dysplasias and reactive lesions.

Hashibe *et al.* (2003) looked for an association between socioeconomic status and OPLs in India. Individuals with a high socioeconomic status and a higher level of education had significantly less OPLs. These associations may be a result of limited or no access to health care, increased high-risk behaviour (less awareness of risk), living environment (outdoor toilet, no refrigerator) and a lack of feeling in control of one's own health.

Poor oral hygiene has also been implicated as a risk for oral cancer (Lissowska *et al.,* 2003; Velly *et al.,* 1998; Sudbø *et al.,* 2001).

I.4. Oral Cancer

I.4.1. Tumourigenesis

There are 3 stages in tumourigenesis: initiation, promotion and progression. Brodland (1997) compares these stages to the 3 stages of childbirth: conception, gestation and birth of a cancer. Initiation is a carcinogen-provoked event, which leads to an irreversible genetic mutation that is passed on to the daughter cells.

Most cells never advance beyond this point. Promotion is the growth or expansion of the initiated cells in the presence of a promoting carcinogen. If the promoting event is removed at this stage tumourigenesis would stop and may even be reversible. The purpose of this stage is to increase the number of initiated cells. As the number of cells grows the probability of more genetic mutations grows. Progression is the accumulation of characteristics that develop into an invasive cancer (Licciardello, Spitz and Hong, 1989). Progression is rare and continues after a malignancy is formed (Brodland, 1997).

The spread of cancer cells from the original site to the surrounding lymph nodes or to a more distant site, via the blood or lymphatic system is called metastasis. The majority of oral cancer metastasis is to the lymph nodes of the head and neck. Distant metastases, although rare, occur most often in the lung (Bettendorf, Piffko and Bankfalvi, *et al.,* 2004).

I.4.2. TNM stage

Tumours are staged according to the globally accepted tumour-node-metastasis (TNM) system. The TNM system illustrates the extent of the tumour spread and is the main determinant to guide treatment and predict outcome (Bettendorf, Piffko and Bankfalvi *et al.,* 2004). The tumour (T_1-T_3) aspect refers to the increasing size of the invasive tumour. T_1 is a tumour less than 2 cm, T_2 is 2 – 4

cm and T_3 is greater than 4 cm in size. The label T_4 is given to a tumour that has invaded an adjoining structure. Carcinoma *in situ* is labelled T_{is}. N reflects the absence or presence of local lymph nodes as well as their number, size and site (Gath and Brakenhoff, 1999; Das and Nagpal, 2002) and M refers to the absence or presence of distant metastasis. The TNM code is then used to stage the tumours. Stage 0 (T_{is}), stage I ($T_1N_0M_0$) and stage II ($T_2N_0M_0$) are referred to as early stage tumours. Stage III ($T_3N_0M_0$ or $T_{1-3}N_1M_0$) and stage IV tumours ($T_{1-3}N_{2-3}M_0$) are called late stage tumours (Sapp, Eversole and Wysocki, 1997). Regardless of T or N stage patients who have distant metastases are ranked as stage IV disease (Vokes, *et al.*, 1993; Sapp, Eversole and Wysocki, 1997). The system reflects prognosis; as clinical stage increases the prognosis decreases. There have been many modifications of this system in an attempt to increase its ability to predict outcome for oral cancer and it has been found that the predictive value of the TNM classification system increases for oral cancer when the site and other histopathological data are considered (Rapidis *et al.*, 1977).

Pathologists also grade tumours according to the percent of the tumour showing incomplete differentiation (Ivkić *et al.*, 2002). When the tumour tissue is similar in shape and structure to the original tissue it is graded well differentiated tissue. As tissue becomes less similar to the original tissue it is labelled moderately differentiated or finally, poorly differentiated SCCs.

I.4.3. Malignancies of the Oral Cavity

Almost 90% of oral cancer originates from the lining epithelium of the oral mucosa and is known as squamous cell carcinoma (SCC) (Hoffman *et al.,* 1998) and hence, the term oral cancer generally refers to oral SCC. Verrucous carcinoma and spindle cell carcinoma are considered well and poorly differentiated variations of SCC, respectively. Approximately 5% of oral carcinomas are VC and are distinguished clinically by their exophytic cauliflower-like appearance (Das and Nagpal, 2002).

The frequency of SCC at different oral sites varies. The sites in decreasing order of frequency, according to Ha and Califano (2004), are the lateroventral tongue, floor of mouth, hard palate, gingiva and buccal mucosa (no geographical parameters listed). In the United States, the most frequent site for oral cancer was the lateroventral tongue and floor of the mouth (Canto and Devesa, 2002). Cancer of the lip is frequently not included in the category of oral SCC because of different etiological factors and prognosis. Lip cancer has a 5-year survival rate of 91.1 - 95% (Hoffman *et al.,* 1998; Reid *et al.,* 2000). The more posterior and inferior the tumour site is within the oral cavity the worse the patient's prognosis (Hoffman *et al.,* 1998).

I.4.4. Treatment of Oral SCC

The most significant prognostic factor in determining survival from oral SCC is the complete removal of the tumour (Brennan *et al.,* 1995). The site and stage of oral cancer determines the treatment (Das and Nagpal, 2002). The majority of oral cancer treatment centres on surgical or radiation therapy, or both, to remove local and regional disease (Vokes *et al.,* 1993). The type of treatment a patient receives is based on the location of the tumour, anticipated post treatment morbidity and the expertise of the specialist involved in the case (Bettendorf, Piffko and Bankfalvi *et al.,* 2004). Stage I and II tumours are considered to be curable with either surgery or radiation alone, while stage III and IV tumours are normally treated with surgery and subsequent radiation (Bettendorf, Piffko and Bankfalvi *et al.,* 2004). The treatment of choice for the majority of oral tumours is surgery (BCCA, 2001a). Treatment of choice for the more posterior tumours of the soft palate, uvula, base of tongue, and tonsils is generally radiation therapy (BCCA, 2001b). Radiation is used at sites which are difficult to access surgically, when the patient refuses surgery or where the tumour is large and surgery may cause extreme morbidity. Radiation is also used in combination with surgery for higher staged tumours when there is advanced nodal disease involved (Vokes *et al.,* 1993) or to reduce risk of recurrence for patients who had positive surgical margins (Loree and Strong, 1990). Patients who have received radiation therapy to the head and neck area may face complications such as xerostomia, erythema, edema and

osteoradionecrosis (Das and Nagpal, 2002). Radiation reactions that occur more than 3 months after the end of treatment are known as late radiation changes and are primarily due to microvascular damage (BCCA, 2001c). A radical neck dissection is often performed at the same time as the original tumour surgery in patients with advanced disease to remove any positive or questionable lymph nodes.

The use of chemotherapy to treat oral SCC is only of palliative value, and is used for late stage SCC with or without radiation.

I.5. Second Oral Malignancies

I.5.1. Recurrence and second primary tumour (SPT) and SOM

Patients who have had an oral SCC are at risk of SCC recurring at the same site or of a second primary cancer (Warren and Gates, 1936; NCIC, 2003; Lippman and Hong, 1989; Jovanovic *et al.,* 1994; van Es *et al.,* 1996). A recurrent oral SCC refers to a SCC occurring at the previous oral site 6 months to 3 years after the treatment of the primary SCC. A tumour is considered persistent if less than 6 months has passed since treatment. Conversely, a second primary tumour (SPT) refers to two entities: a SCC occurring at an oral site that is at least 3 centimetres away from the site of another oral SCC at the same or later time

(Tabor *et al.*, 2002) and a SCC occurring at the prior site of an oral SCC more than 3 years after the primary SCC treatment. There is no universal agreement however, regarding the definition of a second primary tumour and the distance and time between the tumours. Some studies have used 1.5 or 2 centimetres distance instead of 3 cm; while others have used 5 years instead of 3 years time difference (Braakuis, 2003; Hong *et al.*, 1990).

To further illustrate the difficult concepts of recurrences and SPTs, Braakhuis *et al.* (2002) argue for another term "second field tumour (SFT)". This is a tumour that arises beyond the treatment border of the initial tumour but is still part of the larger genetically altered field, a field with multiple foci of genetic alterations. This field can be as large as 7 cm in diameter (Tabor *et al.,* 2002). The difference between a SPT and a SFT, according to Tabor *et al.* (2002) is that a true SPT is not clonally related to the first primary tumour. The SFT arises as a large genetically altered lesion that originated from one altered cell that proliferates and gradually takes over the entire field. As the tissue progresses various sub clones arise with new genetic changes. This leads to a field that may be genetically different but shares the original alteration. The SFT will share some but not all of the molecular markers found in the primary tumour as well as further later changes (Mao, 2002; Rosin, Lam and Zhang, 2003). After the first primary tumour is treated the surrounding field may continue to progress leading to a second tumour (Tabor *et al.,* 2002). Even with radical treatment the area is considered at high-risk for a second tumour (Braakhuis *et al.,* 2003). With

advances in genetic testing determination of what is a SPT, a SFT or a recurrence becomes more complicated. It can be assumed that subsequent tumours that have the same genetic patterns as the primary tumour are recurrent tumours while second tumours with different patterns of loss are SPTs. However, it is unlikely to get complete symmetry between a primary tumour and a subsequent tumour because subsequent molecular changes may have occurred since the primary event and chance alone may have resulted in similar early molecular events (Rosin, Lam and Zhang, 2003). Therefore the differences between a recurrence and a SPT could be very difficult to discern, even with current molecular markers. However, the analysis of the margins of a resected tumour may identify a high-risk group for a SFT (Tabor, *et al.,* 2002).

As mentioned earlier, a SCC occurring at the site of a previous oral SCC could either be a recurrence (more than 6 months but less than 3 years after curative treatment) or a SPT (the tumour occurs more than 3 years after treatment). However, since it is frequently impossible to differentiate between the two, the term second oral malignancy (SOM) has been coined to encompass both recurrences and second primary tumours (Lippman and Hong, 1989; Rosin *et al.*, 2002). This thesis investigates the clinicopathological risk factors that would predict cancer occurring at the sites of previous oral SCC, regardless of whether they are recurrences or second primary tumours at the former cancer site.

I.5.2. Incidence of SOM

Nearly one-third of patients (Shah *et al.,* 2003) with a previous oral cancer will ultimately develop a SOM at a prior cancer site or at other sites within the oral cavity or head and neck region. The majority will arise within the first two years after treatment of the primary cancer (Jones *et al.,* 1992; Lazar *et al.,* 1998).

In a paper by Jovanovic *et al.* (1994) women previously treated for SCC of the oral cavity or lip were found to be at a 74.7-fold increased risk for developing a SOM of the oral cavity or pharynx and men were at a 190.4 fold increased risk. Similarly, Saikawa *et al.* (1991) found a 79.5-fold increased risk of a SOM for their group of oral cavity and lip cancer patients. The risk was highest within the first year it but stayed fairly constant from the second to 14^{th} year of follow-up. Since a SOM, both at the same site or at a second site, can occur years after the curative treatment of the primary tumour it is extremely important to closely monitor previously treated oral cancer patients for a long period of time (Saikawa, *et al.,* 1991; Franco, Kowalski and Kanda; 1991, Day and Blot, 1992; Bouquot and Whitaker; 1994, van der Tol *et al.,* 1999). Follow-up also allows the clinician to deal with any other patient concerns regarding the sequelae of their initial treatment.

I.5.2.1. Incidence of SOM occurring at the site of the previous oral SCC

The rate of SOM at the same site (recurrence) is approximately 15 – 30% (van der Toorn, 2001). It is commonly believed that the majority of recurrences will occur within the first 2 years following treatment of the primary tumour (Hirata *et al.,* 1975; Whitehurst and Droulias, 1977; Boysen *et al.*, 1985; Scholl et al., 1986; Hoffman *et al.,* 1998). Vikram *et al.* (1984) report that recurrence is the main cause of morbidity and mortality for head and neck cancer patients.

Varying rates of local recurrence amongst studies may be partially due to some authors combining local and regional recurrences in their analysis and the varying lengths of follow-up. Table 1 shows the recurrent rates of a variety of studies (13% - 37%) completed over the last 30 years. In 1976, Shah *et al.* reported that more than half of 758 patients in their study suffered either a local (at the primary site) and/or regional (the neck) recurrence. Antoniades *et al.* (2003) found a 55% 3 year locoregional recurrence rate in patients with primary tumours of the anterior faucial pillar – retromolar trigone. Half of the recurrences occurred within the first year.

There have been two theories suggested to explain the high rate of SOM: the field cancerization theory and the cell migration theory.

Table 1. SOM at the same site

	# of oral cancer patients (study total)	Site	Local (L) or Locoregional (R) recurrence [a]	Maximum time followed (years)	Recurrence (%)
Whitehurst and Droulias, 1977	137	tongue	L	30	10.9
Vikram *et al.*, 1984	32 (114)	oral	L	2	19
Hong *et al.*, 1985	103 [b]	Head and neck	R	>2	18 [c]
Boysen *et al.*, 1985	157	Head and neck	L	5	34
Spiro *et al.*, 1986	105	tongue and FOM [d]	L	2	13
Scholl *et al.*, 1986	268	tongue	L	10	16
Loree and Strong, 1990	129 [e]	oral	L	4	36
	269 [f]	oral	L	4	18
Hong *et al.*, 1990	100	Head and neck	L	median 32 months	11
Jones *et al.*, 1992	49	oral	L	2.8	25

	# of oral cancer patients (study total)	Site	Local (L) or Locoregional (R) recurrence [a]	Maximum time followed (years)	Recurrence (%)
Leemans *et al.*, 1994	116 (244)	Head and neck	L	>5	12
Woolgar *et al.*, 1995	123	oral	L	5	13
Hicks *et al.*, 1997	96	FOM [d]	L	20	34 [e]
					13 [f]
Brennan *et al.*, 1995	25	Head and neck	L	2.25	20
Lazar *et al.*, 1998	52	Head and neck	R	Mean 24 months	37
Woolgar *et al.*, 1999	200	oral	L	8	19
Gonzalez-Moles *et al.*, 2002	81	tongue	L	7	23
de Visscher *et al.*, 2002	72	lip	L	9	3
Eckhardt *et al.*, 2004	1000	Head and neck	L	7	19.8

[a] Local-site of primary tumour, locoregional-site of primary tumour or regional lymph nodes of the neck
[b] Stage III and IV SCC
[c] Estimated 5 year recurrence rate was 39%
[d] Floor of mouth (FOM)
[e] Positive surgical margins
[f] Negative surgical margins

I.5.3. Field cancerization and cell migration theory for SOM

I.5.3.1. Field cancerization theory for SOM

In 1953, Slaughter, Southwick and Smejkal published their theory of field cancerization. Field cancerization attributes the high rate of second primary cancers to the exposure of the entire oral cavity (and upper aerodigestive tract) to the same carcinogens (e.g. tobacco) therefore putting all sites at risk for the development of independent oral premalignant lesions and cancers. Hence, precancerous changes could extend far beyond the clinically visible border of the lesion and that areas of premalignant change may occur all over the oral mucosa and the upper aerodigestive tract (Liu *et al.,* 2000). Support for this theory is found when multiple foci of disease within one resection specimen occur or when clinically normal mucosa separates multiple tumours and/or OPLs. Lummerman, Freedman and Kerpel (1995) found evidence to support field cancerization with the presence of 'skip areas' in the dysplastic epithelium with intervening areas of normal or hyperplastic tissue. Thomson *et al.* (2002) performed biopsies on the clinically normal contralateral sites of 26 patients with oral SCC or dysplasia and found that 15 had histologic abnormalities, including dysplasia, carcinoma *in situ (CIS)* and SCC, in clinically normal tissue. All patients had a history of smoking

and drinking. The floor of the mouth (FOM) and lateral and ventral tongue were more prone to dysplastic changes.

I.5.3.2. Cell migration theory for SOM

A second theory proposed by Bedi *et al.* (1996), is premalignant cell migration. In this case, cells detach themselves from a premalignant lesion and migrate to another site where they develop into a tumour. Migration may occur through the tissue or saliva. The new tumour shares a common origin with the initial tumour but develops independently and is separated by a histologically normal field (Braakhuis *et al.,* 2002). Califano *et al.* (2000) further supports this by claiming that clonal outgrowths may travel several centimetres and that this movement of altered clonal populations may involve a significant amount of the mucosa before a malignancy appears.

I.5.4. Factors of primary oral SCC affecting the incidence of SOM

There have been a variety of clinicopathological and primary tumours characteristics that have been studied for their ability to predict a SOM. The status of the primary tumour margins at treatment, the treatment itself, and higher TNM stage (Spiro *et al.*, 1986; Loree and Strong, 1990; Gonzalez-Moles *et al.*, 2002) have all been found by some authors to be associated with a SOM (Shah *et al.*, 1976). Hong *et al.* (1985) found significant associations between

recurrence and the site of the primary tumour, primary treatment type, and advanced nodal involvement. Patients who received radiation alone or in combination with chemotherapy were found to have a higher rate of recurrence than patients who received surgery (Carvalho, Margin and Kowalski, 2003). Tumour stage also significantly affects the rate of recurrence (Leemans et al., 1994). Antoniades et al. (2003) found more than 60% of patients with stages III and IV tumours suffered a recurrence while none of the stage I and II patients they followed had a recurrence.

Complete resection of an oral squamous cell carcinoma is the most important factor in predicting a patient's chances of survival since failure to fully remove the lesion is thought to be the leading cause of oral cancer death (Brennan, *et al.,* 1995). Currently, the gold standard for determining the complete excision of a tumour is the histopathological evaluation of the margins. Jones et al. (1992) found an almost 3 fold increased risk of recurrence in patients with positive margins (CIS or SCC). In a study by Loree and Strong (1990) patients with positive margins had twice as many recurrences as patients with negative margins. Weijers *et al.* (2002) found patients with low-grade dysplasia in the surgical margins had a significantly higher rate of recurrence than patients with tumour margins free of dysplasia. While 1 cm is the generally accepted surgical margin for oral SCC, the actual margin excised may differ dependent on the site, the anatomic structure involved and projected patient morbidity. Bailey, Blanchaert and Ord (2001) suggest at least 5 mm of normal tissue, without any

evidence of SCC, *CIS* or dysplasia, is necessary for a successful surgical margin, while Kerawala *et al.* (2000) suggest that 1 cm may not be a large enough margin to minimize risk of recurrence. de Visscher et al. (2002) concluded that a 3 mm margin was adequate for the removal of early stage SCC of the lower lip with only a 3% recurrence rate in follow-up. Hicks et al. (1997) found a 13% rate of recurrence in patients with FOM tumours excised with at least 5 mm of histologically negative margins.

However, lesions may still recur at sites where the lesion margins were found to be free of dysplastic or malignant cells, possibly due to very small amount of abnormal cells too small to detect histopathologically or the presence of a precursor lesion at the margin of the resected tumour (van der Toorn, 2001). Gath and Brakenhoff (1999) refer to these undetectable tumour cells as 'minimal residual disease'.

The presence of genetic indicators of disease in surgical margins determined to be histologically free of disease is an area of interest for many researchers. Tabor et al. (2004) found evidence of LOH and p53 mutations in the primary tumour margins in 8 of 13 patients who suffered a recurrence. Both Brennan *et al.* (1995) and Partridge *et al.* (2000) found evidence of p53 mutations in histologically clear tumour margins. In the former study, 5 of 13 patients with p53 mutations in the margin suffered a recurrence while none of the patients with negative margins suffered a recurrence.

I.5.5. Survival of patients with SOM at the previous oral SCC site

The development of a SOM at the former oral SCC site greatly affects the overall survival rate. The survival rate of patients with recurrent tumours has been associated with the TNM stage of the recurrent tumour, weight loss, the presence of muscle invasion, tumour site, positive margins and lymph node involvement (Loree and Strong, 1990; Woolgar *et al.*, 1995; Yueh *et al.*, 1998; Woolgar *et al*, 1999; Nyugen *et al.*, 2002). Lacy, Spitznagel and Piccirillo (1999) found survival was affected by the initial tumour TNM stage, tumour grade, initial treatment and the time to, extent of, and treatment of the recurrence. They found that patients who had radiation either alone or in combination with surgery had a worse survival rate than patients treated by surgery alone. This is not surprising since radiation is usually used in conjunction with surgery for high grade tumours or tumours not amenable to surgery. The median survival time for patients with a SOM in Yueh *et al.* (1998) was only 10 months with a one-year survival rate of 54%. Lacy, Spitznagel and Piccirillo (1999) found a 20% 2-year survival rate for patients with a recurrent head and neck cancer.

Nyugen et al. (2002) concluded that weight loss and a history of radiation were stronger predictors of survival prognosis in patients with SOM than TNM staging. The authors found an overall survival rate of 38% following treatment of a SOM.

The best rate was for patients who experienced no weight loss and fell to 12% for those who lost more than 10% of their body weight. TNM staging was not found to be predictive of mortality in patients with recurrent disease in this study.

I.5.6. Treatment of SOM

The treatment of recurrent oral cancer is a dilemma. Surgery is generally the first choice particularly if the recurrence is caught early. Additional surgery, however, will be affected by the sequelae of the primary treatment, the health of the patient and the site and extent of the recurrent tumour involvement (Wong *et al.*, 2003; Eckardt *et al.,* 2003). Patients with a history of radiation may not be able to tolerate more radiation and their tissue, as a result of the primary radiation, may not have the capacity to adequately heal after surgery due to reduced vascularity. In a study of treatment of recurrent head and neck cancer, Wong *et al.* (2003) found none of the patients who received treatment other than surgery survived 5 years. Patients who received radiation and chemotherapy had a mean survival time of 7 months – 2 months longer than patients who received only supportive care. Eckardt *et al.* (2003) had similar results as patients who received surgery only for the treatment of their recurrent tumour had a higher 5-year survival rate than patients who received other modes of therapy.

I.6. Oral Premalignant Lesions (OPLs)

Oral squamous cell carcinomas (SCC) are often preceded by clinically visible premalignant changes (Neville and Day, 2002). A premalignant lesion is defined by the WHO (1978) as "a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart". Oral premalignant lesions often present as a leukoplakia or white patch that cannot be scraped off, erythroplakia or red patch, or a combination of both, known as erythroleukoplakia. Many authors consider the terms leukoplakia and OPL to be interchangeable.

I.6.1. Histopathology of OPLs

I.6.1.1. Hyperplasia

Hyperplasia is a term used to describe thickening of the epithelium either by thickening of the intermediate or prickle layer, known as acanthosis or by thickening of the keratinized layer, hyperkeratosis. Hyperplasia has no cellular or architectural changes. The majority of hyperplasias are the result of an injury or irritation and very few are premalignant, and it is impossible to predict which ones will progress to SCC.

I.6.1.2. Dysplasia

Currently a lesion is considered premalignant when it exhibits changes in the epithelium known as dysplasia (WHO, 1978). Dysplasia, a term meaning disordered or abnormal growth, is graded on numerous changes that can occur in the structure of the epithelium or in the individual cells themselves. These changes are listed in Table 2. Depending on the amount and severity of these changes the pathologist grades the dysplasia as mild, moderate, or severe. The existence and grade of dysplasia is the current gold standard for predicting the malignant risk of OPLs and its presence or absence as well as its severity should always be included on the pathology report (Axéll *et al.,* 1984).

When the changes involve just the basal and parabasal cell layers the dysplasia is graded as mild. Moderate dysplasia occurs when the changes involve half of the cell layers. When two thirds of the cell layers are altered the grade is severe dysplasia and when the cell and architectural changes comprise the whole width of the epithelial layer it is graded as carcinoma *in situ*. Once the changes break through the basement membrane the lesion is considered an invasive cancer.

Table 2. Histological signs of dysplasia [a]

Cellular changes
— loss of polarity of the basal cells — an increased nuclear-cytoplasmic ratio — increased number of mitotic figures — the presence of mitotic figures in the superficial half of the epithelium — cellular pleomorphism — nuclear hyperchromatism — enlarged nucleoli — reduction of cellular cohesion — keratinization of single cells or cell groups in the prickle (intermediate) layer
Architectural changes
— drop-shaped rete processes (bulbous rete pegs) — irregular epithelial stratification — the presence of more than one layer of cells having a basaloid appearance (basal cell hyperplasia)

[a] WHO, 1978

I.6.1.3. Difficulty in diagnosing and grading dysplasia

While histological criteria is a poor cancer risk predictor for low-grade dysplasia, the diagnosis and grading of dysplasia is also difficult and considered to be highly subjective with limited inter-examiner agreement. For example, Abbey *et al.* (1998) compared six oral and maxillofacial pathologists' diagnoses of 120 slides of hyperplasia and dysplasia to a 'gold standard' diagnosis. The authors were interested in determining how adjunctive clinical information (age, gender,

race, lesion site, clinical appearance, size and duration of lesion, smoking history and history of a previous cancer or dysplasia) affected the diagnoses. Only 38.5% of the diagnoses were in exact agreement with the 'gold standard' while 85.4% were within one histological grade. There was a 71.4% agreement on the presence or absence of dysplasia. These results were lower than a previous study involving the same six pathologists and the same slides but without clinical information. The authors concluded that clinical data did not improve accuracy in the diagnosis of oral dysplasia.

I.6.2. Clinical Presentation and OPLs

I.6.2.1. Concept of Leukoplakia

OPLs present most frequently as leukoplakia and occasionally as erythroplakia. Leukoplakia accounts for 85% of oral premalignant lesions (Bouquot and Gorlin, 1986; Bouquot and Whitaker, 1994). Leukoplakia is a definition of exclusion, defined by the World Health Organization (1978) as a "white patch or plaque that cannot be characterized clinically or pathologically as any other disease". This definition was amended in 1984 by Axéll, *et al.,* to include "it is not associated with any physical or chemical causative agent except the use of tobacco." The term, leukoplakia, is most commonly used as a clinical term with no reference to histological factors. In fact, Bouquot and Whitaker (1994) claim

that it is no longer acceptable to presume that microscopic evidence is necessary. Axéll *et al.* (1996) again revised the WHO's definition of leukoplakia to "A predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion; some oral leukoplakia will transform into cancer". The white appearance of the lesion is due to the accompanying epithelial hyperplasia, i.e., hyperkeratinization or thickening of the stratum corneum and/or acanthosis, a thickening of the intermediate layer of the epithelium.

Erythroplakia, similarly, is a red patch that "cannot be characterized clinically or pathologically as being due to any other condition" (WHO, 1978) and its clinical presentation can vary. The red appearance of erythroplakia is due to thinning of the epithelium, allowing the underlying vascular tissue to be more visible.

The clinical presentation of OPLs can vary in colour, size, appearance, texture and margin presentation. Appearance can be homogeneous or non-homogeneous. A homogeneous leukoplakia refers to a leukoplakia that is uniform in both colour (generally white or white-yellow) and in texture (generally smooth or corrugated surface) (Axéll *et al.*, 1984). A non-homogeneous appearance refers to an OPL that is not uniform in either colour (mixed red and white) and/or texture (nodular or verrucous) (Axéll *et al.*, 1984). Lesion margins vary from extremely discrete to very diffuse. OPLs can be a single lesion or multifocal in presentation. When a patient presents with a leukoplakia all attempts to remove possible etiological factors should be made and the patient

should be followed up in 2 to 3 weeks. If the lesion is still present at follow-up a biopsy should be conducted to determine a definitive diagnosis.

A variant of oral leukoplakia is proliferative verrucous leukoplakia (PVL). PVL are not often associated with tobacco use and have a high rate of malignant transformation (Lummerman, Freedman and Kerpel, 1995; Greenspan and Jordan, 2004). These lesions commonly recur after treatment. The lesion is considered persistent and progressive, albeit slow growing. The progression model for PVL begins with a flat leukoplakia that becomes an exophytic verrucous lesion termed verrucous hyperplasia. As the lesion progresses it becomes an exophytic and endophytic verrucous lesion called verrucous carcinoma which could further progress to SCC. PVL is a clinical term and refers to those patients with verrucous lesions involving multiple oral sites or a large diffuse area. Histologically, these lesions then could present at any stage along the continuum from hyperkeratosis without obvious dysplasia, verrucous hyperplasia with or without dysplasia, verrucous carcinoma or SCC. PVL is found more often in older women. In a study by Bagán *et al.* (2004) 63% of patients with PVL went on to develop SCC, with more than half of the SCC patients developing more than one oral SCC. The most common site for a PVL in this study was the gingiva and palate, in contrast to conventional SCC which is most commonly found on the ventrolateral tongue and floor of mouth. Batsakis, Suarez and El-Naggar (1999) consider VH to an irreversible predecessor of VC or SCC.

It should be noted that not all malignant lesions are preceded by a clinically visible premalignant lesion such as leukoplakia or erythroplakia.

I.6.2.2. Prevalence of leukoplakia

The prevalence of leukoplakia varies from study to study, from 0.7% to 24.8% (Axéll *et al.,* 1984) dependent on the definition used, geographical location of the study and whether biopsies were taken to support the clinical diagnosis. For example, in a study by Bouquot and Gorlin (1986), the prevalence rate for leukoplakia in white Americans over the age of 35 was just below 3%. In a survey of more than 20,000 Swedish adults the prevalence rate for any white lesion was 24.8% (Axéll, 1987). However, when just leukoplakia and 'preleukoplakia' were included the prevalence rate was 8.5%. In a study by Bokor-Bratić (2000) the author found the prevalence of leukoplakia amongst nearly 2400 patients who visited the university dental clinic in Yugoslavia to be 2.2%. Bánóczy and Rigó (1991) screened 7820 Hungarian patients resulting in a 1.3% prevalence rate.

The most common site for leukoplakia was the buccal mucosa (Bánóczy and Sugár, 1972; Silverman *et al.,* 1976; Axell *et al.*, 1984; Bouquot and Gorlin, 1986; Jaber *et al.,* 2003). The most common site in Bánóczy and Rigó (1991)

was on the tongue followed by the buccal mucosa. It should be noted that the buccal mucosa is not a common site for dysplasia or cancer and many of these lesions are reactive hyperplasia.

A large epidemiological study associated with the National Health and Nutrition Examination Survey (NHANES III) found an overall weighted prevalence of oral leukoplakia in the US of 0.42 ± 0.08%. Males had a weighted estimate of prevalence of approximately 3 times that of females. The authors found OPLs in only 65 of 16,128 study participants with the majority of the lesions on the gingiva and buccal mucosa (Scheifele, Reichart and Dietrich, 2003). In a review of 23 studies, Petti (2003) estimated that the true global prevalence of leukoplakia is approximately 1.7% - 2.7%. From this he estimated the number of oral cancers from leukoplakia were in the range of 6.2 - 29.1 per 100,000. The only demographic characteristic found to have prevalence was male gender. This supported a large review by Waldron and Shafer (1975) which found leukoplakia slightly more common in males and occurred mainly in the 5th, 6th and 7th decades of life. Bánóczy and Sugár (1972) also found that leukoplakia was more prevalent in the sixth decade of life as did Lummerman, Freedman and Kerpel (1995). Bánóczy *et al.* (1992) found a higher prevalence of leukoplakia in diabetics, especially insulin-dependent diabetics versus healthy controls (6.2% versus 2.2%). The prevalence of leukoplakia in diabetic smokers was notably high at 11.2% in the studied population.

I.6.2.3. Erythroplakia

Erythroplakia generally has a higher risk of malignant transformation and a more severe histological diagnosis. Mashberg (2000) states that the presence of a persistent red area within a lesion is the most significant sign of *CIS* or SCC and the red aspect of the lesion is the area that will have the most cellular change. Mashberg argues that since erythroplakia (or erythroplasia, as he prefers) is associated with a high rate of CIS and SCC it should be considered a cancerous change and not a precancer.

I.6.3. Rate of malignant transformation for OPLs

The progression of a premalignant lesion to cancer, known as malignant transformation, is the result of accumulated genetic damage over time (as reviewed in Silverman and Sugerman, 2000). Malignant transformation rates vary between various studies due to differences in each population's risk factors, for example, differing strengths of tobacco and varying impurities in alcohol in different communities (Shiu *et al.,* 2000). The difficulty in determining an exact malignant transformation rate for OPLs is also influenced by the varying management methods of OPLs, the selection criteria, the different lengths of follow-up time (Silverman, Gorsky and Lozada, 1984) disease definitions, diagnostic criteria and treatment imposed and the presence or absence of

dysplasia (Lind, 1987; Lee *et al.,* 2000; Shah, *et al.,* 2003). The rate of malignant transformation amongst OPLs is also associated with the clinical characteristics and histopathology of the lesion. Table 3 displays the range in malignant transformation rates for OPLs across a variety of studies. Schepman *et al.* (1998) claimed that the accepted rate of malignant transformation of leukoplakia is 5% over an average of 5 years. The WHO (1978) published a 3 – 6% malignant transformation rate for leukoplakia, regardless of the presence of dysplasia. Lind (1987) followed patients with leukoplakia for up to 16 years. The rate of malignant transformation for this group was 8.9%, while dysplasia developed in another 31.8%.

Silverman, Gorsky and Lozada (1984) followed patients with a one centimetre or larger leukoplakia, the majority diagnosed as benign hyperkeratosis. Seventeen and a half percent developed into SCC over an average follow-up time of eight years. Interestingly, almost one third of the OPLs excised recurred. Tradati *et al.* (1997) claim that 10 - 15% of leukoplakia will eventually progress to cancer if left untreated. Bánóczy and Sugár (1972) followed patients with leukoplakia an average of almost 9 years and found that SCC developed in only 5.9% of the patients.

Silverman *et al.* (1976) followed histologically benign leukoplakia in almost 7000 patients for only 2 years, which resulted in a 0.13% malignant transformation rate.

Table 3. Malignant transformation rates for OPL

Author(s)		Country	# of patients	maximum length of follow-up (years)	Malignant transformation rate (%)
Pindborg *et al.*, 1968		Denmark	248	10	4.4
Silverman and Rozen, 1968		USA	117	11	6
Banoczy and Sugar, 1972		Hungary	520	8.7	5.9
Silverman, *et al.*, 1976		India	4762	2	0.1
Banoczy 1977		Hungary	670	30	6
Silverman, *et al.*, 1984		USA	257	8	17.5
Schepman *et al.*, 1998 [a]		Netherlands	59	17.4	5
Gupta, 1980 [b]	Ernakulam district	India	410	10	2.2
	Bhavnagar district		360	10	0.3
Lind, 1987		Norway	157	16	8.9
Hogewind, van der kwast and van der Waal, 1989		Netherlands	84	8	3.6
Shiu *et al.*, 2000		Taiwan	435	10	13.8

[a] No late stage dysplasia. Part of a study of 166 patients.
[b] Population based study

I.6.4. Factors influencing malignant transformation of OPLs

Many studies have investigated factors that influence the malignant transformation of OPLs. Most, if not all, are done on primary OPLs. The following information is mainly from primary OPLs.

I.6.4.1. Dysplasia and malignant transformation of OPLs

Currently, the prediction of the malignant transformation rate of OPLs is based primarily on histopathological factors. The current gold standard to determine risk is the grade of dysplasia found in a histological sample. Simply, the risk of malignant transformation increases with the severity of the grade of dysplasia (see Figure 1). For example, severe dysplasia is considered to be at a greater risk of progressing to SCC versus low-grade lesions (mild and moderate dysplasias) and because of this belief severe dysplasia is treated more aggressively. Lee *et al.* (2000) found the cancer risk of lesions with a histological diagnosis of moderate or severe dysplasia was 2.3 times higher than the risk of OPLs with a diagnosis of mild dysplasia or hyperplasia. Shepman *et al.* (1998) also discovered a greater progression risk in lesions with a diagnosis of moderate or severe dysplasia. In their research, Silverman, Gorsky and Lozada (1984) found more than one third of lesions with dysplasia eventually progressed to cancer and that a very high rate of progression also existed for lesions with a "verrucoid hyperplasic pattern". Table 4 shows the malignant transformation rates of OPLs with a diagnosis of dysplasia. Lummerman, Freedman and Kerpel (1995) reported a 16% malignant transformation rate for OPLs with dysplasia, 20% if the dysplasia progressed to at least *CIS*. The mean transformation time was less than 3 years. Silverman, Gorsky and Lozada (1984) found the greatest proportion of OPLs progressed to oral SCC in the second year of follow-up. The WHO (1978) states that any level of dysplasia, no matter how slight, at a high-

risk location, such as the floor of the mouth and ventral tongue, should be followed very closely.

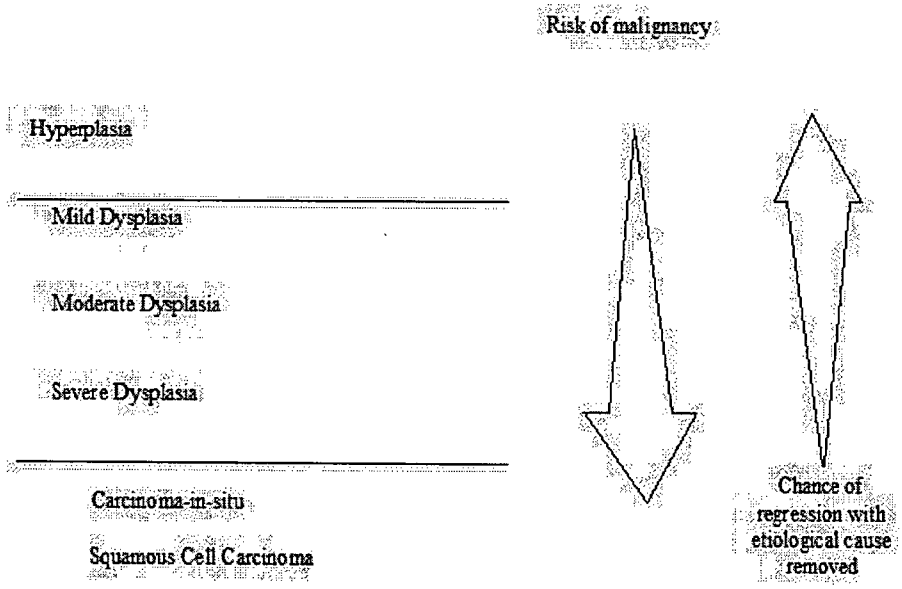


Figure 1. Histological progression model of oral premalignant and malignant lesions

Table 4. Malignant transformation rate for late stage OPL (dysplasia)

Author(s)	Country	# of patients	maximum length of follow-up (years)	Malignant transformation rate (%)
Mincer *et al.*, 1972	USA	56	8	11.1
Silverman *et al.*, 1976	USA	4762	2	7
Bánóczy and Csiba, 1976	Hungary	120	6.3	24
Bánóczy 1977	Hungary	68	20	13.2
Silverman, *et al.*, 1984	USA	22	8.1	36
Lummerman *et al.*, 1995	USA	44	9.4	16
Schepman *et al.*, 1998 [a]	Netherlands	47	17	23
Lee et al., 2000 [b]	USA	70	7	31

[a] Dysplasia group is part of a larger study of 166 patients with leukoplakia.
[b] Includes some former cancer patients

I.6.4.2. Clinical features and malignant transformation of OPLs

Leukoplakia at high-risk sites such as the ventrolateral tongue, floor of the mouth and soft palate (sites that normally have either no or a thin keratinized layer) is associated with an increased risk of progression (Waldron and Shafer, 1975). Other clinical factors such as large size, long duration, non-homogeneous appearance, colour, history of cancer and absence of a high-risk habit have all been found to be associated with an increased cancer risk (Axéll *et al.*, 1984; Bouquot and Whitaker, 1994; Lummerman, Freedman and Kerpel, 1995;

Schepman *et al.,* 1998; Shiu, *et al.,* 2000). Table 5 shows seven features that van der Waal *et al.* (1997) allege increase the risk of malignant transformation.

Sites with the highest rate of malignancy in Bouquot and Gorlin's (1986) study were the tongue, lip and floor of mouth. Küffer and Lombardi (2002) added the soft palate, vermilion border of the lower lip, retromolar pad and adjacent zone of buccal mucosa to sites that are at a high-risk for progression to oral SCC. Bánóczy and Sugár (1972) found malignant transformation was higher for sites on the lateral and base of tongue (~ 40%), females, erosive lesions, the length of time the lesion had been present and tobacco and alcohol use. Both Hogewind, van der Kwast and van der Waal (1989) and Schepman *et al.* (1998) also found a higher rate of progression in females, particularly non-smoking females.

In the aforementioned study by Silverman, Gorsky and Lozada (1984) lesions presenting with a red colour and/or an erosive texture had a four fold increased risk of progressing to cancer. Of interest, almost one quarter of the nonsmokers had lesions that progressed to cancer compared to 16% of the current smokers and 12% of the former smokers. The authors concluded that since there was no known etiological reason for the lesion, nonsmokers with an OPL were at greater risk of malignant transformation.

Jaber *et al.* (2003) followed a large group of patients with dysplasia. The majority of the lesions with a diagnosis of mild dysplasia were found on the buccal mucosa while the severely dysplastic lesions were more likely to found on the floor of the mouth or the lateral border of the tongue. The majority of the white lesions were diagnosed as mildly dysplastic while more than one half of the red or speckled lesions were diagnosed as moderate or severely dysplastic.

Mashberg, Morrissey and Garfinkel (1973) reviewed the clinical signs of asymptomatic early SCC and CIS. More than 90% had a red component to the lesion, which rose to 95% if lesions of the lip were excluded. A white colour was found in more than 60% but only 2.5% were completely white. Excluding lesions on the lip almost 99% of the lesions were found at purported high-risk sites and a majority had a granular or rough texture.

Table 5. Features alleged to associated with increased risk of malignant transformation [a]

Risk factors	Comment
Gender	- particularly female
Long duration of the lesion	
Idiopathic leukoplakia (no known risk factors)	
Location on the floor of mouth (FOM) or tongue [b]	
Non-homogeneous appearance	
Presence of *Candida Albicans*	
Presence of dysplasia	- carries a five fold greater risk than a non-dysplastic lesion - the most important factor

[a] van der Waal *et al.,* 1997
[b] Axell, *et al.,* 1984

I.6.5. Problems with clinicopathological predictors of OPLs

A clinical exam is important to identify lesions that may progress to cancer and whose early removal may prevent cancer occurrence or recurrence. Unfortunately, there are no consistently reliable clinical or histopathological factors to allow the clinician to reliably distinguish clinically between benign and potentially malignant lesions (Tradati *et al.,* 1997).

I.6.5.1. Problems with the histological risk predictors

As mentioned earlier the risk of progression to oral SCC increases with the severity of the histological diagnosis. The majority of OPLs in Lummerman, Freedman and Kerpel's study (1995) were diagnosed with mild dysplasia and 16% still progressed to SCC. Although the majority of OPLs with low-grade dysplasia will not progress to SCC, some, nevertheless, will develop into cancer and it is impossible to determine, based on histology alone, which of this large group of lesions will become cancer. In fact, Lind (1987) feels that the grade of dysplasia is an unreliable prognostic indicator of cancer development.

At the other end of the spectrum, Mashberg (2000) suggests that it is likely that a lesion exhibiting severe dysplasia may have areas of undetected *CIS* and that multiple biopsies are necessary, particularly considering the subjective aspect of histological diagnosis at this stage.

I.6.5.2. Problems with the clinical risk predictors

Although some clinical risk signs are associated with an increased risk of malignant progression, OPLs without evidence of dysplasia at low risk sites with a benign appearance and texture can still progress to SCC.

I.6.5.3. Problems in predicting SOM at sites of previous oral SCC

As previously reported the risk of SOM at sites previously treated for SCC is high and the rate of survival decreases substantially with the development of a recurrence or SPT. The resulting complications from treatment including scar tissue, grafts and/or late radiation changes to the tissue present a very complex problem to the attending clinician. Not only could the effects of treatment mask signs of a new lesion but the clinician may be reluctant to biopsy fragile tissue. It is the objective of this thesis to determine if any clinical signs aid in the prediction of a SOM at the previously treated cancer site.

I.6.6. Staging system for OPLs

Many authors have proposed alternative staging systems for OPLs in the hope that a more uniform reporting of treatment/management results will emerge as well as improve the consistency between pathologists in the grading of samples. Schepman and van der Waal (1995) proposed a new staging system for leukoplakia. This system is based on the cumulative malignant potential of etiological, topographical, clinical and histological risk factors, such as high-risk sites, appearance (non-homogeneous) and the presence of dysplasia. The system stages a leukoplakia according to four variables: size (L), site (S), clinical aspect (C), and histopathology (P). Since histopathology is the current 'gold standard' for malignant potential, leukoplakia with a moderate or severe

dysplasia is automatically classified as stage 4 (the highest stage). A non-homogeneous appearance combined with a high-risk site is labelled as stage 3. In a later paper by this research group stage 4 OPLs were found to have an increased rate of malignant transformation versus stages 1 - 3 (Shepman *et al.*, 1998). van der Waal, Schepman and van der Meij (2000) further modified this system to include just two categories: size and presence of dysplasia. The pathology of the sample is graded as 'no or perhaps mild dysplasia' or 'mild to moderate dysplasia' or 'moderate to severe dysplasia'. Severe dysplasia and *CIS* were considered to be the same. The presence of dysplasia and a size greater than 4 cm led to a higher staged OPL.

Küffer and Lombardi (2002) also made an attempt to introduce a change in terminology into the histological diagnosis of oral precancerous lesions. The authors would like to see lesions that do not show any sign of dysplasia referred to as 'risk lesions' and lesions with dysplasia should be termed 'precursors' of SCC. The authors would also like to eliminate the confusion between the diagnosis of severe dysplasia and *CIS* by using the oral intraepithelial neoplasia staging system (OIN). OIN1 would be the equivalent of mild dysplasia, OIN2 would be the equivalent for moderate dysplasia and OIN3 would be used for severe dysplasia and *CIS*. They later simplified this system by reducing the three grades to two: Low grade OIN (LOIN) is the same as OIN1 and high grade OIN (HOIN) would include both OIN2 and OIN3 (moderate dysplasia through *CIS*).

van der Waal and Axéll (2002) made a third revision of their leukoplakia staging system. In this system the authors use the OIN classification system mentioned above. This system includes only two grades. Grade 0 signifies no or possibly mild dysplasia (OIN-0), grade 1 is the equivalent of OIN1 and OIN2. Again, severe dysplasia and *CIS* were considered synonymous and considered too advanced to be included in the leukoplakia grading system. The authors also advocate treatment for grade 1 or higher dysplasia.

Rosin, Zhang and Poh (2003) proposed a 3 level staging system for OPLs based on pathology and genetic risk. Stage 1 would include lesions that were low risk histologically as well as genetically. Stage 2 would include lesions with no greater than a moderate dysplasia or intermediate genetic risk, while stage 3 would include any lesion with either a severe dysplasia and/or a high-risk genetic pattern.

I.6.7. Treatment of OPLs

The treatment of OPLs is controversial, in particular how and when to treat low-grade dysplasia. Further complicating matters, it is not uncommon for low grade OPLs to regress on their own without any active intervention (Silverman, Gorsky and Lozada, 1984). The primary reason to treat OPLs is the belief that their early recognition and treatment may well thwart the lesions progression to SCC (Pandey *et al.,* 2001) particularly those lesions at a high-risk site with a diagnosis

of dysplasia. Conventional and laser surgeries are the current treatments of choice for OPLs. There is some question that the use of laser on low grade dysplasias may be over treatment since only a small percentage of low grade dysplasia will progress to SCC and that the treatment itself could promote change within the cells possibly leading to lesion progression. However, some researchers such as Silverman, Gorsky and Lozada (1984) and Lummerman, Freedman and Kerpel (1995) claim it is necessary to completely remove all lesions with any level of dysplasia in order to reduce the number of cases that progress to cancer. Conversely, Ephros (1997) feels that the complete excision of all lesions precludes the researchers' ability to monitor the progression of the lesion. Mashberg (2000) recommends surveillance of mild dysplasia, conservative excision of moderate dysplasia and complete excision of severe dysplasia. With the recent advances in molecular markers and risk of progression some authors have based their treatment decisions on the genetic data. Partridge *et al.* (2001), recommend the excision of any lesion with LOH at 2 or more loci. Sudbø, Lippman *et al.* (2004) recommends the excision of all aneuploid dysplasia, even though they found these lesions had a very high rate of recurrence and progression, excised or not. Some authors (Damm, 2004; Sollecito and Alawi, 2004) carry Sudbø's results one step further suggesting that all dysplastic lesions be excised, regardless of ploidy status. Other concerns regarding the treatment of OPLs include the difficulty encountered by pathologists in accurately diagnosing the extent of the histopathology of a

sample received after laser surgery due to the effects of the laser on the lesion margins.

The width of a dysplasia free margin to remove around an OPL is also a topic of debate. As previously mentioned, the accepted margin width when possible in the excision of oral SCC is 1 cm. There is no accepted margin width for OPL with dysplasia. Mao (2000) stated that the genetic lesion is usually larger than both the histopathological lesion and the clinical lesion and the boundaries between normal epithelium and the edge of the genetic lesion are difficult to define. Simply excising the clinical OPL is not apt to cure the disease as the clinically invisible genetic lesion may be spread far out into the surrounding tissue. Molecular testing of the resection margins, which can be minimal in the removal of OPL, particularly when the lesion is large and diffuse, may help reduce the rate of OPL recurrence and malignant transformation (Lippman and Hong, 2001).

Various chemotherapeutic agents have been attempted including bleomycin and vitamin A. However, the success of the chemotherapeutics has been limited and does not appear to correct the genetic damage to the tissue. Often once the chemotherapeutic agent has been withdrawn the OPL recur clinically (Scully, 1995). A recent article by Singh *et al.* (2004) promotes the use of lycopene, the chemical that makes tomatoes red, as a chemotherapeutic in the treatment of leukoplakia. They found that the ingestion of oral lycopene significantly reduced

the clinical size and histological diagnosis of the lesions versus a placebo, without side effects or toxicity.

OPL recurrence is a great concern. Not only for fear of continued progression but the effects of treatment, such as a scar, may make the site more difficult to assess. In a study by Pandey *et al.* (2001) non-homogeneous leukoplakias were excised surgically without mention of the width of the surgical margins. Of the 59 original lesions, 40 were diagnosed with dysplasia. Six lesions recurred (histology unknown) after a minimum follow-up of twelve months and 3 patients developed new lesions at another site. The importance of combining the surgical excision with tobacco cessation therapy as a means to improve outcome was also discussed. Following the laser excision of leukoplakia Ishii, Fujita and Komori (2003) found a leukoplakia recurrence rate of 29.3% and a malignant transformation rate of 1.2%. No mention of the histological diagnoses of the lesions was made. Similarly, Chiesa *et al.* (1993) completed a retrospective study of 167 patients with a history of leukoplakia treated with CO_2 laser. Pre-treatment histological diagnosis was unknown but post-operatively all were found to be dysplasia free. Within five years of the treatment there were 31 local relapses, 27 new lesions and five SCCs. Overall seven SCC developed. The authors concluded that of all demographic and clinical factors recorded only age of leukoplakia onset and size of leukoplakia were found to be significant prognostic factors. Due to the high recurrence rate of OPL it is very important to follow treated patients closely for any signs of the lesion recurring (Bouquot and

Whitaker, 1994) or progressing (Hogewind, van der kwast and van der Waal, 1989).

I.7. Toluidine Blue as an adjunctive diagnostic tool

The introduction of adjunctive diagnostic tools such as the prostate specific antigen test, Pap smear and mammography have led to the earlier detection of prostate, cervical and breast cancers, respectively (Patton, 2003). One of the more common adjunctive tools available for the clinical detection of oral SCC and OPL is toluidine blue (TB).

I.7.1. The Mechanism of Staining and the History of TB

Toluidine blue (TB) also known as Tolonium Chloride, is a metachromatic, acidophilic vital thiazine dye that is soluble in water and alcohol (Dunipace *et al.,* 1992) and is used by clinicians to help in the identification of primary SCC, SOMs and dysplasias. Thiazine is an organic compound made up of a ring of four carbons and two sulphur atoms.

TB was first used approximately 50 years ago as an anitheparin agent for certain types of bleeding disorders. During the 1960s TB began to be studied as an aid in the diagnosis of cervical (Richart, 1963) and oral cancer. Purported

mechanisms of action for TB include binding to the phosphate groups of the nucleic acids and a defective intercellular barrier in neoplastic tissue that allows the dye to penetrate down into the deeper cell layers where there is greater DNA and RNA content. TB has also been reported to bind to acidic tissue components such as sulphates, phosphates and carboxylates which all tend to found in high concentrations, along with DNA and RNA, in neoplastic tissue (Dunipace *et al.*, 1992). Herlin *et al.* (1983) attempted to study the mechanism of TB by staining samples of cancerous and normal squamous epithelium. Normal tissue may have a slight uptake in the superficial cell layer but the cancerous samples stained to a depth of 50 µm. Although the nuclei of the cancerous cells stained dark blue, the authors concluded that the main factor was the permeability of the tissue and membrane. A third possible mechanism of action is the dye is up taken by the mitochondria of malignant and premalignant cells. Mitochondria are the energy source of a cell and become more numerous and acquire a negative charge as the cell progresses along the cancer pathway. Although there is no published research on this mechanism as yet, the Zila pharmaceutical corporation has patented this methodology for its own brand of tolonium chloride (Burkett, 2003).

Mashberg (1983) claimed that there have been no reported reactions or side effects to the topical use of TB. However, Dunipace *et al.* (1992) found TB to have a mutagenic effect using the Ames test.

There are 2 methods for the application of TB: the direct method and the rinse. The direct method involves applying 1% TB solution directly to a gauze dried lesion with a cotton tip applicator, waiting for 30 seconds, wiping the stained area with a cotton tip applicator soaked in 1% acetic acid and finally, a water rinse. This method can be preceded with an initial acetic acid wipe of the lesion prior to TB application. The rinse method entails the patient swishing with 1% acetic acid for 20 seconds, followed by a 1% TB rinse for 20 seconds, another rinse of 1% acetic acid and finally, a water rinse. A stained lesion is considered positive when it maintains the intense dark blue colour after the final acetic acid stage. If the lesion stains weakly it is called 'equivocal', and if no stain remains on the lesion it is considered negative. Mashberg (1980) states that equivocal results should be "considered positive unless proven otherwise."

I.7.2. Uses of TB for Early Oral Cancer and OPL

One of the most difficult challenges for a clinician is deciding when and where to biopsy. Since the histopathology can vary throughout a lesion the importance of determining the site with the highest degree of pathology cannot be overstated. TB can assist the clinician in their decision particularly in large non-homogeneous clinical lesions or areas of field cancerization (Onofre *et al.,* 1995; Shedd *et al.,* 1965) by highlighting those areas with more cellular change. Silverman, Migliorati and Barbosa (1984) claim that the use of TB can accelerate the

clinicians' desire to biopsy and therefore lead to faster diagnosis and treatment. This may be particularly true in patients previously treated for oral cancer. It may be more difficult to assess these patients because of the sequelae of their primary treatment which may mask or mimic new clinical symptoms (Shedd *et al.,* 1965). It can, therefore, be very difficult to assess the group of patients that are at the greatest risk for recurrent disease (Epstein *et al.,* 1997).

TB also helps delineate diffuse and faint lesions and aids in the visualization of lesions that appear clinically normal (Pizer and Dubois, 1979; Epstein, Scully and Spinelli, 1992). TB has also been found to help find second primary lesions, satellite lesions and recurrences (Myers, 1970; Rosenberg and Cretin, 1989) and has been used in surgery to help visualize tumour margins, extensions and satellites at the time of treatment. (Shedd and Gaeta, 1971; Portugal *et al.,* 1996). In a large multicentre study, Epstein, Feldman *et al.* (2003) found that the use of a TB rinse was highly sensitive in the identification of a SOM (*CIS* or SCC) in patients with a previously treated upper aerodigestive tract carcinoma. TB was found to be much more sensitive than clinical examination alone. Ishii, Fujita and Komori (2003) recommend the use of TB prior to laser ablation of an OPL to help establish the lesion margins.

In one of the earliest studies of TB, Shedd *et al.* (1965) using the direct method of TB application, stained a group of patients with oral *CIS* or SCC. All patients retained the dye including two patients with recurrent disease and two patients

with persistent disease post radiation treatment. A second small group of patients with leukoplakia were stained with TB. Only one lesion with a diagnosis of moderate dysplasia stained positive while three patients with lesions considered to be TB negative were found to be malignant upon biopsy.
In a later paper by the same authors (1967) all lesions with a diagnosis of moderate or severe dysplasia, *CIS* and oral SCC stained positive while none of the controls retained the dye. An interesting outcome from this study was the conclusion by the authors that TB helped to discriminate between post radiation changes and disease recurrence.

In two papers Mashberg compared the direct application method with the rinse method. In a 1981 paper he compared cancer patients and patients with "non-malignant" lesions (atypia and benign). The direct application method had less false negatives (sensitivity) results but the rinse method had less false positive (specificity) results. However a very interesting result discovered with the rinse method was the detection of four SCC or *CIS* lesions that were not visible clinically, which led to the conclusion that the rinse method is better in the detection of asymptomatic early cancers. In results published in 1983, Mashberg again compared the two methods and found that the false negative and false positive rates for the rinse method improve when the patients are instructed to swish vigorously allowing the stain to contact the more posterior areas of the mouth.

Moyer, Taybos and Pelleu (1986) evaluated the use of both application methods with poor results. TB rinse was uses on patients with clinically normal appearing tissue as a means of screening for undetected OPL and oral SCC. Patients found to be TB positive returned 10 - 14 days later and were reassessed using the direct application method. Patients who were still TB positive were biopsied and none of the biopsies showed any signs of dysplasia or malignancy.

I.7.3. Sensitivity and Specificity of TB in detection of Early Oral Cancer

Over the last 40 years many studies have been done to determine the sensitivity and specificity of TB in the diagnosis of early oral cancer that may not be distinguishable clinically from reactive lesions or easily visible. Sensitivity (false negatives) relates to how well the dye diagnoses all true disease, while specificity (false positives) refers to the dye's ability to identify the absence of disease (Brunette, 1996). Table 6 offers a summary of some of the available studies. When used by experienced hands for the detection of oral SCC, TB has been found to be highly sensitive in the majority of studies. Warnakulasuriya and Johnson (1996) examined the sensitivity and specificity of TB in a rinse form (OroScan ®) and found a 100% sensitivity rate in the detection of SCC. Specificity varies amongst studies but is found to improve when TB positive lesions are re-stained 10 - 14 days later. This time period allows for the healing of traumatic or inflammatory lesions (Rosenberg and Cretin, 1989). Variations in

the sensitivity and specificity may be the result of how equivocal results are coded in the research, whether the test is used only for cancer or for dysplasia, sample lesion diversity, whether the staining results were confirmed histopathologically and type of application (Patton, 2003). Rosen, Cornish and Edelson (1971) had both low sensitivity and specificity, which may be the result of patient selection. Approximately half of their group suffered from mucositis as a result of excessive alcohol intake. Allen (1998) cautions that the high level of false positives may mislead clinicians and therefore affect patients both emotionally and physically by submitting them to unnecessary procedures. He concluded that TB may only be helpful for those who already have a level of proficiency in assessing oral cancer and precancer. It should be noted that the dorsal surface of the tongue, due to the filiform papillae and the salivary gland openings on the palate, will be the sources of false positives. All authors caution that the use of TB is only an adjunct to a thorough intraoral examination and does not preclude experienced clinical judgment.

TB stain can only be retained by tumours other than SCC as long as they involve mucosal change. Myers (1970) found patients with malignant melanoma, fibrosarcoma and lymphosarcoma (all ulcerated) stained TB positive along with the SCCs. Patients with deeper tumours and no mucosal change did not pick up the stain. Benign ulcers were found to pick up stain but with much less intensity than malignant tissue.

Table 6. Toluidine blue efficacy in the detection of oral SCC

Author(s)	Year	Number of subjects	Toluidine blue application type (rinse or direct) [a]	Sensitivity (%)	Specificity (%)
Single application					
Neibel and Chomet	1964	11	direct	100	NR
Shedd *et al.*	1965	50	direct	100	NR
Shedd *et al.*	1967	62	both	100	NR
Myers	1970	70	direct	100	NR
Rosen *et al.*	1971	45	both	50	50
Vahidy *et al.*	1972	1190	both	86	76
Reddy *et al.*	1973	490	direct	100	NR
Silverman *et al.*	1984	132	direct	98	70
Epstein *et al.*	1992	59	direct	93	63
Onofre *et al.*	1995	44	unknown	92	44
Warnakulasuriya and Johnson	1996	102 (86bx)	rinse	100	100
Epstein *et al.*[b]	1997	46	direct	100	52

Author(s)	Year	Number of subjects	Toluidine blue application type (rinse or direct) [a]	Sensitivity (%)	Specificity (%)
Second application					
Pizer and Dubois [c]	1979	255	direct	NR	99
Mashberg	1980	235	direct	93	92
Mashberg[d]	1981	105	direct	98	93
			rinse	94	93
Mashberg[d]	1983	134 (179 lesions)	direct	98	88
			rinse	89	91
Onofre *et al.* [c]	2001	7 SCC or *CIS*	direct	100	67.5
Epstein *et al.*	2003	81	rinse	96.7	NR

[a] Direct method is applied with a cotton tip applicator; rinse refers to a mouth rinse
[b] Patients with a previously treated SCC
[c] Patients returned 14 days later for second application
[d] Patients received both application methods

I.7.4. Sensitivity and Specificity of TB in detection of OPL

As seen in Table 7 the results of research into the sensitivity of TB in the identification of dysplasia is mixed. Both Epstein *et al.* (1997) and Mashberg (1983) found TB of little help in the detection of low-grade dysplasia. In fact Epstein et al. (1997) found it to be no different than the clinical examination alone. This may be a result of dysplasia not staining as intensely as SCC

resulting in an equivocal result. Epstein, Zhang *et al.* (2003), suggest the variation in TB uptake across dysplasia may be due to molecular differences in TB positive, TB negative, and equivocally stained dysplasias. Their study is discussed later in this introduction. Silverman, Migliorati and Barbosa (1984) found the direct method is highly sensitive in the detection of dysplasia (as well as *CIS* and SCC) however, they also found a high rate of false positives among ulcerated and erythemic benign tissue.

Onofre, Sposto and Navarro (2001) studied the TB results of patients with homogeneous leukoplakia, non-homogeneous leukoplakia, erythroplakia, reticular and erosive lichen planus and suspicious ulcerations. TB staining was completed twice, fourteen days apart, to eliminate lesions that were the result of mechanical trauma, inflammation and potential false-positives. All lesions that remained positive after the second staining (23) were biopsied along with 27 TB negative lesions that the clinicians felt were warranted based on their judgment. While 100% (7/7) of the lesions diagnosed as SCC or *CIS* were TB positive, only 50% (3/6) of the dysplasias (mild or moderate) were TB positive. The remaining 37 lesions (13 false-positives) were confirmed to be benign keratosis, lichen planus or other benign lesions. Martin, Kerawala and Reed (1998) examined the sensitivity of TB in dysplastic lesions in tissue surrounding oral tumours and found that only 17 of 40 (42.5%) moderate and severe dysplasias were found to have stained TB positive.

False positives for OPLs that pick up stain may be associated with their potential to become malignant. Following up on the false positives in the aforementioned studies at a time distant from the actual TB test may find that there were fewer false positives and possibly TB could be showing some predictive value. Interestingly, Mashberg (1980) found nine TB false positive lesions (negative histologically) which eventually were diagnosed as SCC after the second or third biopsy.

Table 7. Toluidine blue efficacy in the detection of dysplasia

	Year	Number of subjects	Toluidine blue application type (rinse or direct)	Sensitivity (%)	Specificity (%)
Single Application					
Mashberg [a]	1983	98	direct	33	90
			rinse	33	93
Silverman *et al*	1984	42	direct	100	NR[b]
Warnakulasuriya and Johnson	1996	102	rinse	79.5	62
Epstein *et al*	1997	45	direct	53	31
Martin *et al*[c]	1998	11 (14 lesions)	NR[a]	58	NR[b]
Second Application					
Onofre *et al*	2001	43	direct	50	65

[a] Patients received both application methods
[a] NR – not reported
[c] Collected at time of tumour surgery to determine extent of dysplasia in surrounding tissues

The use of TB as a screening test for the general population has been found to be unreliable because of the large number of false positives due to trauma or inflammation leading researchers to claim that the stain is better suited for high-

risk populations (Rosen, Cornish and Edelson, 1971; Moyer, Taybos and Pelleu, 1986; Patton, 2003).

I.7.5. TB and surgical margins

Kerawala *et al.* (2000) used toluidine blue rinse on 14 tumours immediately prior to surgery to evaluate the use of the stain in determining margins. Tumours were excised with a 1 centimetre margin beyond the TB positive margin or clinically abnormal mucosa (whichever was wider). TB identified the margins of SCC but missed 10 foci of CIS and severe dysplasia. There were a total of 16 areas of CIS or dysplasia that were TB negative found at the resection margins. Although 1 cm is the accepted margin width for oral SCC excision the authors felt that increasing this distance may lead to fewer margins positive for CIS or dysplasia. The authors concluded that TB at the time of surgery would be of little value in reducing the frequency of recurrence.

I.8. Genetic changes and Oral Cancer

Cancer is a genetic disease that develops when an altered single cell loses its ability to control its growth due to the influence of carcinogens. As the cell continues to cycle it acquires additional genetic changes dependent on the continued exposure to known and unknown etiological factors. These acquired

genetic mutations, as well as possible inherited mutations (Sidransky, 1997) are passed on to the subsequent daughter cells. As the tissue develops, more genetic damage occurs and various subclones arise with additional genetic changes. This is known as multistep carcinogenesis (Hittleman, 2001).

I.8.1. Oncogenes, Tumour Suppressor Genes and Oral Cancer

There are various ways to classify tumour genes. One method of classification is to group the cancer genes into 3 types: the oncogene, the tumour suppressor gene and the DNA-repair gene (Mao, 1997). Oncogenes are derived from proto-oncogenes, which regulate cell cycle growth and differentiation. When a mutation occurs to the proto-oncogene it becomes an oncogene, a gene that is constantly "on", leading to uncontrolled cell growth. Oncogenes found to be involved in oral cancer include the human epidermal growth factor receptor gene (EGFR), ras oncogenes, c-Myc gene and cyclin D1 (Bettendorf, Piffko and Bankfalvi, 2004).

Tumour suppressor genes (TSGs) prevent abnormal proliferation by acting like a brake in regulating the cell cycle (Jorde *et al.,* 2000). The loss of a TSG, due to point mutations, deletions, rearrangements and loss-of-function mutations (Bettendorf, Piffko and Bankfalvi, 2004) results in uncontrolled cell proliferation. These mutations result in a "loss of genetic material from one region of a pair of

chromosomes that are inherited from both parents" (Mao, 1997) known as loss of heterozygosity (LOH).

Many TSGs have been investigated for their role in OPLs and oral cancer. One of the earliest and most common events in head and neck SCC is LOH at chromosome 9p21 (p16), which has been found in tissues with hyperplasia, very early in oral carcinogenesis (van der Riet *et al.,* 1994; Califano *et al.,* 1996; El-Naggar, *et al.,* 1995; Emilion *et al.,* 1996). Other common genes studied for their early role in the development of oral cancer are the FHIT gene found on 3p21 (Roz *et al.,* 1996) and later in tumourigenesis, p53, found on the short arm of the 17th chromosome (17p13). The protein associated with p53 plays a critical role in cell cycle arrest, DNA repair and apoptosis, while the protein associated with p16 plays a role in the inhibition of cell cycle progression (Califano *et al.,* 2000). Other TSG thought to be involved in oral SCC are found on 4q, 5q, 6p, 8p 11q, 13q, 14q and 18q (Mao, 1997, Lippman and Hong, 2001; Irish *et al.,* 2003). Although the loss at 9p21, 3p21 and 17p53 are early indicators of tumourigenesis, it is the accumulation of genetic mutations versus the order of genetic loss that is the more powerful predictor of progression (Mao and Sidranksy, 1994; Califano *et al.,* 1996).

I.8.2. Molecular Assays

An advantage of using genetic data to determine the risk of progression of an oral premalignant lesion is that only a small amount of DNA is necessary to run tests. Cairns and Sidransky (1999) found DNA analysis to be an ideal method for molecular diagnosis because it can endure many of the unfavourable conditions clinical samples undergo and it can be amplified by PCR (polymerase chain reaction) based techniques. Using these PCR techniques, microsatellite analysis can search for LOH and microsatellite instability (a change in the length of the nucleotide repeat) (Braakhuis *et al.,* 2002). Zhang and Rosin (2001) recommend the use of LOH and other molecular tools to identify low grade OPL that are at a high-risk to progress, to identify OPL that are a high-risk in patients with a previous history of oral SCC, to assess and develop strategies for treatment of an OPL and in the development of new treatment itself.

Microsatellites are short DNA repeat sequences that are used for markers to detect change in premalignant or malignant cells. A sample of clinical DNA is compared to a sample of normal DNA to detect allelic imbalance, either the presence of a new allele or a loss of an allele (LOH). The presence of either condition represents altered genetic information. In fact, El-Naggar *et al.* (1995) determined that tumours with significant LOH were more apt to be aneuploid, at an advanced stage and were poorly differentiated.

In situ hybridization (ISH) is an analysis method popular with cytological samples such as exfoliative cells. Chromosomal polysomy, deletions or other chromosomal abnormalities can be easily detected. According to Cairns and Sidransky (1999) FISH (fluorescent in situ hybridization) is probably the most accurate method to assess amplification at the DNA level.

I.8.3. Molecular Research and Oral Cancer

As mentioned earlier, the current 'gold standard' to determine which OPL will progress to SCC is the histopathological diagnosis. Unfortunately, histopathology is a poor predictor for an OPL with low-grade or no dysplasia (Sudbø *et al.,* 2003). The majority of mild and moderate (low-grade) dysplasias do not become squamous cell carcinoma (Rosin *et al.,* 2000). Yet how do we identify the low-grade dysplasia that will progress? There has been exponential growth in DNA research within cancer research. Studies published over the last ten years have found that specific genetic changes or patterns may help identify lesions at risk of progression (Rosin *et al.,* 2000).

I.8.3.1. LOH and Oral Cancer

Early research attempted to find which genetic events were linked to oral SCC. Ah-See, *et al.* (1994) compared loss of heterozygosity between normal and

tumour DNA tissue samples. The authors studied all 22 q limbs and 17 of the p limbs and found that five regions, 3p, 5q, 9q, 11q and 17p, showed a higher rate of LOH in the tumour samples when compared to normal tissue than other regions tested. Also in 1994, Li *et al.* concluded that LOH at more than 2 loci was significantly associated with a poor prognosis.

Genetic research has also increased our understanding of field cancerization and the cell migration theory mentioned earlier in this paper. In 2001, Partridge *et al.* published the results of a study of 11 patients with multiple lesions but without a history of tobacco or alcohol use and concluded that field cancerization may be more widespread than otherwise believed. Tumours greater than 2 centimetres away from the index tumour had identical allelic losses to the index tumour leading the authors to conclude that these subsequent lesions were not true second primary tumours but clonal outgrowths of the original tumour.

I.8.4. LOH and OPL

I.8.4.1. LOH and risk of progression

One of the main goals of researchers over the last 10 years was in the development of a genetic progression model for oral SCC that would supplement the histological diagnosis in the prediction of OPL progression. Califano *et al.*

(1996) devised a genetic model of progression for head and heck cancer by searching for LOH at 10 loci in benign and premalignant lesions. The earliest losses can be found in hyperplastic tissue. As mentioned earlier, the early losses are most commonly found at 9p21, 3p21 and 17p13. Dysplastic tissue had an increased rate of loss versus benign tissue, at the above loci plus additional LOH at other loci. The number of loci involved and the frequency of involvement increase through *CIS* to SCC. The authors also found similar patterns of loss, albeit less, in tissues adjacent to the lesions leading them to conclude that these "clonal outgrowths" in histological normal tissue may be responsible for recurrence of the tumour. These early losses depict the origins of tumourigenesis but for a lesion to become invasive it is the accumulation of multiple losses that is required. The authors investigated their model further in 2000, by compiling serial biopsies of recurrent OPLs over time and affirmed their previous conclusion that the recurrent OPL are a result of clonal outgrowths. Interestingly, it was also reported that the period of time between exposure to a carcinogen and the appearance of a HNSCC may be as long as 25 years and that the genetic changes may significantly precede the histologic and morphologic changes.

Mao *et al.* (1996) found leukoplakic lesions with or without dysplasia that exhibited LOH at 9p21 and 3p14 were much more likely to progress to SCC than leukoplakia, regardless of pathological diagnosis, without LOH at these 2 sites.

Lesions with LOH also progressed to SCC faster than lesions that did not exhibit LOH.

In a retrospective study by Rosin *et al.* (2000), tissue from progressing oral premalignant lesions was compared to nonprogressing cases for changes in LOH. It was found that almost all progressing cases had LOH at 3p and/or 9p. Lesions with this loss had a 3.8-fold increased risk of progressing to cancer compared tp those morphologically similar lesions without such loss. Samples exhibiting a loss at 3p and/or 9p plus at least one other locus exhibited a 33-fold increased risk of becoming malignant as well as a significantly faster rate of malignant transformation compared to those lesions without such loss. The other loci examined in this study were on 4q, 8p, 11q, 13q and 17p. The authors proposed that screening for a loss at 3p and or 9p might be a good initial screening to assess risk of progression.

I.8.4.2. LOH, OPL and other issues

Researchers have begun to combine demographic and clinical data with molecular markers in an oral SCC progression model. Lee *et al.* (2000) combined the molecular markers of progression with demographic and histological markers. Patients with dysplasia, hyperplasia at a high-risk site or a large, symptomatic hyperplasia were part of a chemotherapeutic trial. Nearly one third of the

patients progressed to SCC in a mean time of 4 years. A history of oral cancer, a histological diagnosis of moderate or severe dysplasia (OR=2.3) and chromosomal polysomy were predictive of lesion progression to cancer. The authors then attempted modeling using the combined biomarkers of chromosomal polysomy, p53 expression and LOH at 3p or 9p, along with history of cancer and histology. The combination of the three biomarkers along with histology was found to be very predictive of progression (OR=2.27). Interestingly, more that 40% of the cancer formed at sites separate from either the previous cancer site or the leukoplakia being followed in the study, allowing the authors to conclude that leukoplakia is also a marker for increased cancer risk throughout the oral cavity. The authors concluded the combination of clinical, histopathological and molecular information will give greater power to the prediction of cancer progression.

Zhang *et al.* (2001) found a relationship between the risk of the anatomical site and molecular damage at 3p, 9p and 17p. High-risk sites such as the FOM, ventral and lateral tongue and soft palate, were found to have significantly higher LOH frequency than low risk sites. Loss of 3p and/or 9p was significantly greater at high-risk sites, particularly in mild dysplasias. Loss at more than one arm occurred more frequently at high-risk sites. This research supports the theory that some oral sites are at greater risk of cancer than others.

Guo *et al.* (2001) studied the relationship between TB staining and LOH at three sites, 3p21, 9p21 and 17p13. The authors' biopsied TB-positive stained areas of 46 patients who were between three months and two years post treatment of a head and neck or upper aerodigestive tract cancer. Of the 46 TB-positive biopsies 13 were SCC, 11 were *CIS*, and 22 were histopathologically normal. LOH at one or more of the markers occurred in 76% of all the cases including all the SCC and *CIS* samples. LOH at 9p21 occurred in 69% of the cases. LOH at 3p21, 17p13 or on multiple arms was significantly more common in the SCC samples than in the normal samples. Twenty-five cases had two biopsies taken, one from the TB-positive site and one from at TB-negative site within 5 mm of the TB-positive stained border. Sixteen of the 25 pairs had identical patterns of loss while eight of the remaining nine pairs showed more LOH in the TB-positive sample versus the TB-negative sample. It is not clear however, if the TB-negative biopsy was taken from within the same clinical lesion site as the TB-positive biopsy nor were the histopathological results of the TB-negative biopsies reported.

Epstein, Zhang *et al.* (2003) reported that a significantly higher proportion of LOH was found in TB positive OPL biopsy samples than TB negative OPL samples. Other findings included that TB positive samples were significantly more likely to have loss at more than two arms than TB negative samples. Of interest, none of the TB negative samples showed multiple losses. Of six patients who had multiple biopsies over time, two patients had negative staining

on both occasions, two patients showed a reduction in staining with a reduction in LOH after treatment and two patients showed an increase in TB staining, one with an increase in LOH and the other had persistent LOH change but showed a histologic progression. There was also no significant difference found between strongly staining and weakly (equivocal) staining OPLs and LOH. The authors concluded that TB staining may help identify OPLs with increased risk of progression associated with LOH regardless of histopathological diagnosis.

I.8.5. Molecular markers and SOM

The past 10 years has seen a remarkable amount of research in to the molecular markers that are associated with oral cancer, OPLs and those that predict the risk of malignant transformation. As mentioned in section I.5., the risk of SOM in patients with a history of oral cancer is high and detrimentally affects the 5-year survival rate. Recent research has begun to look for molecular markers that predict SOM at the previously treated cancer sites. Rosin *et al.* (2002) studied the markers, 3p and 9p, which were found to be associated with malignant transformation of OPLs in their earlier research, in patients with an OPL at the former cancer site to see if these same markers would also be associated with risk of SOM. LOH at 3p and/or 9p was found to be associated with a 26.3 fold increased risk of SOM. These losses were found to be much more predictive than the histology of the OPL. Individually, LOH at 3p, 9p and 4q were all found

to be significantly greater in the SOM group. Lesions that progressed to SOM were also found to show more multiple losses.

Patients who were positive for p53 protein expression in their primary tumours were found to develop SOM, both recurrences and SPTs, much faster than patients without p53 protein expression. Shin *et al.* (1996) concluded that p53 expression was a means of identifying patients at a high-risk of SOM.

I.8.6. DNA content and risk of progression

Other methods of genetic change have been investigated in the progression of OPLs to SCC. Ploidy refers to the DNA content of the cell and aneuploidy is the term used for an abnormal number of chromosomes within the cell. Aneuploidy refers to cells that do not have the correct number of chromosomes (diploid). Sudbø *et al.* (2001) found a strong association between ploidy status and risk of progression of OPL. Patients with dysplasia that had aneuploid lesions had a relative risk of progressing to SCC of 27.2 versus diploid OPL. Tetraploid lesions were also found to have a higher risk of progression than diploid lesions. The aneuploid lesions also progressed to SCC at a significantly faster rate than the tetraploid lesions. The authors concluded that OPL aneuploid lesions be treated as a cancerous lesion since the risk for progression is high. In a follow-up to this paper, Sudbø, Lippman *et al.* (2004) found that more than half of the lesions

that progressed to cancer recurred after treatment to cure. The majority of the recurrences were in the patients with aneuploid lesions, the remaining recurrences were in patients with tetraploid lesions. Deaths due to oral cancer were only seen in patients with aneuploid lesions. The authors concluded that the complete resection of the aneuploid OPL does not reduce the risk of progression and death in these lesions.

I.8.7. Molecular markers and surgical margins

Gath and Brakenhoff (1999) refer to histopathologically undetectable tumour cells that spread beyond the margins of excised tumours as the reason behind the high rate of recurrence. This histologically undetectable minimal residual disease may be detected by molecular methods of detection. van der Toorn *et al.* (2001) analyzed the resection margins of patients treated surgically for oral SCC for genetic mutations at p53 and aneuploidy at chromosomes 1 and 7. Genetic change was found in 11 of 20 histologically normal margins. Histologic review of these areas led to modification of the initial histological diagnosis from normal to hyperplasia or low-grade dysplasia. These small foci of residual altered cells could be responsible for the high rate of recurrence in oral SCC.

van Houten *et al.* (2004) found the presence of TP53-mutated DNA in the histologically tumour free surgical margin of tumours to be a significant indicator

of tumour recurrence in head and neck cancer patients. Similarly, the absence of TP53-mutated DNA was significantly correlated with absence of local recurrence.

II. STATEMENT OF PROBLEM

The five year survival rate has improved only marginally in the last decade. The development of second oral malignancies, both at the site of the previously treated tumour or a second primary tumour is one of the primary causes of poor long term survival for oral cancer patients. Although clinicopathological risk predictors exist for primary OPLs little or no research has been done to see if the clinicopathological risk factors of primary OPLs apply to OPLs at sites of previous oral SCC. This study will investigate the clinicopathological risk predictors.

III. OBJECTIVES

To determine clinicopathological features that will predict a SOM at the previously treated cancer site.

IV. HYPOTHESIS

Clinicopathological risk indicators for OPLs from the site of previous oral SCC are similar to those of primary OPLs.

V. MATERIALS AND METHODS

V.1. Patients

The source of patients for this thesis is the Oral Cancer Prevention Longitudinal (OCPL) study funded by the National Institute of Dental Craniofacial Research (NIDCR). The study has at its central core the Provincial Oral Biopsy Service (OBS) of British Columbia. The OCPL study and OBS are described below.

V.1.1. OCPL study

This OCPL study is one of the first cohort studies of patients with oral lesions and is designed to systematically follow changes in clinical, pathological and molecular parameters over time. The study is an ongoing province-wide longitudinal study run jointly by the British Columbia Cancer Agency (BCCA), the University of British Columbia (UBC) and Simon Fraser University (SFU). The objective of the OCPL study is to identify patterns that correlate with malignant transformation (for patients with oral premalignant lesions) or cancer recurrence (for cancer patients) and to use this information to develop a multi-faceted risk model with clinical application. Such studies have not been performed previously due to the difficulty in recruiting such patients to a longitudinal study.

V.1.2. Eligibility for this thesis study

The eligibility criterion for patients for this thesis study included:

1) Aged 18 and over with a diagnosis of oral SCC, *CIS* or VC;
2) Completed and signed informed consent for participating in the study;
3) Able to return to the Oral Oncology/Oral Dysplasia Clinic for regular follow-up;
4) Ability to communicate in English or have had a translator to help in communication;
5) Accrual into the study within 12 months of the treatment of oral SCC (with an intent to cure);
6) At approximately one year post cancer treatment (± 4 months, i.e., 8 - 16 months), the previous cancer site had been carefully examined and an exfoliative cell sample (scrape) taken from the site;
7) At the time of the examination and scrape there was no recurrence or residual tumour present; and
8) The patients had been followed for at least 8 months post cancer treatment.

The OCPL study recruits patients aged 18 and over with a current diagnosis of oral dysplasia or with a current or former diagnosis of oral SCC. Originally the

study was located in the Oral Oncology/Oral Dysplasia Clinic at the Vancouver BCCA site, and now the Oral Oncology/Oral Dysplasia Clinic has expanded to involve other satellite sites: the Oral Oncology/Oral Dysplasia Clinic at the Fraser Valley BCCA site, and the Mouth and Mucosa Disease Clinic at Vancouver General Hospital and UBC Specialty Clinic. The Clinic serves Greater Vancouver and is a referral centre for oral dysplastic lesions and follow-up centre for patients with history of oral SCC. The clinics are staffed by Drs. M. Williams, BC OCPL Study Clinical Director, C. Poh, A. Hovan and P. Gardner.

An Institutional Review Board has approved the OCLP study. Patients with oral dysplasia or history of oral cancer who were referred to the Oral Oncology/ Oral Dysplasia Clinic were given the information on the OCPL study and asked whether they were interested in participating in the study. All patients signed an informed consent form at study entry. Patient participation is on a volunteer basis only and patients were told that they may terminate their participation in the study at any time. Patients involved in the study are given an identification number to ensure confidentiality outside the dental clinic. This identification number is then used to label all patient samples and to identify patients within the study database.

As of January 11, 2004, the cut-off date for this research, the OCPL study had enrolled 202 patients aged 18 and over with a diagnosis of oral SCC, VC or *CIS*, and who are able to communicate and participate in regular follow-up. Figure 2

is a flow chart to demonstrate the patient selection for my study or reasons of exclusion of patients. Of the 202 patients, 49 patients did not have scrape from the prior cancer site at around 8 - 16 months, and another 59 patients had not been in follow-up for 8 months.

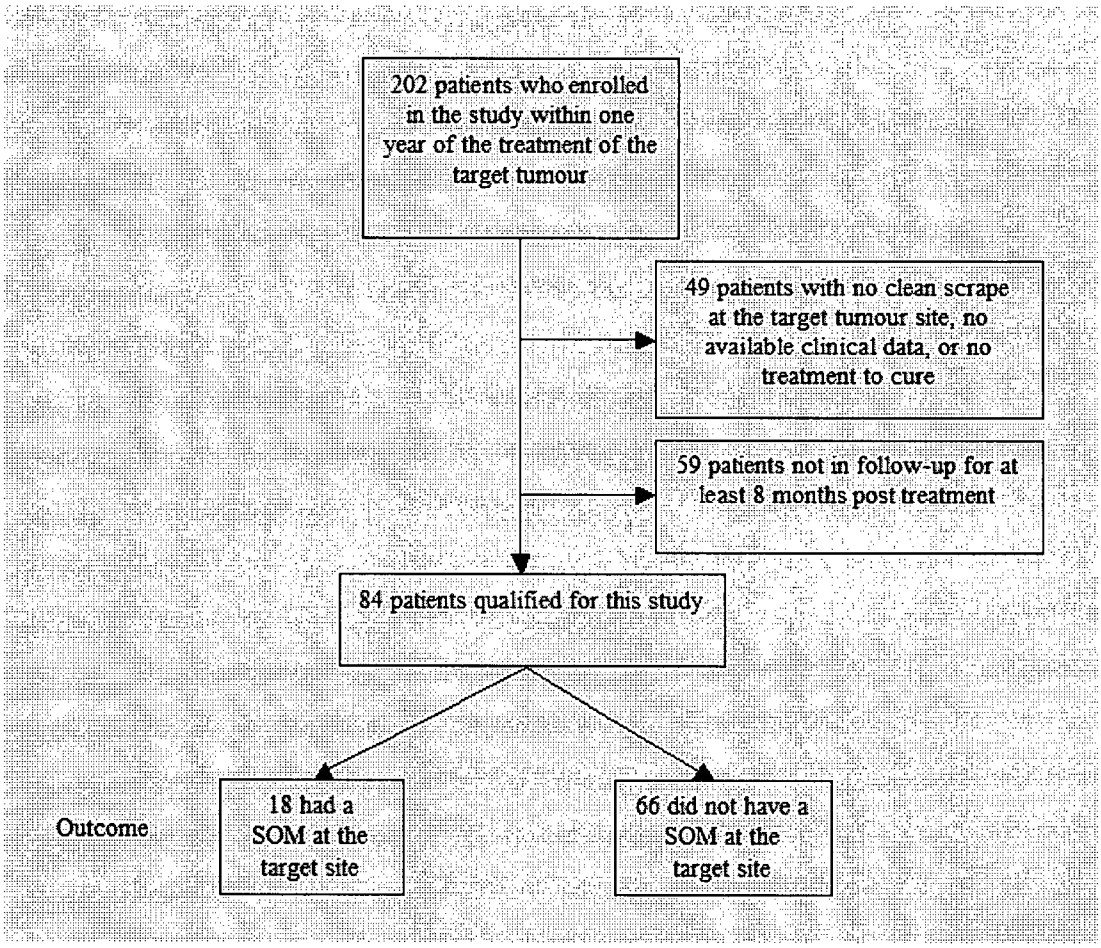


Figure 2. Flow chart of patient selection

V.2. Pathways of data collection and storage

Tables 8 and 9 show the clinical pathway for first and subsequent recall visits within the Oral Oncology/Oral Dysplasia Clinic. Compared to patients who had not consented to participate in the study, patients in the study had the following additional data/samples collected (underlined in Tables 8 and 9):

- Wash -- swish with saline to collect exfoliated cells
- Autofluorescent imaging
- Buccal mucosa brushing
- Lesion and non-lesion brushings
- Mapping brushings

Data collected was entered and stored in a database (MS-ACCESS). As mentioned, the first step when a patient consents and enrols in the study is to give the patient a patient ID number, which is followed by adding the information on the date of entering the study as well as source of patient referrals. This patient ID number is a unique patient identifier.

There are approximately 125 columns of data entry for each patient not including the molecular data. I will discuss the data collection in the order of demographic and habit data, target tumour data, and follow up data. Target tumour is defined as the treated tumour being monitored.

Table 8. OCLP Clinical Pathway -- First Visit

Contact form	personal and contact information
Consent form	
Medical history	current medication -- prescribed and OTC, allergies history of serious and/or complicating illness.
Patient concerns	sensitivity lesion history
Questionnaire	tobacco use alcohol use family history of head and neck cancer

Take initial samples or else re-appoint

Initial samples and data collection:

Wash	swish with saline to collect exfoliated cells	
Clinical exam	Extraoral exam	-- palpation for lymph nodes -- noting any visual abnormalities (i.e., lack of symmetry)
	Intraoral exam	-- examine all intraoral tissues for signs of pathology

Pre-toluidine blue photos of all lesions

Autofluorescent imaging [a]

Toluidine blue staining

Clinical examination of the mouth (tracking sheet)

Post-toluidine blue photo of each lesion

Lesion and non-lesion brushing

Buccal mucosa brushing

Mapping if necessary

Biopsy if indicated (or reappoint for Biopsy)

Rebook and dismiss patient

[a] Autofluorescent imaging will not be discussed in this thesis.

Table 9. OCLP Clinical Pathway – Recall Visit

Medical history	medication changes/additions new allergies hospitalization update general health
Patient concerns	changes in presentation sensitivity
Questionnaire	once a year
Samples and data collection:	
Wash	swish with saline to collect exfoliated cells
Pre photo check	to ensure no new lesions if new lesions include in list to be imaged
Pre-toluidine blue photo	
	Extraoral exam – includes palpation for lymph nodes and noting any visual abnormalities
	Intraoral exam -- examine all intraoral tissues for signs of pathology
Autofluorescent imaging [a]	
Toluidine blue staining	
Clinical examination of the mouth (tracking sheet)	
Post-toluidine blue photo of each lesion	
Lesion and non-lesion brushing	
Buccal mucosa brushing	
Mapping if indicated	
Biopsy if indicated (or reappoint for biopsy)	
Reappoint and dismiss patient	

[a] Autofluorescent imaging will not be discussed in this thesis.

V.3. Collection of data on demographics, medical/family history and habits

Medical history data was collected by the clinician. Demographic, family history and habit data were derived from questionnaires. Study participants filled out the questionnaire upon entry and then on a yearly basis (see appendix 1).

V.3.1. Demographic information

The following information was collected: date of birth, age at the diagnosis of the target oral cancer, gender, ethnicity, and city of residency.

V.3.2. Medical and family history

A thorough medical history of all patient medications, allergies, and other health concerns was obtained by interview between the clinician and the patient. Through the interview, patient's medical oral cancer and dysplasia history were obtained including: history of oral dysplasia; history of head and neck cancer, and number of primary head and neck cancer; family history of head and neck cancer; and diseases that post a patient/sample as biohazard such as HIV infection and hepatitis.

V.3.3. Tobacco and alcohol usage

The initial questionnaire reports on the lifetime use of alcohol, tobacco (both smoked and smokeless), second hand exposure to tobacco smoke, and betel quid use. The yearly questionnaire provides information on the patient's tobacco and alcohol use for the previous year. Smoking is broken down into cigarettes, pipes and cigars.

Cumulative exposure to tobacco smoking was determined via pack year. Pack years were defined as the number of packs of cigarettes (20 cigarettes per pack) smoked per day times the number of years smoked. The initial questionnaire asks for the number of cigarettes, pipes and/or cigars smoked per day in each decade of life from less than 20 years of age to more than 60 years of age. The pack year for each decade of life is then totalled to arrive at the total pack year calculation. In the determination of the statistic pack years, pipes were equivalent to 3 cigarettes and cigars were equivalent to 2 cigarettes, as per BCCA standards. The term "ever smoker" was given to all patients who self-reported the use of smoked tobacco products at least once a week for one year or longer. The term "current smoker" was given to those patients who were still smoking at diagnosis of the initial tumour and "continuing smokers" were still smoking in follow-up according to their most current questionnaire.

Patients recorded on the questionnaire their history of second hand smoke exposure, whether it was at home, work or in a public place. (Appendix 1, question 5). For the purpose of this study 'ever' second hand smoke exposure was any reported exposure to second hand smoke during the patient's lifetime.

V.4. Collection of data on the target tumours

The following information was collected:

1) Tumour size, lymph node involvement and distant metastasis were assessed from the patient chart; and, from this data, when possible, tumour stage was determined;
2) Tumour biopsy number was identified, and the biopsy report and histological slides were retrieved and reviewed. From these, the histological grading of the tumour (carcinoma *in situ*, well differentiated, moderately well differentiated, poorly differentiated SCC) was conducted;
3) Site of the tumour was determined from both the patient chart and thorough clinical examination. The site of the tumour was marked on the tracking sheet (with grid, see Tracking Sheet in Appendix 2 and grid in Appendix 3);
4) Treatment of the tumour was reviewed from the chart, including type of the treatment, time of the treatment (for radiation, this includes starting and ending time) and the name of the clinician for the treatment.

V.5. Collection of clinical data on the site of previous tumour during follow up

This is the most important part of this study as the objective was to determine factors that would help clinicians identify areas of high-risk for SOM. The clinical examination is conducted under the supervision of the attending Oral Medicine specialist. I have played a central role in collecting the following data clinically.

When a new lesion was identified, it was recorded on a tracking sheet. Each lesion had a separate tracking sheet within a patient's file. The tracking sheet also has fields to note which procedures were done and which samples were collected on that date. These include lesion and nonlesion brushings, biopsies, blood draws, images, toluidine blue staining, autofluorescent visualization, a saline wash and brushings of the buccal mucosa for exfoliated cells. Each lesion also has a form for any comments the clinician wants to add that are beyond what is asked in the tracking sheets. The patient's file also includes a grid overlying a schematic drawing of the oral cavity. The clinician draws the patient's lesion(s) onto this grid for future reference and to monitor any shifts in the lesion. All of this information is then uploaded into the OHS database.

It should be noted that since the onset of the study, there have been changes and challenges in how this data is collected and by whom. An attempt has been

made over that last three years to severely limit the number of clinicians involved in the collection of data to maximize inter-examiner reliability. The collection of clinical data is currently limited to six people covering all clinic sites.

V.5.1. Clinical examination with white light

Clinical information gathered at the initial and subsequent recall visits include:

1) Lesion presence: the presence of a lesion in any part of the oral cavity was determined through clinical examination. For the previously treated tumour site, the presence of graft and/or scar was also recorded even in the absence of a lesion. It should be noted the concept 'lesion' referred to high-risk lesions as judged by the clinician (includes leukoplakia, erythroplakia, cancer and ulcer). For example, if there was a lesion clinically regarded as oral lichen planus, this was not recorded as lesion presence.
2) Lesion site: this was recorded and marked on the tracking sheet.
3) Lesion size: the length and width of the lesion was recorded and marked on the tracking sheet. A Marquis colour coded periodontal probe was used to measure the length, width and thickness of a lesion.
4) Lesion appearance: this was determined through the lesion colour, texture and thickness. Colour options are white, predominantly white, predominantly red, red and other. Options available to describe the

texture of a lesion are smooth, verrucous, fissured, nodular, velvety/grainy, ulcerated and other. A lesion was determined as homogeneous leukoplakia if the lesion was thin and homogeneous in its color and uniformly smooth or slightly fissured in texture; otherwise a lesion was called non-homogeneous leukoplakia.

5) Lesion margin: The margin of the lesion was recorded as either discrete (well-defined margin) or diffuse (indistinct margin).

Frequently a patient had multiple oral lesions, and each of the lesions would have the above features recorded at each visit.

V.5.2. Toluidine blue examination

The 1% toluidine blue solution used for this study is made at the BCCA according to the following formula:

- Toluidine blue, 1g.
- Acetic acid, 10 cc.
- Absolute alcohol, 4.19 cc.
- Distilled water, 86 cc.
- NaOH, 125 drops of 2M.
- pH adjusted to 4.5.

The procedure of toluidine blue staining was:

1) The area to be stained was dried with gauze,
2) A 1% TB solution was applied with a cotton tip applicators to the specified/ suspicious area and left to sit on the lesion for 30 seconds;
3) The lesion is swabbed thoroughly with cotton tip applicators soaked in 1% acetic acid and finally;
4) The oral cavity was thoroughly rinsed with water.
5) The level of stain remaining was recorded as positive, equivocal (weak), or negative.

V.5.3. Taking samples

V.5.3.1. Biopsy

When a lesion was regarded as suspicious by the clinician, a biopsy was taken from the lesion, fixed in 10% formalin and submitted to the OBS for pathological assessment. The following information was recorded for the biopsy:

1) The site and size of the biopsy was marked on the tracking sheet.
2) The pathology requisition was completed with information on demographics, habits, history, clinical features of the lesion as well as TB staining and FV status of the lesion.
3) The nature of the biopsy, incisional (wedge or punch) vs. excisional.

4) The remaining clinical lesion size after biopsy (residual length and residual width).

V.5.3.2. Exfoliative cells (scrapes)

Regardless of whether there were clinical lesions or whether a biopsy was to be taken, an exfoliative cell sample was always taken from each lesion, or site of the previous cancer (if no lesion was present at the site) at each visit by using an Arcona cytology brush. A control exfoliative cells sample was taken from normal looking oral mucosa at a high-risk site, if available, at each visit. Finally an exfoliative cell sample was collected bilaterally from the normal looking mucosa of the buccal mucosa.

V.5.3.2. Other samples

Other samples taken include: blood draws and a saline wash. Patients are requested to give one blood sample, through the lab at the BCCA, for the duration of the study. The saline wash involves a patient swishing with 15ml of saline for 15 seconds and expectorating the saline into a large Eppendorf tube.

V.5.4. Coding of clinical lesion/site

The coding of the lesions is a very complex issue, and this is particularly true with the changes lesions go through over time.

The coding of each lesion was called the TL-Code, that is, each lesion site (LS) was given a letter, e.g., the first oral lesion would be designated as LSA, and the second as LSB. The definition of an independent lesion has however changed during the course of the study. Originally each distinct lesion that was not in connection with another lesion was designated as an independent lesion. For example, two distinct lesions (not connected to each other), one on the left anterior lateral tongue and the other on the left posterior lateral tongue would be designated LSA and LSB respectively. However, not infrequently two such lesions would merge over time. This has resulted in changes in the TL-code and in the definition of independent lesions. Lesions in the same field (e.g., one side of the tongue) or lesions within 3 cm from each other are now designated as one field lesion (LSA) with each of the individual lesions within the field called LSA1, LSA2, etc. Also, when multiple small lesions are within the same field the field measurement is being recorded in addition to the sizes of the multiple smaller lesions.

To remedy such inconsistency (LSA and LSB vs. LSA1 and LSA2), another code was given for each lesion, Sort-TL Code. The latter code would designate geographically close lesions (same field or within 3 cm from each other) as one geographical lesion, e.g., LSA and LSB both belong to Sort-TL code A. This task has simplified lesion identification on the database, the downloading of information from the database and the determination of endpoints. The designation of the Sort-TL code was done manually, by me, in consultation with other clinicians, if necessary. This was an extremely time consuming process.

V.5.5. Digital recording of clinical lesion

Intraoral images were taken before and after toluidine blue staining and were taken with a digital camera (Fuji Film FinePix S1 Pro) equipped with Nikon Macro Speedlight SB-29s and AF Micro Nikkor 105 mm 1:2.8D lens at the Vancouver dental clinic. The Fraser Valley dental clinic uses a newer model of the same camera, Fuji Film FinePix S2 Pro, with the same flash and lens as the original camera. Images taken prior to 2002 were taken on a Minolta SRT20 35mm camera with Minolta autobellows rokkor – x 100mm lens.

Clinical images of the suspected oral lesion played an important role in the follow-ups of these patients, allowing the comparison of lesions in re-evaluation

appointments and clinical record audits, and they were essential for case presentation.

V.6. Collection of histopathological data for lesions at previous cancer site

The majority of biopsies from the Oral Oncology/Oral Dysplasia Clinic were read at the OBS, and if not, the slides were reviewed at the OBS. The following histopathological information was recorded: pathology number, biopsy site, and histological diagnosis.

V.7. Treatment of lesions at previous cancer site

Some patients, with an OPL at the former tumour site, received additional treatment to the site after the initial curative treatment was completed. Treatment methods included topical bleomycin, laser ablation or excision and conventional surgery.

V.8. Endpoint for follow up

The endpoints for follow up include: Lost to follow up; death; recurrence at the target tumour site (REC, defined as a tumour occurring less than 3 years after the treatment of the target tumour and within 3 cm of the target tumour); second primary tumour occurring at the target tumour site (PPT, defined as a tumour occurring more than 3 years after the treatment of the target tumour but within 3 cm of the target tumour); second primary tumour occurring at a different site than the target tumour (PP) (more than 3 cm from the primary tumour); and progression (PROG, defined as OPLs that progressed to *CIS* or SCC while the patient was enrolled in the OCPL). I reviewed the database and assigned the appropriate endpoints in conjunction with other OCPL staff. These endpoints were added as an endpoint field to the database. For this thesis, the main endpoint, however, is SOM, including both recurrences and second primary tumour occurring at the former tumour site.

V.9. Statistical Analysis

Differences and associations between different study groups (e.g., SOM vs. non-SOM) were examined and compared. For categorical variables (gender, smoking habit, ethnicity, presence of OPLs, site, and clinical appearance of OPLs, TB

staining, dysplasia, histological diagnosis), Fischer's exact test or Pearson's chi square test, for when more than two categories of data (3 x 2 table or larger) were used. For continuous variables (age, pack year, OPL size), the means were compared with either unpaired (or independent samples) t-tests or a nonparametric Mann-Whitney test if the data failed to have a normal distribution. All the tests were two sided. Survival curves are Kaplan Meier curves. Results were considered statistically significant when $P = 0.05$. Statistical analysis was performed with SPSS software, version 12.0 for Windows, 2003 (SPSS Inc., Chicago, Illinois).

VI. RESULTS

My study focused primarily on clinical indicators. Clinical research is extremely labour intensive involving multiple people working as a team both in the collection of data (sample collection and chart review) in the hospital setting and in the preparation and management of the database. This paper presents the early results of a longitudinal study and not all data collected will be presented at this time. Some of my contributions to the various research projects of the OCPL research team have been presented as abstracts. I will also be a coauthor on studies that are still in progress (Zhang *et al*, in preparation).

Data analysis will be presented in the following order: First, demographic data which includes age, gender, ethnicity, and tobacco use will be described (Section VI.1). Secondly, I will explore the tumour data, which will include risk of site, histology, grade and staging, and the type of treatment used to treat the tumour (Section VI.2). Thirdly, the clinical, histopathological and treatment data relating to any oral premalignant lesion at the previously treated site will be presented (Section VI.3). This will include the presence of an OPL, toluidine blue test results, colour, size, appearance, the presence of other oral OPL, the pathology of the OPL, treatment of OPL and the follow-up times. The fourth section of results (Section VI.4) will compare all the variables between those who developed a second oral malignancy (SOM) and those without a second oral

malignancy (NONSOM). The final section (Section VI.5) will explore the factors that appear to be associated with SOM.

VI.1. Demographic and Habit Information

VI.1.1. Demographics

Table 10 shows the age, gender and ethnicity of the study population with an age distribution at the time of diagnosis shown in Figure 3. The mean and median age of diagnosis of the 84 subjects was 61 (± 13) and 62 years of age with a range of 30 to 87 years. Five (6%) cases were 40 or younger. Identification of the proportion of cases in these younger patients is important because the literature suggests that their etiology and outcome may be different than those diagnosed at an older age (section I.3.5.).

There were 47 (56%) males and 37 (44%) females. The vast majority of patients were Caucasian (62 cases, 74%), with the rest of the population being Asians (16 cases, 19%), Hispanic (3 cases, 4%) and Native Americans (3 cases, 4%).

Table 10. Demographics of study group

Age at tumour diagnosis	
Mean (yrs ± SD)	61 ± 13
Median (yrs)	62
Range (yrs)	30 - 87
Proportion ≤ 40 at diagnosis	6% (5/84)
Gender proportion male	56% (47/84)
Ethnicity	
Caucasian	74% (62/84)
Asian	19% (16/84)
Other [a]	7% (6/84)

[a] Hispanic (3) and Native American (3)

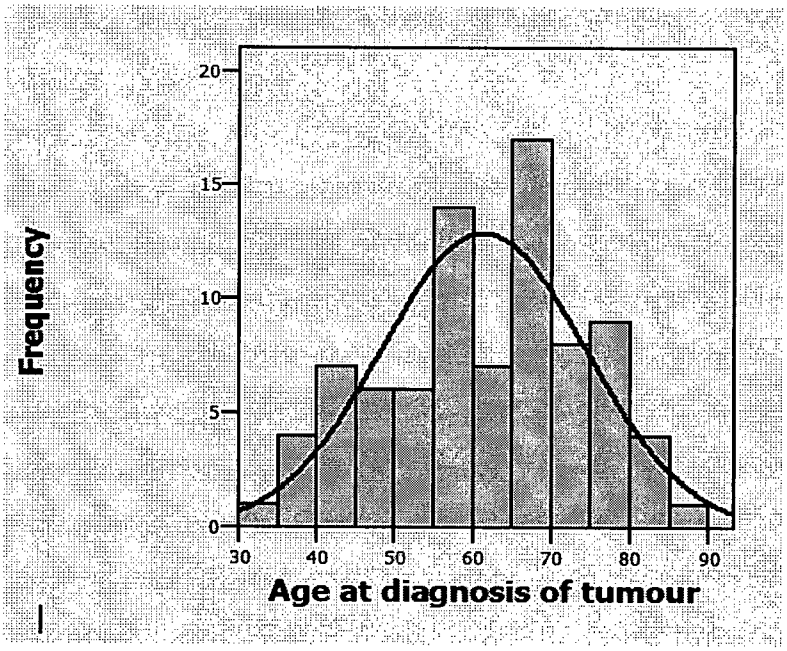


Figure 3. Age at diagnosis of target tumour

The population mean was 61 ± 13 (N=84).

VI.1.2. Tobacco habits

Table 11 summarizes the tobacco use of the study population. Fifty-six (67%) of the 84 subjects in the study had a history of smoking cigarettes, cigars or pipes more than once per week for one year or longer. This group was designated "ever smoker" or "smoker" and those who did not fit into this category were called "never smoker" or "non-smoker". Of the 56 such cases, more than half had quit by the time of their oral cancer diagnosis leaving 26 (46%) still smoking defined as "current smoker" at diagnosis. The majority of current smokers, 19 (34% of ever smokers and 73% of current smokers), continued to smoke after the diagnosis through and up to their most recent questionnaire, defined as "continuing smokers". The mean pack years (as defined in section V.5.) for the ever smokers was 40 ± 38 with a range of 1 to 255 pack years. Nine (11%) individuals had a history of using smokeless tobacco, one of whom was a never smoker. One person (1%), an ever smoker, had a history of chewing betel quid. Of interest, 69 of 83 (83%) respondents reported a history of regular daily exposure (self-reported) to second hand smoke either at home, work or in public places (see appendix 2, question 5). Of these 69 patients with regular exposure to second-hand smoke, 19 were non-smokers and had never used smokeless tobacco. Therefore, of the 84 oral cancer patients, only 12 (14%) had never

smoked, used smokeless tobacco or had regular exposure to second hand smoke.

Table 11. Tobacco use in the study population

All subjects	
Proportion ever smoker (≥ once a week for ≥ 1 year)	67% (56/84)
Proportion current (smokers at diagnosis)	31% (26/84)
Proportion continuing (smokers at most recent questionnaire)	23% (19/84)
Ever smokers only	
Mean pack years (± S.D.) [a,b]	40 ± 38
Median pack years [a,b]	34
Range pack years [a,b]	1 - 255
Proportion current (smokers at diagnosis)	46% (26/56)
Proportion continuing (smokers at most recent questionnaire)	73% (19/26)
Category of smokers	
Light (< 20 pack year)	27% (15/56)
Medium (20 – 40 pack year)	34% (19/56)
Heavy and very heavy (> 40 pack year)	39% (22/56)
Proportion using smokeless tobacco [c]	11% (9/81)
Proportion using betel quid [d]	1% (1/78)
Proportion regular exposure to 2nd hand smoke [e]	83% (69/84)
Proportion of never smokers with no history of smokeless tobacco or exposure to regular second hand smoke	14% (12/84)

[a] Ever smokers only
[b] Pack year: Daily number of packs (20 cigarettes) times years smoked
[c] No questionnaire data for 3 subjects in this category
[d] No questionnaire data for 6 subjects in this category
[e] History of daily exposure to second hand smoke

Figure 4 shows the frequency distribution of the total number of pack years for ever smokers. The majority of ever smokers (96%) reported 100 pack years or

less of tobacco usage, 2% reported between 100 and 125 pack years and one case (2%) reported had a 255 pack year history of tobacco use. Figure 5 shows the frequency distribution without the case of the 255 pack year smoker.

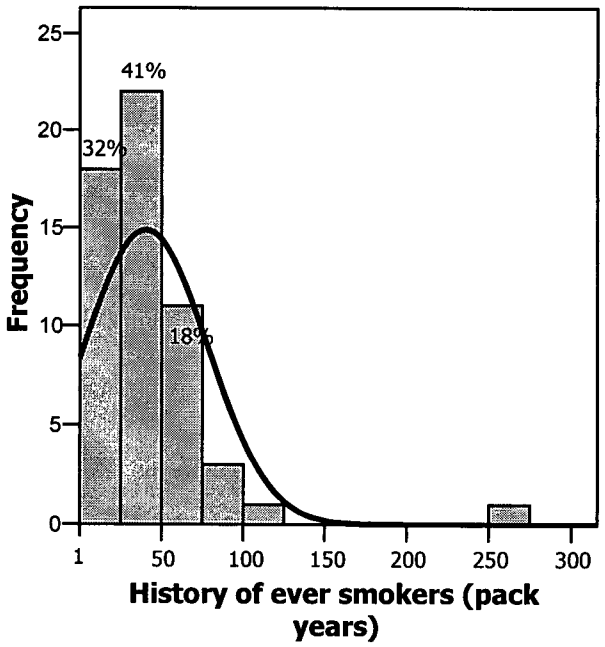


Figure 4. Frequency distribution of pack years smoked for ever smokers (mean 40 ± 38, n = 56).

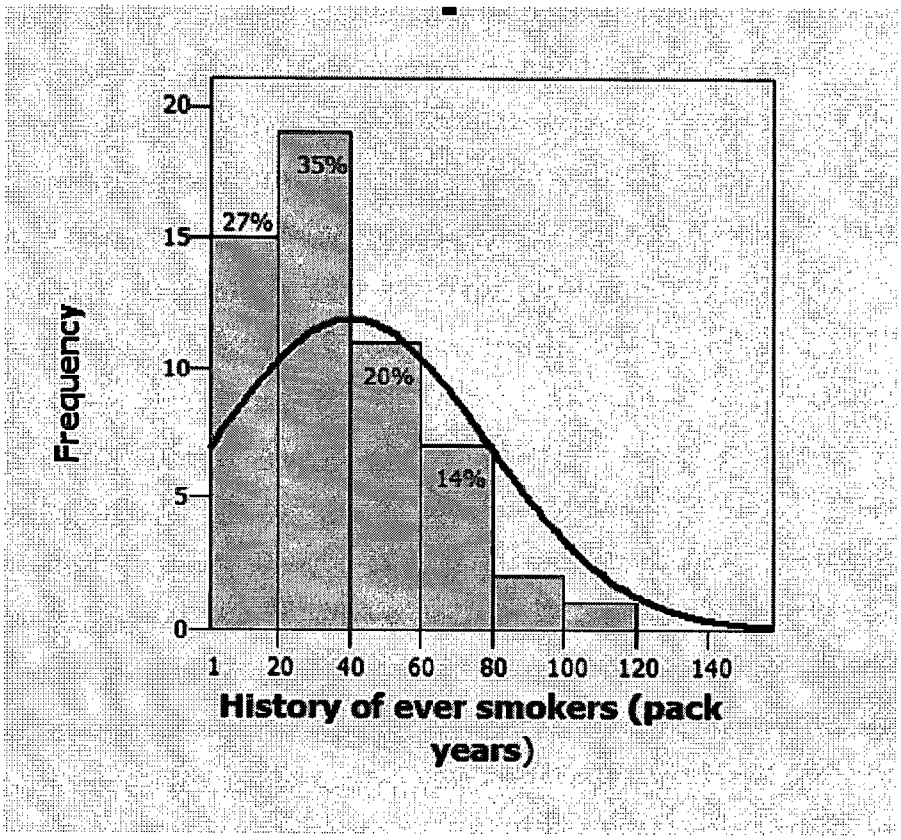


Figure 5. Frequency distribution of pack years smoked for ever smokers without outlier (n = 55).

VI.1.3. Alterations to tobacco habit during follow-up

In Table 12 a comparison is made between patients who were still smoking at their last questionnaire ("continuing smokers") and those who had quit smoking. Individuals who continued to smoke after their diagnosis tended to have smoked more than former smokers (42 pack years versus 39), however, the difference was not significant (*P = 0.15*).

Table 12. Smoking in follow up

	Continuing smokers	Former (non-continuing) smoker	*P value*
Total [a]	N = 19	N = 37	
Mean pack years (± SD)	42 ± 21	39 ± 44	*0.15*
Proportion exposed to 2nd smoke	100% (19/19)	92% (34/37)	*0.54*

[a] Ever smokers only

VI.2. Target Characteristics

Table 13 summarizes data collected on histopathological features, clinical history of patients and treatment for the study population. Target tumour is defined as the treated tumour being monitored.

Sixty-one (73%) of the 84 patients in this study had tumours that were located at sites in the oral cavity that are classified as high-risk sites for cancer development in Western countries (see section I.3.3. of introduction). This included 38 on the ventrolateral surface of the tongue, 15 on the floor of the mouth and 8 on the soft palate complex. The remaining 23 tumours were on the buccal mucosa, gingiva, hard palate and other low-risk sites.

The majority of the target tumours were primary oral tumours (69 cases, 82%). Of the 15 patients (18%) with a history of a previously treated oral cancer, 40%

(6/15) of the target tumours were at the same site as the previously treated tumour and 60% (9/15) were located at other oral sites.

Eighty of the target tumours had staging information available. Thirty-four percent were CIS (27 stage 0 cases), 44% were early stages (35 stage I and II cases) and 23% were late stages (18 stage III and IV cases). Histologically, of the 80 cases with data available, 34% (27) were *CIS,* 55% (44/80) were well to moderately well differentiated SCC and 11% (9/80) were poorly differentiated. Of the 44 well to moderately well differentiated carcinomas 4 were verrucous carcinomas.

In all cases, treatment was performed with intent to cure, and none of the 84 cases had clinical or histological evidence of residual disease at the end of treatment. The majority of tumours were treated with surgery only (56 cases, 67% of total), with 20 cases (24%) received radiation only and 8 (10%) cases received both radiation and surgery. Surgery was performed on 76% (64/84) of the tumours while 33% (28/84) of the cases received radiation.

Table 13. Clinicopathological features and treatment of the target

Proportion at high-risk site [a]	73% (61/84)
Proportion with a diagnosis of SCC/VC	68% (57/84)
Proportion with a previous oral cancer prior to the target	18% (15/84)
At same site	40% (6/15)
At different site	60% (9/15)
Histological diagnosis	
CIS	32% (27/84)
SCC	63% (53/84)
VC	5% (4/84)
Target tumour stage [b]	
CIS	34% (27/80)
I and II (early stage)	44% (35/80)
III and IV (late stage)	23% (18/80)
Tumour histology [c]	
CIS	34% (27/80)
Well and moderately well differentiated SCC	55% (44/80)
Poorly differentiated SCC	11% (9/80)
Treatment of target tumour	
Proportion surgery	76% (64/84)
Proportion radiation	33% (28/84)
Proportion both	10% (8/84)

[a] High-risk sites for oral cancer: Floor of mouth, lateral and ventral tongue, soft palate
[b] Lack of complete staging for 4 of the 84 cases.
[c] N = 80. No data for 4 SCC cases.

VI.2.1. Smoking and target tumour characteristics

Next, tumour features were studied for differences between ever and never smokers. As shown in Table 14, a greater proportion of ever smokers had

previously treated oral SCCs, particularly at a different site, and these differences were significant (27% versus 0, *P = 0.002,* 16% versus 0, *P = 0.026,* respectively). Likewise, ever smokers had a greater proportion of stage III and IV tumours (29% versus 12%) and more poorly differentiated cancers (15% versus 4%) although these differences were not statistically significant.

Table 14. Comparison of target tumour characteristics between smokers and non-smokers

	Ever smoker	Never smoker	*p value*
Total	N = 56	N = 28	
Proportion at high-risk site [a]	77% (43/56)	64% (18/28)	*0.30*
Proportion with a diagnosis of SCC/VC	68% (38/56)	68% (19/28)	*1*
Proportion with a previous oral cancer prior to the target	27% (15/56)	0	***0.002***
At same site	11% (6/56)	0	*0.17*
At different site	16% (9/56)	0	***0.026***
Histological diagnosis			
CIS	32% (18/56)	32% (9/28)	
SCC	63% (35/56)	64% (18/28)	*0.94*
VC	5% (3/56)	4% (1/28)	
Target tumour stage [b]			
CIS	34% (18/53)	33% (9/27)	
I and II (early stage)	38% (20/53)	56% (15/27)	*0.142*
III and IV (late stage)	28% (15/53)	11% (3/27)	
Tumour histology [c]			
CIS	33% (18/54)	35% (9/26)	
Well and moderately well differentiated SCC	52% (28/54)	62% (16/26)	*0.28*
Poorly differentiated SCC	15% (8/54)	4% (1/26)	
Treatment of target tumour			
Proportion surgery	73% (41/56)	82% (23/28)	*0.426*
Proportion radiation	34% (19/56)	32% (9/28)	*1*
Proportion both	7% (4/56)	14% (4/28)	*0.431*

[a] High-risk sites for oral cancer: Floor of mouth, lateral and ventral tongue, soft palate complex.

[b] N = 80. Lack of staging data for 4 cases (3 ever smokers and 1 never smoker)

[c.] N =80. No data for 4 cases. (2 ever smokers and 2 never smoker)

VI.3. Post-treatment description of clinical alterations to former tumour site

Since the thesis objective was to determine clinicopathological features that predict a SOM at the previously treated cancer site, the outcome investigated is the development of a SOM. This section contains clinicopathological data collected for the post-treatment tumour site during follow-up. Two approaches were used. The first focused on a description of clinical changes at approximately one year after cancer treatment (range 8 - 16 months), with a goal of identifying features that could later be examined for ability to predict development of second oral malignancy (SOM) in that time frame. The second approach involved an examination of the most severe clinical pathology observed ever during follow-up of each patient. The follow-up time was from treatment to either development of SOM or date of last study visit for the nonSOM group.

Table 15 presents data for the entire population. Table 16 presents data analyzing associations between clinical alterations at the former tumour site and smoking habits. Tables 17 through 22 compare the clinical alterations at the former tumour site and the risk of site, prior history of oral cancer, invasive cancer, cancer stage, tumour grade and history of radiation treatment for the target tumour.

VI.3.1. Post-treatment tumour site manifestation for the study population

Table 15 summarizes the data available on clinical changes occurring at the former cancer site at approximately one year (8 to 16 months) post-treatment and ever in follow-up (six months post treatment to SOM or last follow-up visit). Figure 6 displays the frequency distribution of the target date (one year) examinations. Figures 7 and 8 display the frequency distribution for time from treatment to SOM (n = 18) and the last follow-up examination for the nonSOM patients (n = 66).

Data was available at one year for 74 of the 84 cases for toluidine blue retention. Fifteen (20%) of the 74 cases were positive for this stain, with positive lesions including those that were both clearly positive and equivocal/weak in staining. The decision to include equivocal staining in the positive category was based on earlier studies done in our laboratory which suggest that even when equivocal/weak, stain retention is associated with outcome (see section I.8.).

In 23 (27%) of the 84 cases, a clinical leukoplakia (OPL) was observed at the site of the former tumour at one year post-treatment. There was a wide variation in the size of the OPLs. Figure 9 shows a frequency distribution of the largest dimension of the OPL at one year, with a mean and median of 11 and 6 mm respectively, and a range of 2 - 60 mm. More than one third (8/23) of the

lesions were larger than 10 mm length or width. The mean area of the OPLs was 68 mm. Ten (46%) of 22 lesions were non-homogeneous in appearance. Of note, almost one-third of the patients (26 cases) had more than one lesion at one year. Six biopsies were taken at the one year follow-up, all on OPLs at the former cancer sites with OPLs (6/23). Five of the biopsies were found to be hyperplasia or low-grade dysplasia. Only 2 OPL were treated with either surgery or laser at one year post tumour treatment.

Table 15 also shows data for the most severe clinical pathology observed during all of the follow-up visits of each patient. Slightly more than one third (34%) of the 82 of the patients showed TB stain retention at the former cancer site during follow-up and 36 (43%) of the 84 patients developed a clinical leukoplakia (OPL). More than half (21) of the 36 lesions had a length or width that was greater than 10mm. More than half of the OPLs (21 of 36 cases, 58%) that developed at former cancer sites were non-homogeneous in appearance. Multiple lesions were found in one third (28/84) of the patients during follow-up. More than half of the OPL at a former cancer site that were monitored in follow-up were biopsied (21/36). Four biopsies (3 hyperplasias and 1 severe dysplasia) were completed at sites without an OPL (no reasons listed). The majority of biopsies were hyperplasia or low grade dysplasia (16/21). Five of the lesions received either laser or surgical treatment in follow-up.

Eighteen patients (21%) developed a second oral malignancy at the former cancer site (SOM). The median time to develop a SOM was less than 2 years. Twelve percent of the patients were reported to have suffered metastasis and 12 patients (14%) died, 3 (25%) from oral cancer.

Table 15. Post-treatment tumour site manifestation for the study population

Follow up time (months)	
Time from tumour treatment to one year examination point	
Mean (± SD)	13 ± 2
Median	13
Range	8 - 16
Time from tumour treatment to last follow-up examination (non SOM)	
Mean (± SD)	28 ± 15
Median	25
Range	8 - 81
Time from tumour treatment to second oral malignancy (SOM)	
Mean (± SD)	26 ± 14
Median	23
Range	12 – 63

Toluidine blue staining	
At one year (proportion positive) (n = 74)	20% (15/74)
Ever during follow up (proportion positive) (n = 82)	34% (28/82)
Presence of an oral premalignant lesion (OPL) at former tumour site	
At one year	27% (23/84)
Ever during follow up	43% (36/84)
Size	
At one year (n = 23)	
Mean largest dimension (mm) (± SD)	11 ± 13
Median (mm)	6
Range (mm)	2 - 60
Area (mm^2) (± SD)	68 ± 97
Proportion of lesions with the largest dimension ≥ 10 mm	35% (8/23)
Ever during follow up (n = 36)	
Mean largest dimension (mm) (± SD)	16 ± 13
Area (mm^2) (± SD) [c]	213 ± 340
Proportion of lesions with the largest dimension ≥ 10	58% (21/36)
Appearance of OPL at former tumour site (Proportion non-homogeneous)	
At one year (n = 22) [a]	46% (10/22)
Ever during follow up (worst)	58% (21/36)
Proportion with multiple OPLs in the oral cavity	
At one year	31% (26/84)
Ever during follow up	33% (28/84)

Biopsy	
At one year	
Proportion of all former cancer site biopsied	7% (6/84)
Proportion of OPL at former cancer site (n = 23) biopsied	26% (6/23)
Ever during follow up	
Proportion of all former cancer site biopsied	30% (25/84)
Proportion of OPL at former cancer site (n = 36) biopsied	58% (21/36)
Mean # (± SD) of biopsies per cancer site	0.6 ± 1
Mean # of biopsies per OPL	1 ± 1
Pathology (worst pathology per OPL)	
At one year (n = 6)	
Hyperplasia	33% (2/6)
Mild and moderate dysplasia	50% (3/6)
Severe dysplasia	17% (1/6)
Ever during follow up (n = 21)	
Hyperplasia	38% (8/21)
Mild and moderate dysplasia	38% (8/21)
Severe dysplasia	24% (5/21)
Proportion of OPL at former tumour site treated by surgery or laser	
At one year	9% (2/23)
Ever during follow up (n = 36)	14% (5/36)
Outcome	
Proportion with second oral malignancy (SOM)	21% (18/84)
Proportion with distant metastasis	12% (10/84)

Proportion dead	14% (12/84)
Dead of disease (DOD, dead of oral cancer)	25% (3/12)
Dead of cancer, not oral (DOC)	42% (5/12)
Dead not cancer (DNC)	33% (4/12)

[a] N = 22. No data for one lesion.

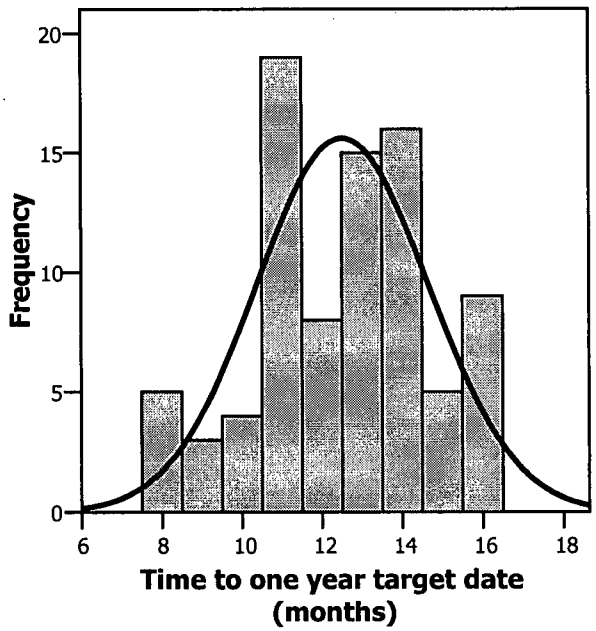


Figure 6. Frequency distribution of time to one year target date

(mean = 13 ± 2 months, n = 84)

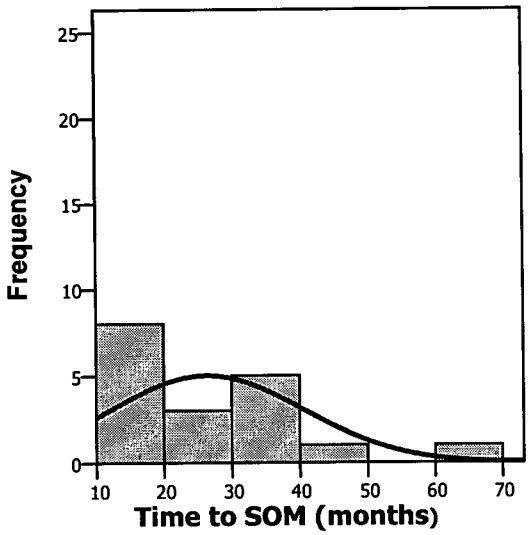


Figure 7. Frequency distribution of time to SOM

(mean = 26 ± 14, n = 18)

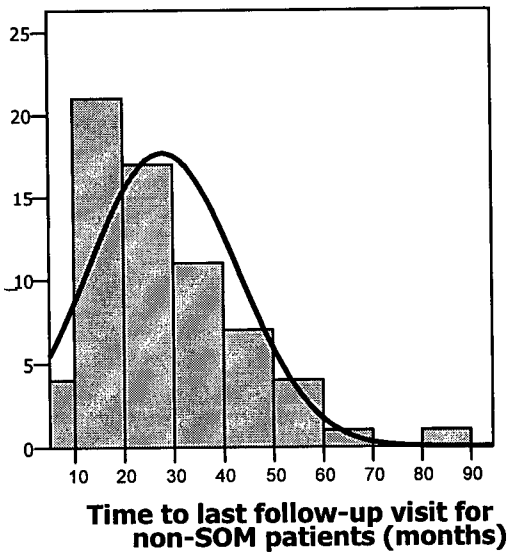


Figure 8. Frequency distribution of time to last follow-up visit for non-SOM patients

(mean = 28 ± 15, n = 66)

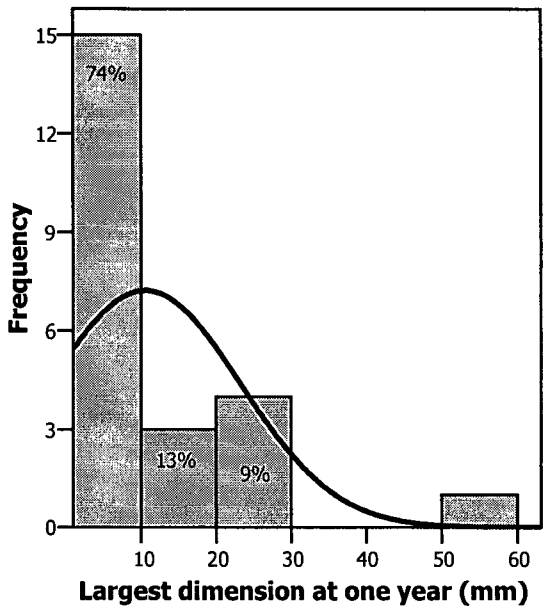


Figure 9. Frequency distribution of largest dimension at the one year target date (mean = 11 ± 13, n = 23)

VI.3.2. Tobacco Habits and post-treatment tumour site manifestation

Table 16 compares clinical changes at the former cancer site in smokers (ever) and non-smokers (never smokers). A greater proportion of smokers had toluidine blue positivity at the one year target date (8 – 16 months), but this trend was not significant (26% of smokers versus 8.3% of non-smokers, $P = 0.122$). This difference between the two groups becomes even less significant when comparing toluidine blue results ever in follow-up. Similarly, a higher percentage of smokers have a lesion at one year but the difference between the

two groups decreases during follow-up. Only one comparison was significant – the presence of multiple lesions at one year was greater in smokers than in non-smokers (39% versus 14%, *P = 0.024*).

Table 16. Comparison of post treatment tumour site manifestations between smokers and nonsmokers.

	Ever Smoker	**Never smoker**	***P value***
Number of cases	56	28	
Toluidine blue staining			
At one year (proportion positive) (n = 74)	26% (13/50)	8% (2/24)	*0.122*
Ever during follow up(proportion positive) (n = 82)	38% (21/55)	26% (7/27)	*0.328*
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	32% (18/56)	18% (5/28)	*0.202*
Ever during follow up	46% (26/56)	36% (10/28)	*0.483*
Size of OPL at former tumour site			
At one year (n = 23)			
Mean largest dimension (mm) (± SD)	12 ± 14	5 ± 2	*0.325*
Median (mm)	6	5	
Range (mm)	2 - 60	3 - 8	
Area (mm^2) (± SD)	80 ± 107	25 ± 23	*0.745*
Proportion of lesions with the largest dimension ≥ 10 mm	44% (8/18)	0% (0/5)	*0.122*

	Ever Smoker	Never smoker	*P value*
Ever during follow up (n = 36)			
Mean largest dimension (mm) (± SD)	17 ± 14	12 ± 9	*0.271*
Area (mm^2) (± SD)	242 ± 383	137 ± 184	*0.337*
Proportion of lesions with the largest dimension ≥ 10 mm	62% (16/26)	50% (5/10)	*0.709*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	47% (8/17)	33% (2/6)	*0.660*
Ever during follow up (worst)	54% (14/26)	70% (7/10)	*0.468*
Proportion with multiple OPLs in the oral cavity			
At one year	39% (22/56)	14% (4/28)	***0.024***
Ever during follow up	39% (22/56)	21% (6/28)	*0.141*
Biopsy of OPLs			
At one year (n = 23)	22% (4/18)	40% (2/5)	*0.576*
During the entire follow up	54% (14/26)	70% (7/10)	*0.468*
Pathology (worst pathology per OPL)			
At one year (n = 4)[a]			
Hyperplasia	25% (1/4)	0	Ø
Mild and moderate dysplasia	50% (2/4)	1	
Severe dysplasia	25% (1/4)	0	

	Ever Smoker	Never smoker	*P value*
Ever during follow up (n = 21)[a]			
Hyperplasia	36% (5/14)	43% (3/7)	Ø
Mild and moderate dysplasia	36% (5/14)	43% (3/7)	
Severe dysplasia	29% (4/14)	14% (1/7)	
Proportion of OPL at former tumour site treated by surgery or laser			
At one year	11% (2/18)	0% (0/5)	*1*
Ever during follow up (n = 36)	19% (5/26)	0% (0/10)	*0.293*

[a] Numbers too small for statistical analysis.

VI.3.3. Target tumour characteristics and post-treatment tumour site manifestation

VI.3.3.1. Risk sites and post-treatment tumour site manifestation

The only significant findings when comparing post treatment clinical changes at the former tumour site, as seen in Table 17, were the proportion of multiple lesions at both one year and ever in patients whose previous tumour was at a low risk site. At the one year target date 57% of the patients with a former cancer at a low risk site had multiple lesions versus 21% in patients with a former tumour at a high-risk site (*P = 0.003*). This statistic remained significant

when comparing multiple lesions between the two groups ever during follow-up (23% high-risk site versus 61% low risk site, *P = 0.002*).

Table 17. Comparison of post treatment tumour site manifestation at high–risk and low-risk sites

	Lesions at high-risk site [a]	Lesions at low-risk sites	*P value*
Number of cases	61	23	
Toluidine blue staining			
At one year (proportion positive) (n = 74)	17% (9/54)	30% (6/20)	*0.213*
Ever during follow up(proportion positive) (n = 82	34% (21/61)	33% (7/21)	*1*
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	26% (16/61)	30% (7/23)	*0.785*
Ever during follow up	41% (25/61)	48% (11/23)	*0.626*
Size of OPL at former tumour site			
At one year (n = 23)			
Mean largest dimension (mm) (± SD)	9 ± 14	14 ± 9	*0.135*
Median (mm)	6	20	
Range (mm)	2 - 60	3 - 25	
Area (mm^2) (± SD)	35 ± 45	144 ± 141	*0.118*
Proportion of lesions with the largest dimension ≥ 10 mm	25% (4/16)	57% (4/7)	*0.182*

	Lesions at high-risk site [a]	Lesions at low-risk sites	*P value*
Ever during follow up (n = 36)			
Mean largest dimension (mm) (± SD)	17 ± 15	14 ± 10	*0.787*
Area (mm^2) (± SD) [c]	245 ± 390	141 ± 181	*0.761*
Proportion of lesions with the largest dimension ≥ 10 mm	60% (15/25)	55% (6/11)	*1*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	44% (7/16)	43% (3/7)	*1*
Ever during follow up (worst)	60% (15/25)	55% (6/11)	*1*
Proportion with multiple OPLs in the oral cavity			
At one year	21% (13/61)	57% (13/23)	***0.003***
Ever during follow up	23% (14/61)	61% (14/23)	***0.002***
Biopsy of OPLs			
At one year (n = 23)	25% (4/16)	29% (2/7)	*1*
Ever during follow up (n = 36)	56% (14/25)	64% (7/11)	*0.729*
Pathology (worst pathology per OPL)			
At one year [b]			
Hyperplasia	25% (1/4)	50% (1/2)	
Mild and moderate dysplasia	50% (2/4)	50% (1/2)	*Ø*
Severe dysplasia	25% (1/4)	0	

	Lesions at high-risk site [a]	Lesions at low-risk sites	*P value*
Ever during follow up (n = 25)			
Hyperplasia	21% (3/14)	71% (5/7)	
Mild and moderate dysplasia	43% (6/14)	29% (2/7)	*Ø*
Severe dysplasia	36% (5/14)	0% (0/9)	
Proportion of OPL at former site treated by surgery or laser			
At one year	13% (2/16)	0% (0/7)	*1*
Ever during follow up (n = 36)	16% (4/25)	9% (1/11)	*1*

[a] High-risk sites for oral cancer: Floor of mouth, lateral and ventral tongue, soft palate complex.
[b] Numbers too small for statistical analysis.

VI.3.3.2. Prior cancer history and post-treatment tumour site manifestation

Table 18 compares the post treatment changes between patients with and without a prior history of oral cancer. No significant findings were found between the two groups when comparing treatment of the target tumour, toluidine blue positivity and the presence of an OPL in follow-up. However, the largest dimension of an OPL at one year was greater in the group with a prior history of oral cancer. This result was approaching significance statistically (21 mm versus 7 mm, *P = 0.053*). Multiple OPLs were significantly more likely to be found in the group of patients with a prior history of oral cancer both at one year

and ever in follow-up (80% versus 20%, *P<0.001*, and 80% versus 23%, *P<0.001*, respectively). Patients with a prior history of oral cancer were also more likely to have been treated with surgery or laser at the former cancer site both at one year and ever during follow-up (33% versus 0%, *P = 0.053*, and 38% versus 7%, *P = 0.061*, respectively).

Table 18. Comparison of post treatment tumour site manifestation between patients with and without a history of oral cancer

	With prior history of oral cancer	**Without prior history of oral cancer**	***P value***
Number of cases	15	69	
Treatment of target tumour			
Proportion with radiation	40% (6/15)	32% (22/69)	*0.558*
Proportion with surgery	73% (11/15)	77% (53/69)	*0.747*
Proportion with both surgery and radiation	13% (2/15)	9% (6/69)	*0.629*
Toluidine blue staining			
At one year (proportion positive) (n = 74)	27% (4/15)	19% (11/59)	*0.488*
Ever during follow up (proportion positive) (n = 82)	40% (6/15)	33% (22/67)	*0.764*
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	40% (6/15)	25% (17/69)	*0.337*
Ever during follow up	53% (8/15)	41% (28/69)	*0.400*

	With prior history of oral cancer	Without prior history of oral cancer	*P value*
Size of OPL at former tumour site			
At one year time (n = 23)			
Mean largest dimension (mm) (± SD)	21 ± 21	7 ± 6	*0.053*
Median	16	5	
Range	3 - 60	2 - 60	
Proportion greater than 10mm	67% (4/6)	24 % (4/17)	*0.131*
Area	150 ± 147	39 ± 54	*0.135*
Ever during follow up (n = 36)			
Mean largest dimension (mm) (± SD)	22 ± 18	14 ± 11	*0.168*
Area (mm^2) (± SD)	409 ± 596	157 ± 208	*0.231*
Proportion of lesions with the largest dimension ≥ 10 mm	75% (6/8)	54% (15/28)	*0.424*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	50% (3/6)	44% (7/16)	*1*
Ever during follow up (worst)	50% (4/8)	60% (17/28)	*0.694*
Proportion with multiple OPLs in the oral cavity			
At one year	80% (12/15)	20% (14/69)	***<0.001***
Ever during follow up	80% (12/15)	23% (16/69)	***<0.001***

	With prior history of oral cancer	Without prior history of oral cancer	*P value*
Biopsy of OPLs			
At one year (n = 23)	33% (2/6)	24% (4/17)	*0.632*
During the entire follow up (n = 36)	75% (6/8)	54% (15/28)	*0.424*
Pathology (worst pathology per OPL)			
At one year (n = 6) [a]			
Hyperplasia	0	50% (2/4)	*Ø*
Mild and moderate dysplasia	50% (1/2)	50% (2/4)	
Severe dysplasia	50% (1/2)	0	
Ever during follow up (n = 21)			
Hyperplasia	17% (1/6)	47% (7/15)	*0.258*
Mild and moderate dysplasia	50% (3/6)	33% (5/15)	
Severe dysplasia	33% (2/6)	20% (3/15)	
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 23)	33% (2/6)	0% (0/17)	*0.059*
Ever during follow up (n = 36)	38% (3/8)	7% (2/28)	*0.061*

[a] Numbers too small for statistical analysis.

VI.3.3.3. Target tumour stage and post-treatment tumour site manifestation

There were significant differences found when comparing the treatment of the target tumour between invasive cancer (SCC) and *CIS*. As seen in Table 19, a much larger proportion of SCC patients received radiation than the CIS patients (49% versus 7%, $P<0.001$) while a much greater proportion of CIS patients received surgery than the patients with SCC (96% versus 66%, $P = 0.002$). Only one patient with CIS received radiation only for the treatment of the target tumour.

Table 19. Comparison of post treatment tumour site manifestation between patients with invasive and non-invasive cancer [a]

	SCC	**CIS**	***P value***
Number of cases	53	27	
Treatment of target tumour			
Proportion with radiation	49% (26/53)	7% (2/27)	***<0.001***
Proportion with surgery	66% (35/53)	96% (26/27)	***0.002***
Proportion with both surgery and radiation	13% (7/53)	4% (1/27)	*0.255*

	SCC	CIS	*P value*
Toluidine blue staining			
At one year (proportion positive) (n = 71)	18% (8/45)	23% (6/26)	*0.758*
Ever during follow up(proportion positive) (n = 78)	31% (16/51)	37% (10/27)	*0.623*
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	26% (14/53)	30% (8/27)	*0.795*
Ever during follow up	40% (21/53)	52% (14/27)	*0.345*
Size of OPL at former tumour site			
At one year time (n = 22 [b])			
Mean largest dimension (mm) (± SD)	12 ± 16	6 ± 3	*0.920*
Median	6	6	
Range	2 - 60	3 - 11	
Proportion greater than 10mm	36% (5/14)	25% (2/8)	*1*
Area (mm^2) (± SD)	80 ± 109.0	24 ± 18	*0.664*
Ever during follow up (n = 35 [b])			
Mean largest dimension (mm) (± SD)	17 ± 15	13 ± 10	*0.495*
Area (mm^2) (± SD) [c]	276 ± 423	111 ±134	*0.434*
Proportion of lesions with the largest dimension ≥ 10 mm	62% (13/21)	50% (7/14)	*0.511*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	50% (7/14)	29% (2/7)	*0.642*
Ever during follow up (worst)	62% (13/21)	50% (7/14)	*0.511*

	SCC	CIS	*P value*
Proportion with multiple OPLs in the oral cavity			
At one year	30% (16/53)	26% (7/27)	*0.797*
Ever during follow up	32% (17/53)	30% (8/27)	*1*
Biopsy of OPLs			
At one year (n = 22[b])	29% (4/14)	25% (2/8)	*1*
During the entire follow up (n = 35 [b])	62% (13/21)	50% (7/14)	*0.511*
Pathology (worst pathology per OPL) [c]			
At one year (n = 6)			
Hyperplasia	1 (25%)	1 (50%)	Ø
Mild and moderate dysplasia	2 (50%)	1 (50%)	
Severe dysplasia	1 (25%)	0	
Ever during follow up (n = 20)			
Hyperplasia	7 (54%)	1 (14%)	Ø
Mild and moderate dysplasia	4 (31%)	3 (43%)	
Severe dysplasia	2 (15%)	3 (43%)	
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 22[b])	0% (0/8)	14% (2/14)	*1*
Ever during follow up (n = 35 [b])	14% (3/21)	14% (2/14)	*1*

[a] Not including 4 VC patients.
[b] 1 OPL was at a former VC site
[c] Numbers too small for statistical analysis.

Table 20 compares post treatment OPL information and the stage of the target tumour. There were no statistically significant differences between the two groups although the early stage group did show a trend towards having a greater number of patients with multiple lesions at one year compared to the late stage patient group (40% versus 11%, *P = 0.056*).

Table 20. Comparison of post treatment tumour site manifestation between patients with early and late stage cancer [a]

	Late Stage (III + IV)	**Early Stage (I + II)**	***P value***
Number of cases	18	35	
Treatment of target tumour			
Proportion with radiation	61% (11/18)	40% (14/35)	*0.162*
Proportion with surgery	56% (10/18)	71% (25/35)	*0.359*
Proportion with both surgery and radiation	17% (3/18)	11% (4/35)	*0.678*
Toluidine blue staining			
At one year (proportion positive) (n = 45)	14% (2/14)	19% (6/31)	*1*
Ever during follow up(proportion positive) (n = 51)	31% (5/16)	29% (10/35)	*1*
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	17% (3/18)	31% (11/35)	*0.333*
Ever during follow up	22% (4/18)	43% (15/35)	*0.226*

	Late Stage (III + IV)	Early Stage (I + II)	*P value*
Size of OPL at former tumour site			
At one year time (n = 14)			
Mean largest dimension (mm) (± SD)	8 ± 10	13 ± 17	*0.368*
Median	3	6	
Range	2 - 20	2 - 60	
Proportion greater than 10 mm	33% (1/3)	36% (4/11)	*1*
Area (mm^2) (± SD)	50 ± 78	88 ± 118	*0.456*
Ever during follow up (n = 19)			
Mean largest dimension (mm) (± SD)	21 ± 13	16 ± 16	*0.307*
Area (mm^2) (± SD)	193 ± 155	314 ± 492	*0.530*
Proportion of lesions with the largest dimension ≥ 10 mm	100% (4/4)	53% (8/15)	*0.245*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	33% (1/3)	55% (6/11)	*1*
Ever during follow up (worst)	75% (3/4)	60% (9/15)	*1*
Proportion with multiple OPLs in the oral cavity			
At one year	11% (2/18)	40% (14/35)	*0.056*
Ever during follow up	17% (3/18)	40% (14/35)	*0.123*
Biopsy of OPLs			
At one year (n = 14)	33% (1/3)	27% (3/11)	*1*
During the entire follow up (n = 19)	50% (2/4)	60% (9/15)	*1*

	Late Stage (III + IV)	Early Stage (I + II)	*P value*
Pathology (worst pathology per OPL) [b]			
At one year			
Hyperplasia	100% (1/1)	0	
Mild and moderate dysplasia	0	67% (2/3)	Ø
Severe dysplasia	0	33% (1/3)	
Ever during follow up			
Hyperplasia	100% (2/2)	56% (5/9)	
Mild and moderate dysplasia	0	33% (3/9)	Ø
Severe dysplasia	0	11% (1/9)	
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 14)	0% (0/3)	18% (2/11)	*1*
Ever during follow up (n = 19)[a]	0% (0/4)	20% (3/15)	*1*

[a] No VC or CIS included.
[b] Numbers too small for statistical analysis.

VI.3.3.4. Target tumour histology and post-treatment tumour site manifestation

As shown in Table 21 there were no significant differences in the post treatment clinical and histological information between well to moderately well differentiated SCC and poorly differentiated SCC groups. The well to moderately well differentiated SCC group had more biopsies completed both at one year (4

versus 0) and ever during follow-up (11 versus 1) but the results were too small for statistical analysis.

Table 21. Comparison of post treatment site information between well to moderately well differentiated and poorly differentiated invasive cancer

	Poorly	**Well to moderately**	***P value***
Number of cases (not including *CIS*)	9	48	
Treatment of target tumour			
Proportion with radiation	56% (5/9)	43% (19/44)	*0.715*
Proportion with surgery	67% (6/9)	71% (31/44)	*1*
Proportion with both surgery and radiation	22% (2/9)	11% (5/44)	*0.588*
Toluidine blue staining			
At one year (proportion positive) (n = 45)	29% (2/7)	18% (7/38)	0.614
Ever during follow up(proportion positive) (n = 51)	25% (2/8)	35% (15/43)	0.703
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year (n = 53)	11% (1/9)	30% (13/44)	0.416
Ever during follow up (n = 53)	22% (2/9)	41% (18/44)	0.456

	Poorly	Well to moderately	*P value*
Size of OPL at former tumour site			
At one year time (n = 14)			
Mean largest dimension (mm) (± SD)	4 ± 0	14 ± 16	0.400
Median	4	8	
Range	4 - 4	2 - 60	
Proportion of lesions with the largest dimension ≥ 10 mm	0% (0/1)	46% (6/13)	*1*
Area (mm^2) (± SD)	8 ± 0	104 ± 117	*1*
Ever during follow up (n = 20)			
Mean largest dimension (mm) (± SD)	19 ± 23	19 ± 15	0.800
Area (mm^2) (± SD)	440 ± 616	291 ± 420	*0.400*
Proportion of lesions with the largest dimension ≥ 10 mm	50% (1/2)	72% (13/18)	*0.521*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	100% (1/1)	54% (7/13)	1.000
Ever during follow up (worst)	50% (1/2)	72% (13/18)	1.000
Proportion with multiple OPLs in the oral cavity			
At one year	22% (2/9)	36% (16/44)	0.701
Ever during follow up	22% (2/9)	39% (17/44)	0.463

	Poorly	Well to moderately	*P value*
Biopsy of OPLs			
At one year (n = 14)	0% (0/1)	31% (4/13)	1.000
During the entire follow up (n = 20)	50% (1/2)	61% (11/18)	1.000
Pathology (worst pathology per OPL) [a]			
At one year (n = 14)			
Hyperplasia	0	25% (1/4)	
Mild and moderate dysplasia	0	50% (2/4)	Ø
Severe dysplasia	0	25% (1/4)	
Ever during follow up (n = 20)			
Hyperplasia	100% (1/1)	55% (6/11)	
Mild and moderate dysplasia	0% (0/1)	27% (3/11)	Ø
Severe dysplasia	0% (0/1)	18% (2/11)	
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 14)	0% (0/1)	8% (1/13)	1.000
Ever during follow up (n = 20)	0% (0/2)	11% (2/18)	1.000

[a] Numbers too small for statistical analysis.

VI.3.3.5. Radiation and post-treatment tumour site manifestation

Table 22 compares post treatment information between patients who had their target tumour treated with radiation, either alone or in combination with surgery, and patients who received no radiation in the treatment of their primary tumour. None of the comparisons were found to be statistically significant except for the appearance of OPLs at one year. A significantly greater percentage of non-homogeneous OPLs were found in patients who had received radiation versus those who did not receive any radiation treatment (75% versus 27%, *P = 0.039*).

Table 22. Comparison of post treatment tumour site information between patients treated with and without radiation

	With radiation [a]	**Without radiation**	***P value***
Number of cases	28	56	
Toluidine blue staining			
At one year (proportion positive) (n = 74)	19% (5/27)	21% (10/47)	*1*
Ever during follow up(proportion positive) (n = 82)	29% (8/28)	37% (20/54)	*0.474*

	With radiation [a]	Without radiation	*P value*
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	29% (88)	27% (15/56)	*1*
Ever during follow up	43% (12/28)	443% (24/56)	*1*
Size of OPL at former tumour site			
At one year time (n = 23)			
Mean largest dimension (mm) (± SD)	8 ± 8	12 ± 15	*0.413*
Median	5	6	
Range	2 - 21	2 - 60	
Proportion greater than 10mm	25% (2/8)	40% (6/15)	*0.657*
Area (mm^2) (± SD)	65 ± 100	69 ± 99	*0.492*
Ever during follow up (n = 36)			
Mean largest dimension (mm) (± SD)	14 ± 12	17 ± 14	*0.636*
Area (mm^2) (± SD)	154 ± 174	243 ± 399	*0.636*
Proportion of lesions with the largest dimension ≥ 10 mm	58.% (7/12)	58% (14/24)	*1*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	75% (6/8)	27% (4/15)	***0.039***
Ever during follow up (worst)	64% (9/14)	46% (12/26)	*0.333*

	With radiation [a]	Without radiation	*P value*
Proportion with multiple OPLs in the oral cavity			
At one year	29% (8/28)	332% (18/6)	*0.804*
Ever during follow up	31% (9/28)	34% (19/56)	*0.811*
Biopsy of OPLs			
At one year (n = 23)	38% (3/8)	20% (3/15)	*0.621*
During the entire follow up (n = 36)	58% (7/12)	58% (14/24)	*1*
Pathology (worst pathology per OPL) [b]			
At one year (n = 6)			
Hyperplasia	33% (1/3)	33% (1/3)	
Mild and moderate dysplasia	67% (2/3)	33% (1/3)	*Ø*
Severe dysplasia	0	33% (1/3)	
Ever during follow up (n = 21)			
Hyperplasia	43% (3/7)	36% (5/14)	
Mild and moderate dysplasia	57% (4/7)	29% (4/14)	*Ø*
Severe dysplasia	0	36% (5/14)	
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 23)	13% (1/8)	7% (1/15)	*1*
Ever during follow up (n = 36)	8% (1/12)	17% (4/24)	*0.646*

[a] Radiation only or in combination with surgery.
[b] Numbers too small for statistical analysis.

VI.4. Second Oral Malignancy (SOM)

For the following analysis the study population was divided into one of two outcome variables, SOM or second oral malignancy at the previously treated cancer site and non-SOM, no second oral malignancy at the previously treated cancer site. There were 18 patients who had a SOM occur by the January 11, 2004 cutoff date. The remaining 66 patients were placed in the non-SOM group.

VI.4.1. Demographics, tobacco habits and SOM

Table 23 displays the comparison of demographic and tobacco variables between the two outcome groups. There were no significant differences found in mean age of diagnosis, gender, ethnicity or tobacco use. Of the 18 patients who had a SOM 78% occurred within 3 years after curative treatment.

Table 23. Comparison of demographics and tobacco habits between SOM and non-SOM groups

	SOM	Non-SOM	*P value*
Number of cases	18	66	
Age at tumour diagnosis			
Mean (yrs ± SD)	61 ± 12	61 ± 13	*0.961*
Median	63	62	
Range	37 – 80	30 – 87	
Proportion male	56% (10/18)	56% (37/66)	*1*
Ethnicity			
Proportion Caucasian	78% (14/18)	73% (48/66)	*0.770*
Proportion Asian	11% (2/18)	21% (14/66)	*0.503*
Proportion Other [a]	11% (2/18)	6% (4/66)	*0.604*
Smoker			
All subjects			
Proportion ever smoker (≥ once a week for ≥ 1 year)	61% (11/18)	68% (45/66)	*0.583*
Proportion current (smokers at diagnosis)	33% (6/18)	30% (20/66)	*0.782*
Proportion continuing (smokers at most recent questionnaire)	27% (5/18)	21% (14/66)	*0.540*

	SOM	Non-SOM	*P value*
Ever smokers only			
Mean pack years (± S.D.)	36 ± 17	42 ± 41	*0.959*
Median pack years	31	36	
Range pack years	15 - 61	1 - 255	
Proportion current (smokers at diagnosis)	55% (6/11)	44% (20/45)	*0.738*
Proportion continuing (smokers at most recent questionnaire)	83% (5/6)	70% (14/20)	*1*
Category of smokers			
Light (< 20 pack year)	33% (3/9)	26% (12/47)	
Medium (20 – 40)	44% (4/9)	32% (15/47)	*0.518*
Heavy and very heavy (> 40)	22% (2/9)	43% (20/47)	
Proportion smokeless tobacco use history (n = 81)	6% (1/18)	13% (8/63)	*0.676*
Proportion betel quid use history (n = 78)	5.9% (1/17)	0/61	*0.218*
Proportion with regular exposure to 2nd hand smoke (n = 83)	83% (15/18)	83% (54/65)	*1*
Proportion with no history of tobacco use (smoked or smokeless) and without regular exposure to 2nd hand smoking	11% (2/18)	15% (10/66)	*1*

[a] Hispanic (3) and Native American (3)

VI.4.2. Target tumour characteristics and SOM

Table 24 shows that there are no significant factors found when comparing outcome with the target tumour characteristics, such as site risk, pathology, histology, staging and treatment.

Table 24. Comparison of tumour characteristics between SOM and non-SOM groups

	SOM	Non-SOM	*P value*
Number of cases	18	66	
Proportion at high-risk site [a]	67% (12/18)	74% (49/66)	*0.558*
Proportion with target diagnosis of SCC [b]	71% (12/17)	65% (41/63)	*0.778*
Proportion with prior oral cancer	17% (3/18)	18% (12/66)	*1*
Tumour stage (n = 80) [c]			
CIS	29% (5/17)	35% (22/63)	
I and II (early stages)	53% (9/17)	41% (26/63)	*0.683*
III and IV (late stages)	18% (3/17)	24% (15/63)	
Tumour Histology (n = 80) [b]			
CIS	28% (5/18)	36% (22/62)	
Well and moderately well differentiated SCC	61% (11/18)	53% (33/62)	*0.817*
Poorly differentiated SCC	11% (2/18)	11% (7/62)	
Treatment			
Proportion with radiation	33% (6/18)	35% (23/66)	*1*
Proportion with surgery	72% (13/18)	77% (51/66)	*0.756*
Proportion with both radiation and surgery	6% (1/18)	11% (7/66)	*1*

[a] High-risk sites for oral cancer: Floor of mouth, lateral and ventral tongue and soft palate complex

[b] Not including VC (4).

[c] No data for 4 cases.

VI.4.3. Post treatment tumour site manifestation and SOM

Outcome results were then compared with the clinical data collected and the results are shown in Table 25. Analysis of the clinical data collected at the one year target date (8 – 16 months) found a significantly larger percentage of the SOM group (50% versus 11%, $P = 0.001$) displayed toluidine blue positivity. The SOM group were also found to be significantly more likely to have a lesion present at the one year follow-up (72% versus 15%, $P < 0.001$). Both of these results remained highly significant during the entire follow-up. The presence of toluidine blue positivity at the former tumour site ever during follow-up was significantly greater in the SOM group (67% versus 25%, $P = 0.002$), as was the presence of an OPL at the former tumour site ever during follow-up (72% versus 35%, $P = 0.007$). Kaplan Meier survival curves for the above results are shown in Figure 10.

The mean largest dimension and area of the OPL in the SOM group at one year were larger than the nonSOM group (14 mm versus 6 mm, $P = 0.067$ and 92 mm^2 versus 36 mm^2, $P = 0.088$), with the results approaching significance statistically. However, a comparison of the mean largest dimension and area 'ever' during follow-up resulted in the SOM group having statistically larger lesions than the nonSOM group (23 mm versus 11 mm, $P = 0.006$ and 422 mm^2 versus 95 mm^2, $P = 0.003$, respectively).

Significantly more biopsies were performed 'ever' during follow-up at the former tumour sites on patients in the SOM group than the nonSOM group both among the entire study population and those who presented with an OPL ever (72% versus 18%, *P<0.001* and 85% versus 44%, *P = 0.033,* respectively).

There were no significant differences found when comparing OPL appearance, multiple lesions in the oral cavity, pathology of the OPL biopsied, metastasis and death between the two groups.

Table 25. Comparison of post treatment tumour site manifestation between SOM and non-SOM groups

	SOM	Non-SOM	*P value*
Number of cases	18	66	
Toluidine blue staining			
At one year (proportion positive) (n = 74)	50% (9/18)	11% (6/56)	***0.001***
Ever during follow up(proportion positive) (n = 82)	67% (12/18)	25% (16/64)	***0.002***
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	72% (13/18)	15% (10/66)	***< 0.001***
Ever during follow up	72% (13/18)	35% (23/66)	***0.007***

	SOM	Non-SOM	*P value*
Size of OPL at former tumour site			
At one year (n = 23)			
Mean largest dimension (mm) (± SD)	14 ± 16	6 ± 5	*0.067*
Median (mm)	8	5	
Range (mm)	3 – 60	2 – 20	
Area (mm^2) (± SD)	92 ± 114	36 ± 61	*0.088*
Proportion of lesions with the largest dimension ≥ 10 mm	46% (6/13)	20% (2/10)	*0.379*
Ever during follow up (n = 36)			
Mean largest dimension (mm) (± SD)	23 ± 16	11 ± 10	***0.006***
Area (mm^2) (± SD)	422 ± 489	95 ± 116	***0.003***
Proportion of lesions with the largest dimension ≥ 10 mm	77% (10/13)	48% (11/23)	*0.159*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year (n = 22)	50% (6/12)	36% (4/10)	*0.680*
Ever during follow up (worst) (n = 36)	69% (9/13)	48% (12/23)	*0.484*
Proportion with multiple OPLs in the oral cavity			
At one year	44% (8/18)	27% (18/66)	*0.249*
Ever during follow up	50% (9/18)	29% (19/66)	*0.101*

	SOM	Non-SOM	*P value*
Biopsy of OPLs			
At one year (n = 23)	23% (3/13)	30% (3/10)	*1*
During the entire follow up			
Proportion of all former sites biopsied (n = 84)	72% (13/18)	18% (12/66)	***<0.001***
Proportion of OPL at former tumour sites biopsied (n = 36)	85% (11/13)	44% (10/23)	***0.033***
Pathology (worst pathology per OPL) [a]			
At one year (n = 23)			
Hyperplasia	33% (1/3)	33% (1/3)	Ø
Mild and moderate dysplasia	33% (1/3)	67% (2/3)	
Severe dysplasia	33% (1/3)	0	
Ever during follow up (n = 36)			
Hyperplasia	55% (7/11)	33% (2/10)	Ø
Mild and moderate dysplasia	23% (3/11)	42% (5/10)	
Severe dysplasia	23% (3/11)	25% (3/10)	
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 23)	8% (1/13)	10% (1/10)	*1*
Ever during follow up (n = 36)	23% (3/13)	9% (2/23)	*0.328*

	SOM	Non-SOM	*P value*
Outcome			
Proportion with metastasis	11% (2/18)	12 % (8/66)	*1*
Proportion dead	11% (2/18)	15% (10/66)	*1*

[a] Numbers too small for statistical analysis.

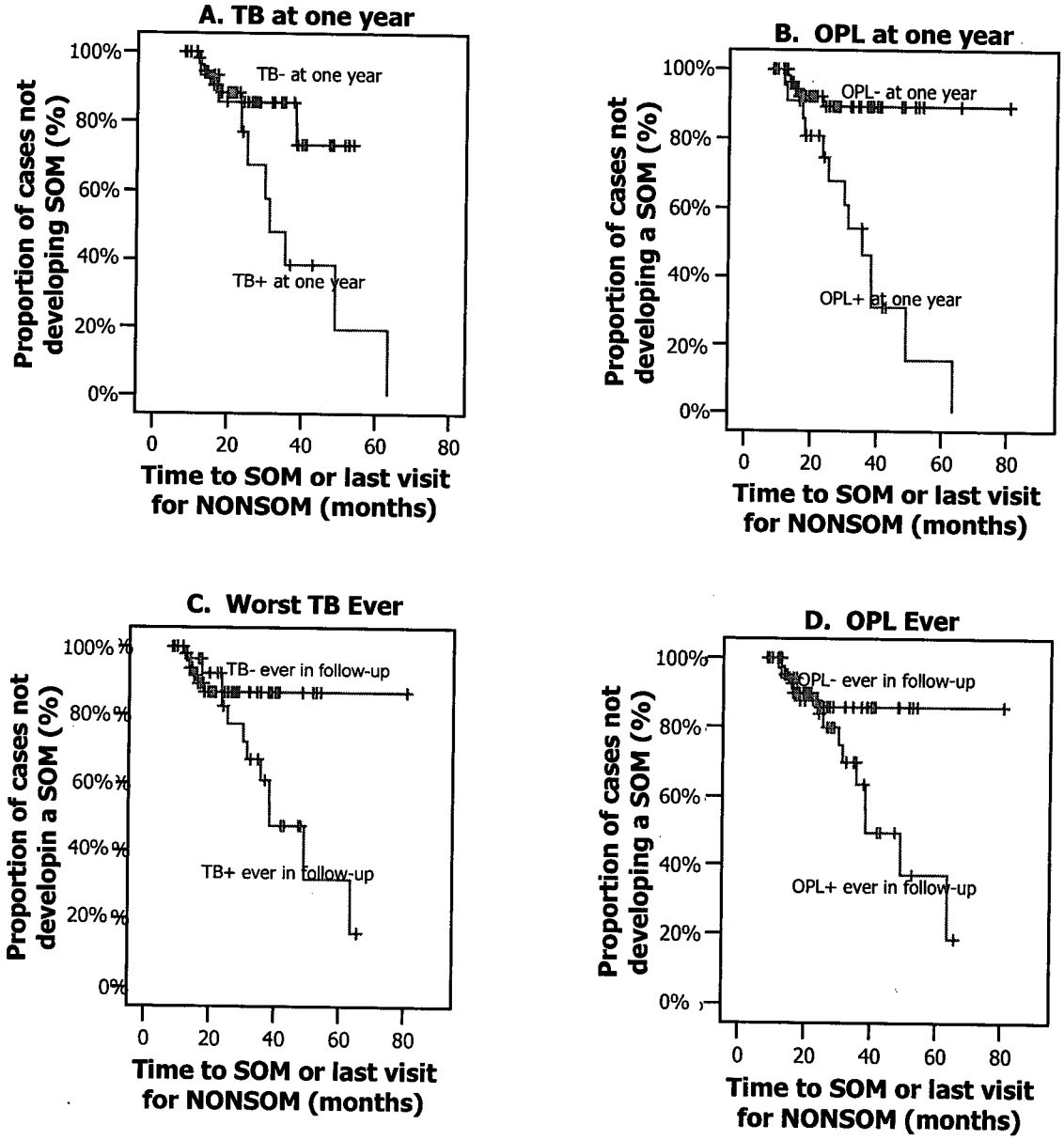


Figure 10. Probability of developing a SOM at the former target tumour site, according to clinical risk factors. A, progression as a function of toluidine blue result at the target date (15 TB+, 59 TB-). B, progression as a function of the presence of an OPL at the target date (23 OPL+, 61 OPL-). C, progression as a function of toluidine blue result, ever during follow-up (28 TB+, 54 TB-). D, progression as a function of the presence of an OPL, ever during follow-up (41 OPL+, 43 OPL-).

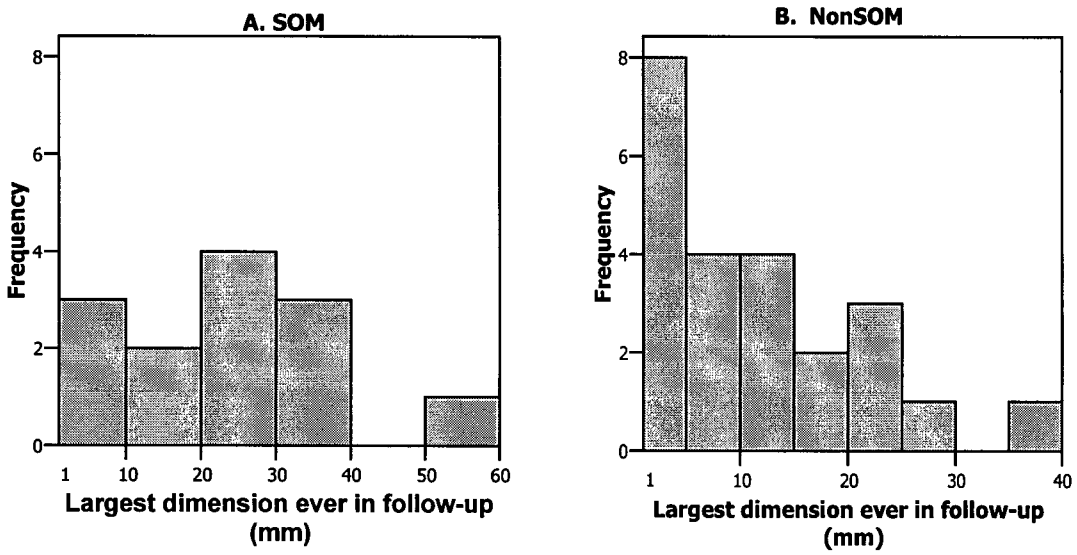


Figure 11. Frequency distribution of the means of the largest dimension ever during follow up.

A, SOM (mean = 23 ± 16 mm, n= 13) and

B, Non-SOM (mean = 11 ± 10mm, n = 23).

VI.5. Factors associated with second oral malignancies (SOM)

As shown in Table 25, several clinical parameters seemed to be associated with appearance of SOM, including TB positive staining, presence of OPL, and large size of OPLs. In this section, these three parameters will be examined.

VI.5.1. Toluidine blue (TB) staining.

The following section compares the demographic, tobacco and post treatment characteristics between toluidine blue positive and negative lesions.

VI.5.1.1. Demographics, tobacco habits and TB

Tables 26 and 27 compare the toluidine blue results at one year and ever, respectively, with the demographic and tobacco habits of the patients.

VI.5.1.1.1. Demographics, tobacco habits and TB at one year

Table 26 compares age at diagnosis, gender, ethnicity and tobacco use in cases which were toluidine blue positive (TB+) and negative (TB-) at one year. There were no significant differences in age at diagnosis, or gender between the two

groups. The proportion of "other" (3 Hispanic and 3 Native American) in the TB+ group was approaching significance (20% versus 3%, *P = 0.054*). There was no significant difference between the proportion of ever smokers between the two groups. The proportion of current smokers (smoking at tumour diagnosis) was close to being significant in the TB+ group (53% versus 25%, *P = 0.059*) and the proportion of continuing smokers in the TB+ group (smoking in follow-up) was significantly greater than the TB- group (47% versus 19%, *P = 0.040*). The amount of tobacco use (pack years exposure) was not significantly different between the two groups.

Table 26. Comparison of demographics and tobacco habits between TB positive and negative lesions at one year

	TB positive	**TB negative**	**P value**
Number of cases	15	59	
Age at tumour diagnosis			
Mean (yrs ± SD)	62 ± 11	62 ± 13	*0.901*
Median	63	62	
Range	43 - 84	37 - 87	
Proportion male	53% (8/15)	56% (33/59)	*1*
Ethnicity			
Proportion Caucasian	67% (10/15)	74% (44/59)	0.531
Proportion Asian	13% (2/15)	22% (13/59)	0.721
Proportion Other [a]	20% (3/15)	3% (2/59)	0.054

	TB positive	TB negative	P value
Smoker			
All subjects			
Proportion ever smoker (≥ once a week for ≥ 1 year)	87% (13/15)	63% (37/59)	*0.122*
Proportion current (smokers at diagnosis)	53% (8/15)	25% (15/59)	*0.059*
Proportion continuing (smokers at most recent questionnaire)	47% (7/15)	19% (11/59)	***0.040***
Ever smokers only			
Mean pack years (± S.D.)	33 ± 18	45 ± 44	*0.558*
Median pack years	31	36	
Range pack years	11 - 61	1 - 255	
Proportion current (smokers at diagnosis)	62% (8/13)	41% (15/37)	*0.215*
Proportion continuing (smokers at most recent questionnaire)	88% (7/8)	73% (11/15)	*0.621*
Category of smokers			
Light (< 20 pack year)	31% (4/13)	24% (9/37)	
Medium (20 – 40)	31% (4/13)	32% (12/37)	*0.670*
Heavy and very heavy (> 40)	39% (5/13)	43% (16/37)	
Proportion smokeless tobacco use history (n = 71)	7% (1/14)	12% (7/57)	*1*
Proportion betel quid use history (n = 68)	7% (1/14)	0% (0/54)	*0.206*
Proportion with regular exposure to 2nd hand smoke (n = 73)	93% (13/14)	81% (48/59)	*0.440*
Proportion with no history of tobacco use (smoked or smokeless) and without regular exposure to 2nd hand smoking	7% (1/15)	15% (9/59)	*0.676*

[a] Hispanic (3) and Native American (3)

VI.5.1.1.2. Demographics, tobacco habits and TB at one year ever in follow-up

As shown in Table 27, there were no demographic or tobacco habit differences found between TB+ and TB- patients ever during follow-up. However, there was a greater percentage of "other" ethnicity (3 Hispanic and 3 Native Americans) in the TB+ group that approached significance (18% versus 2%, *P = 0.054*).

Table 27. Comparison of demographics and tobacco habits between TB positive and negative lesions ever during follow-up.

	TB positive	**TB negative**	**P value**
Number of cases	28	54	
Age at tumour diagnosis			
Mean (yrs ± SD)	61 ± 13	62 ± 13	*0.864*
Median	62	62	
Range	43 - 84	30 - 87	
Proportion male	57% (16/28)	64% (29/54)	*0.818*
Ethnicity			
Proportion Caucasian	68% (19/28)	78% (42/54)	0.425
Proportion Asian	14% (4/28)	20% (11/54)	0.721

	TB positive	TB negative	P value
Proportion Other [a]	18% (5/28)	2% (1/54)	0.054
Smoker			
All subjects			
Proportion ever smoker (≥ once a week for ≥ 1 year)	75% (21/28)	63% (34/54)	*0.328*
Proportion current (smokers at diagnosis)	39% (11/28)	26% (14/54)	*0.312*
Proportion continuing (smokers at most recent questionnaire)	32% (9/28)	19% (10/54)	*0.179*
Ever smokers only			
Mean pack years (± S.D.)	41 ± 26	40 ± 44	*0.436*
Median pack years	36	34	
Range pack years	7 - 104	1 - 255	
Proportion current (smokers at diagnosis)	52% (11/21)	41% (14/34)	*0.578*
Proportion continuing (smokers at most recent questionnaire)	82% (9/11)	71% (10/14)	*0.661*
Category of smokers			
Light (< 20 pack year)	29% (6/21)	37% (9/34)	
Medium (20 – 40)	24% (5/21)	38% (13/34)	*0.652*
Heavy and very heavy (> 40)	48% (10/21)	35% (12/34)	
Proportion smokeless tobacco use history (n = 79)	12% (3/26)	11% (6/53)	*1*
Proportion betel quid use history (n = 76)	4% (1/25)	0% (0/51)	*0.329*
Proportion with regular exposure to 2nd hand smoke (n = 81)	78% (21/27)	87% (47/54)	*0.341*
Proportion with no history of tobacco use (smoked or smokeless) and without regular exposure to 2nd hand smoking	14% (4/28)	13% (7/54)	*1*

[a] Hispanic (3) and Native American (3)

VI.5.1.2. Target tumour characteristics and TB

See section VI.3.3. No differences were seen in tumour information including location, prior cancer history, stage, histology and treatment between TB positive and negative lesions at one year or ever in follow-up.

VI.5.1.3. Post-treatment tumour site manifestation and TB

Tables 28 and 29 display the clinical information comparing TB+ and TB- sites at one year and worst ever during follow-up, respectively.

VI.5.1.3.1. Post-treatment tumour site manifestation and TB at one year

Table 28 examines TB+ and TB- lesions for association with other clinical indicators at one year. As expected, a significant percentage of TB+ cases (93%) had the stain retained when a clinical lesion (OPL) was present. The one site which stained TB + without a lesion had an area of denture irritation that stained TB equivocal. TB status of this area at the following visit was negative. In comparison, leukoplakia was present in only 14% of the TB- lesions ($P < 0.001$). In contrast, only one (7%) case had a TB+ former cancer site without

apparent clinical lesion. TB+ lesions were found to have a significantly greater percentage of lesions with a non-homogeneous than TB- lesions (79% versus 40%, *P = 0.038*).

There was a tendency for TB+ lesions to be larger in size, both in largest dimension (14 mm versus 6 mm, *P = 0.070*) and area (91 mm^2 versus 34 mm^2, *P = 0.145*) at one year, but these results were not significant. Similarly, TB+ lesions at one year had a greater mean dimension (21 mm versus 12 mm, *P = 0.066*) and area 'ever' in follow-up (352 mm^2 versus 127 mm^2, *P = 0.025*). The latter was significant.

Not surprisingly, TB+ lesions which were TB+ at one year were more apt to be biopsied both at one year and 'ever' in follow-up than TB- lesions. At one year the results were very close to significant (43% versus 0%, *P = 0.051*), while the results ever during follow-up were found to be significant (79% versus 40%, *P = 0.039*).

Table 28. Comparison of post-treatment tumour site manifestation between TB positive and negative lesions at one year

	TB positive	TB negative	*P value*
Number of cases	15	59	
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	93% (14/15)	14% (8/59)	***<0.001***
Ever during follow up	93% (14/15)	34% (20/59)	***<0.001***
Size of OPL at former tumour site			
At one year time (n = 22)			
Mean largest dimension (mm) (± SD)	14 ± 15	6 ± 6	*0.070*
Median	7	4	
Range	3 - 60	2 - 20	
Proportion of lesions with the largest dimension ≥ 10 mm	43% (6/14)	25% (2/8)	*0.649*
Area (mm^2) (± SD)	91 ± 115	34 ± 48	*0.145*
Ever during follow up (n = 34)			
Mean largest dimension (mm) (± SD)	21 ± 16	12 ± 11	*0.066*
Area (mm^2) (± SD)	352 ± 486	127 ± 165	***0.025***
Proportion of lesions with the largest dimension ≥ 10 mm	71% (10/14)	50% (10/20)	*0.296*

	TB positive	TB negative	*P value*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	50% (7/14)	43% (3/7)	*1*
Ever during follow up (worst)	79% (11/14)	45% (9/20)	*0.053*
Proportion with multiple OPLs in the oral cavity			
At one year	47% (7/15)	31% (18/59)	*0.359*
Ever during follow up	53% (8/15)	32% (19/59)	*0.145*
Biopsy of OPLs			
At one year (n = 22)	43% (6/14)	0% (0/8)	*0.051*
During the entire follow up (n = 34)	79% (11/14)	40% (8/20)	***0.038***
Pathology (worst pathology per OPL) [a]			
At one year (n = 6)			
Hyperplasia	33% (2/6)	0	Ø
Mild and moderate dysplasia	50% (3/6)	0	
Severe dysplasia	17% (1/6)	0	
Ever during follow up (n = 19)			
Hyperplasia	36% (4/11)	50% (4/8)	Ø
Mild and moderate dysplasia	36% (4/11)	25% (2/8)	
Severe dysplasia	27% (3/11)	25% (2/8)	

	TB positive	TB negative	*P value*
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 22)	7% (1/14)	13% (1/8)	*1*
Ever during follow up (n = 34)[a]	29% (4/14)	5% (1/20)	*0.135*

[a] Numbers too small for statistical analysis.

VI.5.1.3.2. Post-treatment tumour site manifestation and TB ever in follow-up

A further comparison was made between worst TB status ever in follow-up and the clinical indicators and lesion characteristics and the results can be seen in Table 29. Similar to the TB results at one year, there was a strong association with TB+ and OPLs both at one year and ever during follow-up (64% versus 9%, *P<0.001* and 82% versus 24%, *P<0.001*). The 5 TB+ results that were not associated with an OPL were all classified as TB equivocal at the site of a scar, graft or denture irritation. Four were found to be TB- at the next follow-up visit by the Oral Medicine specialist or Oral Medicine/Oral Pathology resident. The remaining patient suffered a recurrence 4 months later. There was a tendency for the largest mean dimension and area to be greater in the TB+ ever group but these results were not significant (12 mm versus 6 mm, *P = 0.080* and 77 mm^2 versus 34 mm^2, *P = 0.094,* respectively). OPLs which stained TB+ ever in

follow-up were significantly more likely to have a non-homogeneous appearance (70% versus 29%, *P = 0.024*).

Table 29. Comparison of post-treatment tumour site manifestation between TB positive and negative lesions ever

	TB positive	**TB negative**	***P value***
Number of cases	28	54	
Presence of an oral premalignant lesion (OPL) at former tumour site			
At one year	64% (18/28)	9% (5/54)	***<0.001***
Ever during follow up	82% (23/28)	24% (13/54)	***<0.001***
Size of OPL at former tumour site			
At one year time (n = 23)			
Mean largest dimension (mm) (± SD)	12 ± 14	6 ± 8	*0.080*
Median	6	3	
Range	3 - 60	2 - 20	
Proportion of lesions with the largest dimension ≥ 10 mm	39% (7/18)	20% (1/5)	*0.621*
Area (mm^2) (± SD)	77 ± 105	34 ± 60	*0.094*
Ever during follow up (n = 36)			
Mean largest dimension (mm) (± SD)	18 ± 14	12 ± 11	*0.100*
Area (mm^2) (± SD)	77 ± 105	34 ± 60	*0.094*
Proportion of lesions with the largest dimension ≥ 10 mm	65% (15/23)	46% (6/13)	*0.310*

	TB positive	TB negative	*P value*
Appearance of OPL at former tumour site (Proportion non-homogeneous)			
At one year	44% (8/18)	40% (2/5)	*1*
Ever during follow up (worst)	70% (16/23)	39% (5/13)	*0.071*
Proportion with multiple OPLs in the oral cavity			
At one year	36% (10/28)	30% (16/54)	*0.622*
Ever during follow up	39% (11/28)	32% (17/54)	*0.624*
Biopsy of OPLs			
At one year (n = 23)	33% (6/18)	0% (0/5)	*0.272*
During the entire follow up (n = 36)	78% (18/23)	23% (3/13)	***0.002***
Pathology (worst pathology per OPL) [a]			
At one year (n = 6)			
Hyperplasia	33% (2/6)	0	Ø
Mild and moderate dysplasia	50% (3/6)	0	
Severe dysplasia	17% (1/6)	0	
Ever during follow up (n = 19)			
Hyperplasia	33% (6/18)	67% (2/3)	Ø
Mild and moderate dysplasia	39% (7/18)	33% (1/3)	
Severe dysplasia	28% (5/18)	0	

	TB positive	TB negative	*P value*
Proportion of OPL at former tumour site treated by surgery or laser			
At one year (n = 23)	6% (1/18)	20% (1/5)	*0.395*
Ever during follow up (n = 36)	17% (4/23)	8% (1/13)	*0.634*

[a] Numbers too small for statistical analysis.

VI.5.2. Presence of oral premalignant lesions (OPL) at the former cancer site.

VI.5.2.1. Comparison of demographics and tobacco habits between patients with an OPL and those without

Tables 30 and 31 compare the demographic and tobacco habits in patients with an OPL at one year (Table 30) and ever (Table 31).

VI.5.2.1.1. Demographics, tobacco habits and OPL at one year

Table 30 compares age at diagnosis, ethnicity and tobacco habits between patients who had an OPL present at one year and those who did not. Age at diagnosis and ethnicity were not found to be significantly different between the two groups. Smoking habit was associated with OPL status. OPLs were more

frequently present among those cases that were smokers at the time of their oral cancer diagnosis. There was a tendency for more current smokers (48% versus 25%, *P = 0.063*) and continuing smokers (44% versus 15%, *P = 0.008*) to have an OPL although only the latter comparison was statistically significant.

Table 30. Comparison of demographics and tobacco habits between patients with OPL at prior cancer sites and patients without OPL at the sites at one year

	OPL	No OPL	P value
Number of cases	23	61	
Age at tumour diagnosis			
Mean (yrs ± SD)	61 ± 13	62 ± 13	*0.819*
Median	62	62	
Range	40 - 84	30 - 87	
Proportion male	65% (15/23)	53% (32/61)	*0.333*
Ethnicity			
Proportion Caucasian	26% (6/23)	26% (16/61)	1
Proportion Asian	13% (3/23)	21% (13/61)	0.538
Proportion Other [a]	13% (3/23)	5% (3/61)	0.339
Smoker			
All subjects			
Proportion ever smoker (≥ once a week for ≥ 1 year)	78% (18/23)	62% (38/61)	*0.202*
Proportion current (smokers at diagnosis)	48% (11/23)	25% (15/61)	*0.063*
Proportion continuing (smokers at most recent questionnaire)	44% (10/23)	15% (9/61)	***0.008***

	OPL	No OPL	P value
Ever smokers only			
Mean pack years (± S.D.)	35 ± 17	43 ± 44	*0.993*
Median pack years	36	32	
Range pack years	11 - 73	1 - 255	
Proportion current (smokers at diagnosis)	61% (11/18)	40% (15/38)	*0.159*
Proportion continuing (smokers at most recent questionnaire)	91% (10/11)	60% (9/15)	*0.178*
Category of smokers			
Light (< 20 pack year)	22% (4/18)	29% (11/38)	
Medium (20 – 40)	39% (7/18)	32% (12/38)	*0.791*
Heavy and very heavy (> 40)	39% (7/18)	40% (15/38)	
Proportion smokeless tobacco use history (n = 81)	5% (1/22)	14% (8/59)	*0.432*
Proportion betel quid use history (n = 78)	5% (1/21)	0% (0/57)	*0.269*
Proportion with regular exposure to 2nd hand smoke (n = 83) b	82% (18/22)	84% (51/61)	*1*
Proportion with no history of tobacco use (smoked or smokeless) and without regular exposure to 2nd hand smoking	13% (3/23)	15% (9/61)	*1*

[a] Hispanic (3) and Native American (3)
[b] History of daily second hand smoke exposure.

VI.5.2.1.2. Demographics, tobacco habits and OPL worst ever in follow-up

As shown in Table 31 there were no significant differences in age at diagnosis or ethnicity between patients who had an OPL ever at the former tumour site and those who did not. Smoking status also appeared to be a factor in the presence of an OPL during follow-up. A larger percentage of patients with an OPL ever in

follow-up were current smokers (42% versus 23%, *P = 0.095*) and continuing smokers (33% versus 15%, *P = 0.064*). However, neither result was significant.

Table 31. Comparison of demographics and tobacco habits between patients with OPL at prior cancer sites and patients without OPL at the sites ever

	OPL	No OPL	P value
Number of cases	36	48	
Age at tumour diagnosis			
Mean (yrs ± SD)	62 ± 13	61 ± 13	*0.769*
Median	63	62	
Range	40 - 87	30 - 84	
Proportion male	58% (21/36)	54% (26/48)	
Ethnicity			
Proportion Caucasian	72% (26/36)	75% (36/48)	0.806
Proportion Asian	17% (6/36)	21% (10/48)	0.781
Proportion Other [a]	11% (4/36)	4% (2/48)	0.395
Smoker			
All subjects			
Proportion ever smoker (≥ once a week for ≥ 1 year)	72% (26/36)	63% (30/48)	*0.483*
Proportion current (smokers at diagnosis)	42% (15/36)	23% (11/48)	*0.095*
Proportion continuing (smokers at most recent questionnaire)	33% (12/36)	15% (7/48)	*0.064*

	OPL	No OPL	P value
Ever smokers only			
Mean pack years (± S.D.)	37 ± 24	43 ± 46	*0.928*
Median pack years	33	34	
Range pack years	5 - 104	1 - 255	
Proportion current (smokers at diagnosis)	58% (15/26)	37% (11/30)	*0.179*
Proportion continuing (smokers at most recent questionnaire)	80% (12/15)	64% (7/11)	*0.407*
Category of smokers			
Light (< 20 pack year)	27% (7/26)	27% (8/30)	
Medium (20 – 40)	35% (9/26)	33% (10/30)	*0.934*
Heavy and very heavy (> 40)	39% (10/26)	40% (12/30)	
Proportion smokeless tobacco use history (n = 81)	9% (3/34)	13% (6/47)	*0.727*
Proportion betel quid use history (n = 78)	3% (1/32)	0% (0/46)	*0.410*
Proportion with regular exposure to 2nd hand smoke (n = 83) [b]	83% (29/35)	83% (40/48)	*1*
Proportion with no history of tobacco use (smoked or smokeless) and without regular exposure to 2nd hand smoking	11% (4/36)	17% (8/48)	*0.543*

[a] Hispanic (3) and Native American (3)
[b] History of daily second hand smoke exposure.

VI.5.2.2. Target tumour characteristics and the presence of an OPL

See section VI.3.3. No differences were seen in tumour information including location, prior cancer history, stage, histology and treatment between patients with or without OPLs at the former cancer site at one year or ever during follow-up.

VI.5.3. Size of oral premalignant lesions (OPL)

This section compares the size of OPLS and the demographic, tobacco habits and post treatment manifestations at the former cancer site.

VI.5.3.1. Demographics, tobacco habits and size of OPL

VI.5.3.1.1. Demographics, tobacco habits and size of OPL at one year

As shown in Table 32 there were no significant differences in the age at diagnosis, tobacco habits or ethnicity between OPLs equal to or greater than 10mm and OPLs less than 10mm.

Table 32. Comparison of demographics and tobacco habits in patients with OPL ≥ 10 mm and those patients with OPL < 10 mm at one year

	OPL ≥ 10mm	OPL < 10mm	*P value*
Number of cases	8	15	
Age at tumour diagnosis			
Mean (yrs ± SD)	62 ± 11	60 ± 14	*0.815*
Median	62	62	
Range	46 - 81	40 - 84	
Proportion male	63% (5/8)	67% (10/15)	*1*
Ethnicity			
Proportion Caucasian	75% (6/8)	73% (11/15)	*1*
Proportion Asian	13% (1/8)	13% (2/15)	*1*
Proportion Other [a]	13% (1/8)	13% (2/15)	*1*
Smoker			
All subjects			
Proportion ever smoker (≥ once a week for ≥ 1 year)	100% (8/8)	67% (10/15)	*0.122*
Proportion current (smokers at diagnosis)	50% (4/8)	47% (7/15)	*1*
Proportion continuing (smokers at most recent questionnaire)	50% (4/8)	40% (6/15)	*0.685*

	OPL ≥ 10mm	OPL < 10mm	*P value*
Ever smokers only			
Mean pack years (± S.D.)	33 ± 21	37 ± 15	*0.515*
Median pack years	25	36	
Range pack years	15 - 73	11 - 61	
Proportion current (smokers at diagnosis)	50% (4/8)	70% (7/10)	*0.630*
Proportion continuing (smokers at most recent questionnaire)	100% (4/4)	86% (6/7)	*1*
Category of smokers			
Light (< 20 pack year)	38% (3/8)	10% (1/10)	
Medium (20 – 40)	25% (2/8)	50% (5/10)	*0.421*
Heavy and very heavy (> 40)	38% (3/8)	40% (4/10)	
Proportion smokeless tobacco use history (n = 22)	0% (0/7)	7% (1/15)	*1*
Proportion betel quid use history (n = 21)	14% (1/7)	0% (0/14)	*0.333*
Proportion with regular exposure to 2nd hand smoke (n = 22) [b]	100% (7/7)	73% (11/15)	*0.263*
Proportion with no history of tobacco use (smoked or smokeless) and without regular exposure to 2nd hand smoking	0% (0/8)	20% (3/15)	*0.526*

[a] Hispanic (3) and Native American (3)
[b] History of daily second hand smoke exposure.

VI.5.3.1.2. Demographics, tobacco habits and largest size of OPL ever in follow-up

The age at diagnosis and ethnicity were not found to be significantly related to the largest size of an OPL ever. Interestingly, the size of the lesion was found to be significantly associated with the amount of tobacco used. Patients with the smaller lesions smoked more than the patients with the larger lesions (50 pack year versus 29 pack year, *P = 0.012*). This result was also found to be significant when comparing categories of smoking amounts. There was a greater percentage of heavy smokers in the OPL < 10mm group (*P = 0.017*).

Table 33. Comparison of demographics and tobacco habits in patients with OPL ≥ 10 mm and those patients with OPL < 10 mm ever

	OPL ≥ 10mm	**OPL < 10mm**	**P value**
Number of cases	21	15	
Age at tumour diagnosis			
Mean (yrs ± SD)	64 ± 67	59 ± 14	*0.246*
Median	67	56	
Range	43 - 84	40 - 87	
Proportion male	67% (14/21)	47% (7/15)	*0.310*

	OPL ≥ 10mm	OPL < 10mm	P value
Ethnicity			
Proportion Caucasian	67% (14/21)	80% (12/15)	0.468
Proportion Asian	19% (4/210	13% (2/15)	1
Proportion Other [a]	14% (3/21)	7% (1/15)	0.626
Smoker			
All subjects			
Proportion ever smoker (≥ once a week for ≥ 1 year)	76% (16/21)	67% (10/15)	*0.709*
Proportion current (smokers at diagnosis)	33% (7/21)	53% (8/15)	*0.310*
Proportion continuing (smokers at most recent questionnaire)	29% (6/21)	40% (6/15)	*0.499*
Ever smokers only			
Mean pack years (± S.D.)	29 ± 22	50 ± 22	***0.012***
Median pack years	22	44	
Range pack years	5 - 77	30 – 104	
Proportion current (smokers at diagnosis)	44% (7/16)	80% (8/10)	*0.109*
Proportion continuing (smokers at most recent questionnaire)	86% (6/7)	75% (6/8)	*1*
Category of smokers			
Light (< 20 pack year)	44% (7/16)	0% (0/10)	
Medium (20 – 40)	31% (5/16)	40% (4/10)	***0.017***
Heavy and very heavy (> 40)	25% (4/16)	60% (6/10)	
Proportion smokeless tobacco use history (n = 34)	5% (1/20)	14% (2/14)	*0.555*
Proportion betel quid use history (n = 32)	5% (1/20)	0% (0/12)	*1*
Proportion with regular exposure to 2nd hand smoke (n = 35) [b]	85% (17/20)	80% (12/15)	*1*
Proportion with no history of tobacco use (smoked or smokeless) and without regular exposure to 2nd hand smoking	14% (3/21)	7% (1/15)	*0.626*

[a] Hispanic (3) and Native American (3)

[b] History of daily second hand smoke exposure.

VI.5.3.2. Target tumour characteristics and the size of OPL

See section VI.3.5. No differences were seen in tumour information including location, prior oral cancer history and treatment between patients with an OPL ≥ 10 mm or < 10 mm.

VI.5.3.3. Post-treatment tumour site information and the size of OPL

Tables 34 and 35 display the post treatment site information at one year and worst ever, respectively for OPL size greater than 10 mm and less than 10 mm.

VI.5.3.3.1. Post-treatment tumour site information and the size of OPL at one year

The size of the OPL at one year was only associated significantly with the presence of multiple OPLs within the oral cavity (75% versus 27%, *P = 0.039*). Otherwise, TB positivity, appearance and biopsies performed were not associated with these size categories.

Table 34. Comparison of post-treatment tumour site manifestation in patients with OPL ≥ 10 mm and those patients with OPL < 10 mm at one year

	Size of OPL ≥ 10 mm	Size of OPL < 10 mm	*P value*
Number of cases	8	15	
Proportion Toluidine blue positive (n = 22)	75% (6/8)	57% (8/14)	*0.649*
Appearance of OPL (Proportion non-homogeneous)	50% (4/8)	43% (6/14)	*1*
Proportion with multiple OPLs in the oral cavity	75% (6/8)	27% (4/15)	***0.039***
Biopsy of OPLs	13% (1/8)	33% (5/15)	*0.369*
Pathology (worst pathology per OPL)			
Hyperplasia	0	40% (2/5)	
Mild and moderate dysplasia	0	60% (3/5)	*Ø*
Severe dysplasia	100% (1/1)	0	
Proportion of OPL at former tumour site treated by surgery or laser	13% (1/8)	7% (1/15)	*1*

VI.5.3.3.2. Post-treatment tumour site information and the size of OPL ever during follow-up

Interestingly, as shown in Table 36, the presence of an OPL greater than or equal to 10mm ever during follow-up is strongly associated with a non-homogeneous appearance (81% versus 27%, $P = 0.002$). Toluidine blue positivity, multiple lesions, rate of biopsy and associated pathology were not found to be related to these size categories.

Table 35. Comparison of post-treatment tumour site manifestation in patients with OPL ≥ 10 mm and those patients with OPL < 10 mm ever

	Size of OPL ≥ 10 mm	Size of OPL < 10 mm	*P value*
Number of cases	21	15	
Proportion Toluidine blue positive (n = 36)	71% (15/21)	53% (8/15)	0.310
Appearance of OPL (proportion non-homogeneous)	81% (17/21)	27% (4/15)	***0.002***
Proportion with multiple OPLs in the oral cavity	48% (10/21)	27% (4/15)	*0.302*
Biopsy of OPLs	57% (12/21)	60% (9/15)	*1*
Pathology (worst pathology per OPL)			
Hyperplasia	42% (5/12)	33% (3/9)	
Mild and moderate dysplasia	25% (3/12)	56% (5/9)	*0.691*
Severe dysplasia	33% (4/12)	11% (1/9)	
Proportion of OPL at former tumour site treated by surgery or laser	19% (4/21)	7% (1/15)	*0.376*

This study has a power of 99% for detecting differences in the proportion of OPLs found in patients who developed an SOM and those that did not (non-SOM). The difference in the proportions of TB positive patients between the

same two groups has a power of 95% (1 sided test, alpha level of 5%). This calculation is performed by a web-based statistics program (Lenth 2004) that runs on the Windows® XP professional platform.

VII. DISCUSSION

The mortality rate for oral cancer is high primarily due to late diagnosis, frequent recurrence and the development of second primary tumours. Although patients with a history of oral cancer are monitored extensively, it is not uncommon for a SOM to appear suddenly, right under the clinician's watchful eyes. The identification of high-risk OPLs in patients with a prior history of cancer before these lesions progress into SOM would be critical for early intervention and improving the prognosis of patients with oral cancer.

Currently, clinicians use a number of clinicopathological criteria, derived from primary OPL research, to predict the risk of OPLs for a SOM. Factors associated with risk of progression for primary OPL include the site and size of the lesion, clinical appearance of the lesion and pathological presence and degree of dysplasia (Axéll *et al.,* 1984; Bouquot and Whitaker, 1994; Lummerman, Freedman and Kerpel, 1995; Schepman *et al.,* 1998; Shiu, *et al.,* 2000; Lee *et al.,* 2000). However, little or no research has been done to see if these clinicopathological risk factors of primary OPLs apply to OPLs at sites of previous oral SCC. A clinician's ability to predict which lesion is at a high-risk for a SOM prior to a second cancer diagnosis would be very advantageous. With this information treatment can be performed with a minimal amount of morbidity and with less emotional distress to the patient. This thesis investigated the

clinicopathological risk predictors in 84 patients with a history of oral cancer in a longitudinal study setting. In addition, the risk predicting value of toluidine blue, a visual aid, was also studied since recent retrospective studies from both primary OPLs and OPLs in patients with a history of oral cancer have shown cancer risk predictive value (Epstein et al., 2003; Guo et al., 2001). Two approaches were used. The first was to look at these risk factors at one year post tumour treatment, which is a critical point for clinical identification since the majority of SOM occur within the first two or three years of tumour treatment. The second was to look at these risk factors throughout the duration of follow-up (ever). To exclude the confounding effects of other parameters on SOM, the demographic, habit information and target tumour information were compared between the SOM group and non-SOM group.

During the study period, 18 of the 84 (21%) patients with a history of oral SCC developed a SOM at the previously treated cancer site, 50% within 2 years and 78% within 3 years. The study investigated the relationship between SOM and the following parameters: demographic features, tobacco habits, target tumour information, and clinical features of post-treatment tumour sites. Of these parameters, it was found that SOM was related to the uptake of TB at the prior cancer site, and the presence and large size of OPLs at the previously treated cancer site.

VII.1. Demographic characteristics and SOM

In this study, demographic characteristics including gender, age at diagnosis of oral SCC, and ethnicity did not affect the outcome of development of SOM. It should be noted that the literature has suggested that patients diagnosed with oral SCC at a young age will have a poorer outcome than older patients. This thesis did not examine this factor, due to the limited number of patients of a young age ($\leq$ 40 years old).

VII.2. Tobacco usage and SOM

As discussed in the literature review, tobacco usage is the most important etiological factor for oral SCC, and continued usage of tobacco products after a diagnosis of oral SCC is associated with increased incidence of SOM. For example, Day *et al.* (1994b) found that patients who quit more than one year prior to the diagnosis of their primary tumour were at significantly less risk of developing a SOM. Silverman (2003) stated that oral cancer patients who do not change their habits are at a much greater risk of developing a SOM.

These results did show that smokers were more likely to have a history of prior oral cancer, i.e., the current target cancer is already a SOM for those patients (27% vs. 0% for patients who never smoked, $P = 0.002$). However, during the

study follow up period smoking status was not found to relate to the development of SOM, although smoking was related to 2 of the 3 risk factors that predict SOM: the presence of OPL and uptake of TB staining (discussed in Sections VI.4.3.).

Presence of OPLs at the prior cancer site was found to predict SOM. Increased tobacco usage was noted to be associated with increased incidence of OPLs at the prior cancer site. Current smokers (smoking at tumour diagnosis) showed a tendency to have a higher incidence of OPLs (which predict SOM) than patients who had quit smoking prior to tumour diagnosis either at the one year examination time (48% versus 25%, $P = 0.063$) or for the whole follow-up time (42% versus 23%, $P = 0.095$). In addition, a greater percentage of smokers who continued to smoke in follow-up were more likely to develop OPLs at the prior cancer site than those that quit smoking both at one year (44% versus 15%, $P = 0.008$) and ever during the entire follow up (33% versus 15%, $P = 0.064$, approaching significance).

Uptake of TB at the former cancer site was another risk factor that predicts SOM. Increased tobacco usage was noted to be associated with increased uptake of TB by OPLs at the former cancer site. Patients who were still smoking at the time of tumour diagnosis showed a trend towards a higher incidence of TB-positive OPLs at one year time (53% versus 25%, $P = 0.059$). In addition smokers who

continued to smoke in follow-up were more likely to develop TB positive OPLs at the previously treated cancer site one year post treatment than those had quit smoking (47% versus 19%, $P = 0.040$).

Large size of OPLs was the 3rd risk factor that predicts SOM. Smoking status however did not affect the size of OPLs at the one-year examination period. However, during the entire follow-up period, patients with OPLs < 10mm were more likely to be smokers and heavy smokers as compared to patients with OPLs ≥ 10mm. This data could be confounded by a few extreme outliers.

In summary, smoking did affect some of the risk predictors for SOM, and the direct effects of smoking on SOM may need a longer follow up period.

VII.3. Target tumour and SOM

The majority of research regarding prediction of a SOM has focused on the target tumour characteristics such as positive margin status at treatment, use of radiation for treatment, and TNM stage (Spiro *et al.*, 1986; Loree and Strong, 1990; Gonzalez-Moles *et al.*, 2002). Carvalho, Margin and Kowalski (2003) found that patients who had received radiation for their initial treatment had a higher rate of recurrence than patients who were treated with surgery. Section I.5.4.

cites many papers that indicate that the presence of tumour or dysplasia at the resection margin and late TNM staging are related to increased incidence of SOM

The current thesis only dealt with patients treated with curative intent; hence patients with obvious tumour involvement at the margin without subsequent radiation treatment were not included in the study. Therefore, the effect of margin status and the development of a SOM will not be discussed here.

There were no statistical differences found when comparing the site of the target tumour, prior history of oral cancer, tumour stage, tumour histology or treatment of the primary tumour. As this study involved mainly early stage cancer, late stage cancer (III and IV) only accounted for 21% of the cases, the effect of tumour stage on the outcome of SOM may not be obvious due to the small number of late stage tumours.

Interestingly, patients with a prior history of oral cancer were significantly more likely to have multiple lesions both at one year and ever in follow-up (80% versus 20%, $p<0.001$, and 80% versus 23%, $p<0.001$).

VII.4. Clinical characteristics of post-treatment tumour sites and SOM

There are 3 major risk factors that are used for the identification of high-risk primary OPLs: the site, size and appearance of OPLs. All of these 3 factors were investigated in this longitudinal study for their value in predicting the cancer risk of OPLs at previous cancer sites.

VII.4.1. Site of OPLs at prior cancer site and SOM

Certain oral sites termed high-risk sites have also been associated with increased malignant transformation (Waldron and Shafer, 1975; Bouquot and Gorlin, 1986). High-risk sites include the floor of the mouth, the lateroventral tongue, and the soft palate complex. Location of primary OPLs is one of the major clinical risk factors for predicting the cancer risk of these lesions. The site of OPLs in patients with a history of oral cancer, however, offers little help in differentiating the low-risk from high-risk lesions. First of all, the site of previous oral cancer is a high-risk site, regardless where the location is; secondly the majority (around 70 to 90%) of oral SCC occurs in the high-risk region, naturally OPLs occurring at prior cancer sites would be mostly located at the high-risk site. The results of this study showed that 73% of the target oral SCCs were located at a high-risk region. Not surprisingly the location of the OPLs did not predict

the development of SOM (67% of SOM were located at the high-risk region versus 74% of non-SOM, *P = 0.558*).

The theory for the increased incidence of high-risk OPLs at high-risk regions remains speculative. The two most popular hypotheses are a) the epithelial lining is thinner and nonkeratinized, hence easier for carcinogens to penetrate and target the basal epithelial cells; b) the high-risk regions are located at the lower portion of the oral cavity, where saliva pools, and therefore the epithelia in these regions are more likely to be exposed to carcinogen dissolved in saliva longer.

The study showed that patients with the target tumour at high-risk site versus low-risk site showed a trend of higher pack years of tobacco usage (44 ± 41 pack year versus 28 ± 21 pack year, *P=0.079*, data not shown). Whether this suggests that patients with oral SCC at low risk regions had a higher susceptibility remains speculative.

Interestingly, patients whose target tumour was at a high-risk site were less likely to have multiple oral lesions both at one year and ever in follow-up (21% versus 57%, *P=0.003*; 23% versus 61%, *P=0.002*). The reasoning for this is unclear but again suggests that patients with cancer at a low-risk region may have a wider field of cancerization and more widespread genetic instability.

VII.4.2. Appearance of OPLs at prior cancer site and SOM

Another major risk factor for predicting cancer risk of primary OPLs is the appearance of OPLs. The literature indicates that primary OPLs with a non-homogeneous appearance have a much higher cancer risk than those primary OPLs with a homogeneous appearance (section I.6.2.). This thesis has, for the first time, shown that clinical appearance of OPLs can not be relied upon to differentiate high-risk from low-risk lesions, either at the one-year examination period or over the entire follow-up period.

The sites of a previous tumour are generally fragile because of intensive treatment such as aggressive surgery/laser and radiation. Consequently these sites could easily become inflamed, red or ulcerated. Such reactive changes could be very difficult to differentiate from non-homogenous leukoplakia. The study results showed that 6/8 (75%) of OPLs from previously irradiated sites were non-homogeneous in appearance as compared to 4/15 (27%) of OPLs from tumour sites without radiation ($P = 0.039$). Such results support the theory that tumour treatment could induce reactive changes resembling non-homogeneous appearance. OPL ≥ 10 mm were also associated with the presence of nonhomogenous appearance ever during follow-up (81% versus 27%, $P = 0.002$).

VII.4.3. Size of OPLs at prior cancer site and SOM

Large size of OPLs has been found to be a major risk factor for primary OPLs. In this study, the development of SOM was noted to be associated with a larger size of OPLs at both one year examination period (average size of OPLs that later developed into SOM were 14 mm versus 6 mm for OPLs that did not develop into SOM, $p = 0.067$, approaching significance) and during the whole follow-up period (23 mm versus 11 mm, $p = 0.006$).

Patients with lesions that were ≥ 10 mm were more likely to have multiple lesions (75% versus 27%, $P = 0.039$). This may be due to a wider underlying field of cancerization.

VII.4.4. Presence of OPLs at prior cancer site and SOM

An unexpected finding of this study was that the presence of an OPL at the former cancer site predicted SOM. The presence of an OPL at the former tumour site, both at one year and ever during follow-up, was found to be a highly significant indicator of recurrence. Seventy-two percent of the patients that had an eventual recurrence had an OPL at the former tumour site at their one-year

follow-up versus 15% of the non-SOM group (*P<0.001*). The presence of an OPL ever during follow-up was also very significant (72% versus 35%, *P=0.007*).

The majority of primary OPLs do not progress into cancer, malignant transformation rates vary from <1% to >30% dependent on the presence of dysplasia and geographical differences. The presence of OPLs at previous cancer sites showed a much higher malignant transformation rate. Identification of OPLs at the one-year examination showed a malignant transformation rate in more than half of patients with OPLs at the previous cancer sites (57%, 13/23). The results suggest that presence of OPLs, particularly large lesions, should be immediately biopsied and followed very closely regardless of their location and clinical appearance.

The presence of any OPL following the curative treatment of the target tumour would be an easy clinical indicator for clinicians to judge in most cases. For those OPLs that may be difficult to differentiate from treatment sequelae (scar tissue, grafts, late radiation changes) other clinical indicators, such as TB or increasing size, could help the clinician judge the recurrence risk.

VII.4.5. Toluidine blue (TB) and SOM

TB as a sensitive visual aid in the identification of early SCC has been reported in many studies, but its value in the identification of high-risk OPLs has generally been regarded as low because many low-grade dysplastic lesions do not stain with TB. Recent studies using molecular markers, however, have shown that the selective staining of some low grade OPLs with TB, in fact, could be used to differentiate high-risk OPLs from the low-risk OPLs. Molecular retrospective studies have shown that TB positive OPLs are more likely to contain high-risk molecular clones compared to TB negative OPLs with similar histopathological appearance (Epstein, Zhang *et al.,* 2003; Guo et al., 2001).

This is the first study to investigate the value of TB in predicting SOM in a longitudinal setting. The results showed that uptake of TB stain at either one year post treatment or over the entire follow up period were significantly associated with the development of a SOM regardless of the histology of the OPL. At one year 50% of the OPLs that would eventual progress to an SOM were found to be TB positive versus 11% of the non-SOM group (*P=0.001*) with a similar level of significance for TB positive ever during follow-up (67% versus 25%, *P=0.002*).

All these suggest that TB, in experienced hands, could be a powerful tool in the identification of high-risk OPLs that could later progress into a SOM.

VII.5. Histopathology and treatment of OPLs at previous cancer sites in relation to SOM

For primary OPLs, the presence and degree of dysplasia is currently the gold standard in the prediction of cancer risk. In the current study, there were 23 visible OPLs noted at the previous cancer sites at the time of the one year examination. Of these, 6 were biopsied (3 from sites later progressing into a SOM, and 3 from sites that did not become SOM), the number of biopsies was too small for comparison of the value of histology between the SOM and non-SOM group.

During the entire follow-up period, 36 OPLs were noted. Of these, 23 were biopsied (13 from SOM group, and 10 non-SOM group). Although a significantly greater percentage of biopsies were taken from the SOM group (72% versus 18%, $P < 0.001$), no correlation was found between SOM and the presence and degree of dysplasia. There was no significant differences when comparing the proportion of severe dysplasias between the SOM and non-SOM groups (data not shown, 23% versus 25%, $P = 1$).

The results here need to be interpreted with much caution because of the small number of biopsies. However, the effects of aggressive treatment of the target tumours could again produce reactive changes resembling dysplastic changes histologically that render histopathology less effective, particularly in those with low-grade dysplasias.

Of course, treatment of the OPLs at the former cancer sites could confound the previously discussed risk factors. The study results however did not show a difference in the treatment of OPLs at former cancer sites between the SOM and nonSOM group. Only a small percentage of OPLs at both SOM and non-SOM groups were treated at either one year examination period (1/3 versus 1/10, $P = 1$) and during the entire follow-up period (3/13 versus 2/23, $P = 0.328$).

VII.6. Study Limitations

Longitudinal studies are extremely difficult studies due to many factors. This paper presents early results from the longitudinal study. Some of the patients for this thesis were amongst the very first patients enrolled in the longitudinal study. Over the years, there have been revisions and improvement in various aspects of the study. Consequently, it is generally presumed that results from patients enrolled later would have fewer flaws in their follow-up compared to patients enrolled early in the study. Patients who were enrolled early were also

more likely to be missing data. It is hoped that at the end of the longitudinal study the one year examination range can be shortened to 10 – 14 months. The larger number of patients expected to be part of the final results will allow further breakdown of the data.

For example, one of the limitations of this study was inter-examiner reliability. In the early stages of this study data was collected from a number of clinicians working at the BCCA Oral Oncology/Oral Dysplasia clinic. The General Practise Resident (GPR) turnover in the clinic is approximately every 3 months, and the limited experience of these residents could produce potential problems in the data collection, such as judgment of TB staining results and measurement of the lesion size. As mentioned in the section I.7. TB sensitivity and specificity improves when used by experienced personnel. To control the interexaminer variation, we have for the past 3 years limited the data retrieval and lesion scrapings to a small number of calibrated OCPL study personnel (Oral Medicine specialists and oral medicine residents and study clinical personnel). As mentioned in section I.7.3. TB specificity improves when patients who stain positively return 10 – 14 days later for allow for inflammatory or trauma induced lesions to heal. Due to limited clinic space and time this was not always possible in our clinic and left up to the clinician's judgment. The TB+ result will remain in the database even when patients return and are found to be TB- 2 weeks later.

There were a limited amount of biopsies performed in follow-up early in the study. It is now study protocol to biopsy an OPL every 2 years, with the patient's consent, to track progression that may not be reflected in the clinical appearance of the lesion.

As mentioned in section V.5.5., the coding of the lesions early in the study was not uniform and may have led to some confusion. A coding system protocol was developed and implemented and the entire database of lesions was assigned a lesion sort code based on geographical location and whether the lesion(s) were part of a field.

A selection bias was also present as all patients were part of a hospital based research project. Tobacco data was collected via a questionnaire and may not accurately reflect the true habit of the patient.

VII.7. Future research

Future research should include variables not included in this thesis such as alcohol use, as well as multivariate analysis of variables to look for interaction between the factors involved. It would be interesting to look for clinical indicators of second primary tumours. LOH at 3p and/or 9p has been found to be associated with a 26.3 fold increased risk of SOM at the former cancer site

(Rosin *et al.*, 2002). Combining this information with the results of this thesis could lead to the development of a model of both clinical and genetic risk of recurrence. Earlier research from this lab (Epstein *et al.*, 2003) determined a link between molecular markers and TB status in OPLs.

VII.8. Summary

The majority of studies on the outcome of oral SCC have been done on the tumour characteristics, such as tumour staging and grade. There was a dearth of research into clinical indicators of SOM at a previously treated site. This is the first longitudinal study that has investigated the clinicopathological characteristics of post-treatment tumour sites that could predict the development of SOM.

The results showed that site and appearance of OPLs, two major risk predictors for primary OPLs were not good predictors of a SOM in patients with a history of oral cancer. However 3 risk factors found at previously treated cancer sites have been found to predict SOM: the presence and large size of OPLs and the uptake of TB at either one year after the completion of treatment or during the entire follow up time. Data collection is still ongoing and if the results are upheld they could have a great impact on the ability of clinicians to assess the risk of SOM and lead to early intervention. Treatment of SOM at the former tumour site has a poor 5-year survival rate and is hampered by the sequelae of the initial

treatment (Wong *et al.*, 2003). Comparatively, management of a high-risk patient prior to the development of a SOM could possibly prevent the SOM with less morbidity. Clinicians may also follow-up the patient at risk more frequently. It is hoped that this information will decrease the rate of SOM at the former tumour site, which in turn may reduce the 5-year mortality rate that is so high for oral cancer.

These study results will affect the clinical management of patients in the Oral Oncology/ Oral Dysplasia Clinic. Future data could provide information on the impact of these study results on the outcome of the patients.

VIII. APPENDICES

Appendix 1. Oral Health Study Questionnaire

ORAL STUDY QUESTIONNAIRE

1. In addition to being Canadian or a landed immigrant, what is your ethnic or cultural heritage?

(Check one box only):

- ☐ White
- ☐ East or South-east Asian (eg. China, Japan, Indonesia, Philippines, Vietnam)
- ☐ South Asian (eg. India Pakistan, Sri Lanka)
- ☐ First Nations
- ☐ Black
- ☐ Other (Please Specify)_____

2. a) What is the highest grade (or year) of high school or elementary school that you have completed?

Grade ___ Never attended school ____

b) How many years of post-secondary school have you completed (college, university)?

Years ___ None __

3. a) Have you ever used chewing tobacco?

Yes ☐ No ☐

b) Have you ever used betel nut?

Yes ☐ No ☐

4. Have you ever regularly smoked cigarettes, cigars or pipes more than once per week for one year or longer? Yes ☐ No ☐

If Yes, please specify:

a) At what age did you begin smoking:

Cigarettes? ____
Cigars? ____
Pipes? ____

b) Do you currently smoke:

Cigarettes?	Yes	☐	No	☐
Cigars?	Yes	☐	No	☐
Pipes?	Yes	☐	No	☐

c) If you have quit smoking, at what age did you permanently stop:

Cigarettes? ____
Cigars? ____
Pipes? ____

d) Looking back over your entire life, on average, how many did you usually smoke *per day*?

	Before Age 20 years	In your 20's	In your 30's	In your 40's	In your 50's	60's & older
Cigarettes	____	____	____	____	____	____
Cigars	____	____	____	____	____	____
Pipes	____	____	____	____	____	____

5. Looking back over the last year, please think about your exposure to the smoke of others, either at home, at work, and in public places (such as restaurants, recreational facilities).

Are you regularly exposed to smoke of others:

At home?	Yes	☐	No	☐
At work?	Yes	☐	No	☐
In public places?	Yes	☐	No	☐

If Yes, to any of the above, please specify:

How often are you regularly exposed to smoke of others:

	Never	Less than once a month	More than once a month but less than once a week	At least once a week	Daily
At home?	☐	☐	☐	☐	☐
At work?	☐	☐	☐	☐	☐
In Public Places?	☐	☐	☐	☐	☐

6. Looking back over your entire life, please check the age periods in which you were daily exposed to the smoke of others.

Before Age 20 years	In your 20's	In your 30's	In your 40's	In your 50's	60's & older
☐	☐	☐	☐	☐	☐

7. Have you ever regularly consumed alcoholic beverages more than once per month for one year or longer? Yes ☐ No ☐

If Yes, please specify:

a) At what age did you begin drinking:

Beer? ____
Wine? ____
Spirits (liquor)? ____

b) Do you currently drink:

Beer?	Yes	☐	No	☐
Wine?	Yes	☐	No	☐
Spirits (liquor)?	Yes	☐	No	☐

c) If you have quit drinking, at what age did you permanently stop:

Beer? ____
Wine? ____
Spirits (liquor)? ____

d) On average, how much did you usually drink *per week*:

Beer	____	bottles
Wine	____	glasses
Spirits (liquor)	____	(shots – 1 oz.)

8. Have any of your immediate family members (parents, brothers/sisters, daughters/sons, grandparents, aunts/uncles related by birth not marriage) had cancer in the head and neck region (excluding skin cancer)? Yes ☐ No ☐

If Yes, please specify all who had head and neck cancer:

☐ Parents
☐ Brothers/sisters
☐ Daughters/sons
☐ Grandparents
☐ Aunts/uncles related by birth not marriage

20020218

Appendix 2. Lesion Tracking Sheet.

To be completed at initial visit and each follow-up visit

PLEASE USE A TRACKING SHEET FOR EACH LESION: lesion _____site_____

Patient Surname, First Initial: _____; Study ID# _____

	DATE (yyyy/mmdd)							
LESION	**VISIT NUMBER (visit 1, 2, etc. , bx, surgery……)**							
	LESION PRESENT: 0 = No or 1= Yes							
	CLINICAL DESCRIPTION OF LESION- Specify, use code sheet for Lesion= 0 or 1- **ie. use codes 1-10**							
	LESION SITE: (refer to code sheet) the general description. N/C= No change							
	LESION GRID LOCATION: Specify grid site. N/C= no change							
	LESION TYPE- Indicate if diffuse, discrete, scar only etc.							
	LENGTH (MM):							
	WIDTH (MM):							
	THICKNESS (MM):							
	COLOR: 0 = Normal; 1= White; 2=Predominantly (>50%) white; 3=Predominantly (>50%) red; 4 = Red; 5= Other, specify in memo							
	APPEARANCE: 1 = Homogenous 2 = Nonhomogenous							
	TEXTURE: (Record all that apply) 1=Ulcerated; 2=Smooth; 3 =Velvety/Grainy; 4=Nodular; 5=Verrucous; 6= Fissured; 7=Other ; N/C= No Change							
	TOLUIDINE BLUE RESULTS: 0 = Neg 1 = Pos 2 =Equiv 3= Not done							
	BLOOD SAMPLE: 1 = Yes 0= No 3= declined If declined, please state reason							
TX	**INTERIM THERAPY** (REFER TO CODE SHEET)- Between appointments (dates on comment or treatment sheet)							
	INTERIM SMOKING: 1= No change; 2= Increase; 3= Decrease; 4=Stopped (REFER TO CODE SHEET)							
BX	**BIOPSY :** 1 = Yes 0 = No. If yes, then use the Biopsy Tracking Sheet							
	BIOPSY CONCURRENT TO SCRAPE: 1 = Yes 0 = No							

Study ID:	Name:	Lesion Code

	DATE (YYYY/MMDD)							
SAMPLE	LESION SCRAPE DONE: B=CYTOBTUSH							
	NORMAL SCRAPE DONE: B= CYTOBRUSH							
	NORMAL SCRAPE GRID LOCATION: (SPECIFY WHERE ON GRID) > 3 CM AWAY FROM LESION (PREFER TONGUE/FOM							
	PHOTO DONE: 1 = YES 0 = NO							
	WASH: 1 = YES 0 = NO							
	CRYOBRUSH : 1 = YES 0 = NO							
	CRYOBRUSH GRID LOCATION: (SPECIFY WHERE ON GRID) (PREFER R+L BUCCAL MUCOSA)							
GOGGLE	GOGGLES- : 0=NEG; 1=POS; 2=EQUIV; 3=NOT DONE; 4=N/A (NOT APPLICABLE); 5= TRIAL OBSERVATION ONLY- OLD							
	GOGGLE GRID LOCATION: (SPECIFY WHERE ON GRID)							
	GOGGLE LENGTH (MM):							
	GOGGLE WIDTH (MM):							
	PRESENCE OF ORANGE FLUORESCENCE: 1 = YES 0 = NO							
	GOGGLE DIGITAL IMAGE TAKEN: 1 = YES 0 = NO							
	GOGGLE COMMENT: 1=YES 0=NO COMMENT. IE. COMMENT ON THE POSITIVE AREA RELATED TO THE							
map	MAPPING CODE- DENISE'S STUDY ONLY, FIRST COLUMN IS THE LESION SITE CODE, THE FOLLOWING ARE THE MAP							
	MAPPING DISTANCE FROM LESION- CODE 5= 5 MM; 10= 10 MM							
	WORRISOME: 1=INCREASE IN SIZE; 2= COLOUR CHANGE; 6= OTHER, STATE							
	SCRAPER- WHO DID THE SCRAPE PROCEDURES?							
	CLINICIAN DOING THE EVALUATION- USE INITIALS							

updated: 20040525

LESION SITE CODES

LOCATION: (ie. record Tongue/Anterior as 1A)

1 = Tongue: Lateral border
2 = Tongue: Ventral surface
3 = Tongue: Dorsal surface
4 = FOM (floor of mouth)
5 = Gum
6 = Soft palate
7 = Hard palate
8 = Buccal mucosa
9 = Labial mucosa
10= Retromolar Trigone

ORIENTATION:

R = Right	L = Left
A = Anterior	P = Posterior
U = Upper	W = Lower
M= Midline	

TEXTURE:

1 – Ulcerated
2 – Smooth
3 – Velvety, grainy: Non-elevated change in surface texture
4 – Nodular: raised due to submucosal, or intraepithelial thickening
5 – Verrucous: irregular, grainy, pointy projections with elevated above the surface of the adjacent unaffected mucosa.
6 – Fissured: cracks or fissures within the lesion.
7- Other, please specify in memo
N/C- No change

INTERIM THERAPY CODES:

1=	Surgery- Excision
2=	Surgery- Laser
3=	Radiation- External
4=	Radiation- Gold Seed
5=	Radiation- Radium
6=	Chemotherapy- Bleomycin
7=	Chemotherapy- Vitamin A/BCar
9=	Chemotherapy- Topsyn
10=	Other- (please specify)
11=	Surgery- Incisional Bx (not a tx)
88= N/A	99= Unknown

INTERIM SMOKING:

S= Smoker/ Chewing tobacco
NS= Nonsmoker
FS= Former Smoker
*** first visit status only

LESION = 0

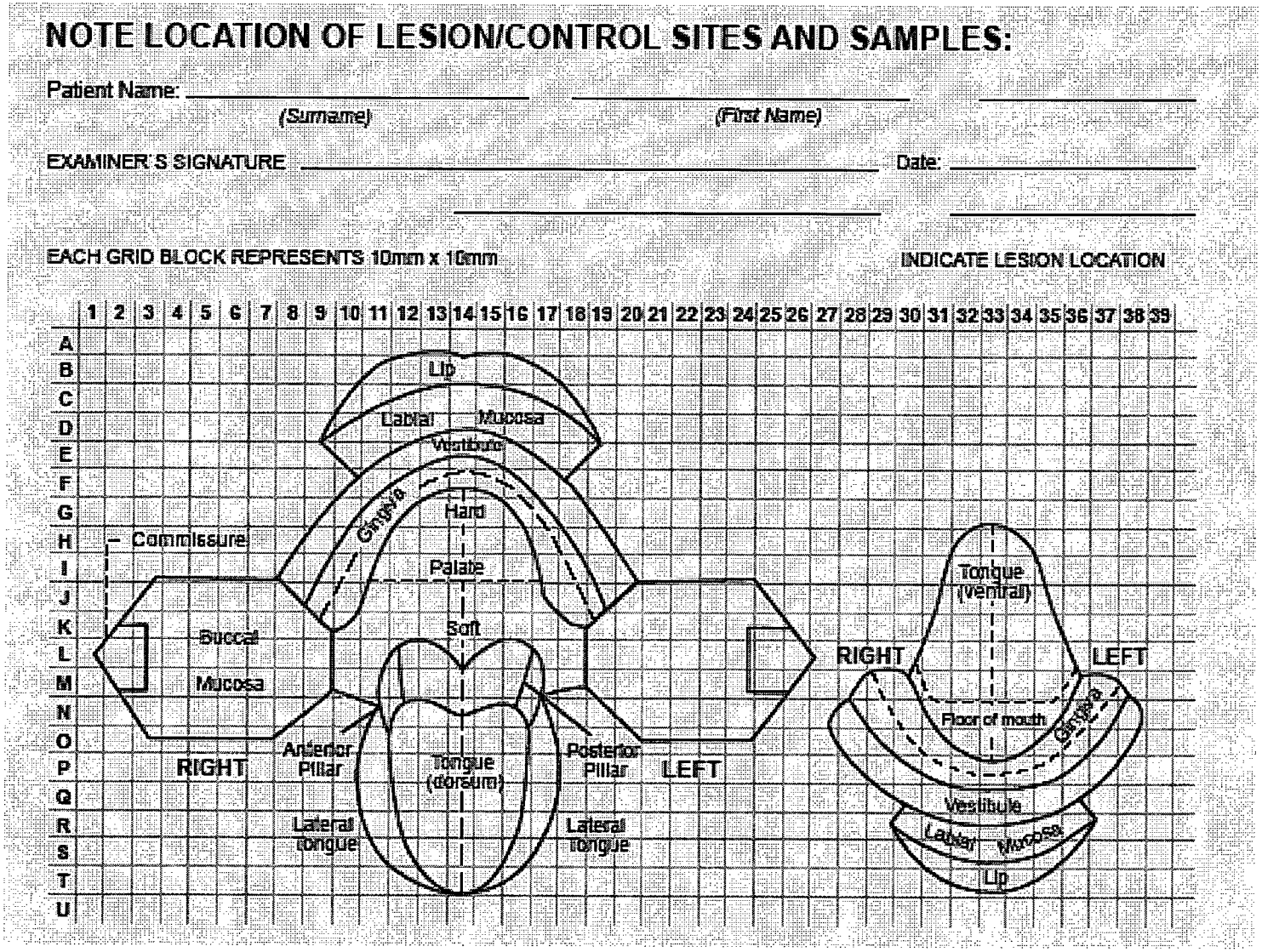
1= Scar
2= Graft
3= Normal epithelium (no associated erythma or ulceration around scar)
4= Fibroepithelial polyp
5= Reactive change (erythmatous change)
7= Other
11= Unrelated ulcer at other site (not former site)

LESION = 1

6= Lichen Planus
7= Other
8= Leukoplakia (white
9= Erythroplakia (red)
10= Ulcer at former cancer site or dysplasia site

*** All entries must be complete, any unchanged variable must be recorded as N/C. 20021106**

Appendix 3. Lesion Grid



IX. REFERENCES

Abbey, L. M., Kaugars, G. E., Gunsolley, J. C., Burns, J. C., Page, D. G., Svirsky, J. A., Eisenberg, E., and Krutchkoff, D. J. The effect if clinical information on the histopathologic diagnosis of oral epithelial dysplasia. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics, *85:* 74-77, 1998.

Ah-See, K. W., Cooke, T. G., Pickford, I. R., Soutar, D., and Balmain, A. An allelotype of squamous carcinoma of the Head and Neck Using Microsatellite Markers. Cancer Research, *54:* 1617-1621, 1994.

Allen, C. Toluidine blue: Proceed with caution? Oral Surgery, Oral Medicine, Oral Pathology, *86:* 255, 1998.

American Cancer Society Cancer facts and figures 2004. 2004. Access date: 2004/05/30. www.cancer.org

Antoniades, K., Lazaridis, N., Vahtsevanos, K., Hadjipetrou, L., Antoniades, V., and Karakasis, D. Treatment of squamous cell carcinoma of the anterior faucial pillar-retromolar trigone. Oral Oncology, *39:* 680-686, 2003.

Axéll, T., Holmstrup, P., Kramer, I., Pindborg, J., and Shear, M. International seminar on oral leukoplakia and associated lesion related to tobacco habits. Community Dentistry and Oral Epidemiology, *12:* 1-154, 1984.

Axéll, T. Occurrence of leukoplakia and some other oral white lesions among 20333 adult Swedish people. Community Dentistry and Oral Epidemiology, *15:* 46-51, 1987.

Axéll, T., Pindborg, J., Smith, C., van der Waal, I., and Lesions, I. C. G. o. O. W. Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18-21 1994. Journal of Oral Pathology and Medicine, *25:* 49-54, 1996.

Bagán, J., Murillo, J., Poveda, R., Gavalda, C., Jimenez, Y., and Scully, C. Proliferative verrucous leukoplakia: unusual locations of oral squamous cell carcinomas and field cancerization as shown by the appearance of multiple OSCCs. Oral Oncology, *40:* 440-443, 2004.

Bailey, J., Blanchaert, RH Jr, Ord, RA Management of Oral Squamous Cell Carcinoma Treated with Inadequate Excisional Biopsy. Journal of Oral and Maxillofacial Surgery, *59:* 1007-1010, 2001.

Batsakis, J. G., Suarez, P., and El-Naggar, A. Proliferative verrucous leukoplakia and its related lesions. Oral Oncology, *35:* 354-359, 1999.

Bánóczy, J. and Sugár, L. Longitudinal studies in oral leukoplakias. Journal of Oral Pathology, *1:* 265-272, 1972.

Bánóczy, J. and Csiba, A. Occurence of epithelial dysplasia in oral leukoplakia. Oral Surgery, Oral Medicine, Oral Pathology, *42:* 766-774, 1976.

Bánóczy, J. Follow-up studies in oral leukoplakia. Journal of Maxillofacial Surgery, *5:* 69-75, 1977.

Bánóczy, J. and Rigo, O. Prevalence study of oral precancerous lesions within a complex screening system in Hungary. Community Dentistry and Oral Epidemiology, *19:* 265-267, 1991.

BCCA, General Principles of Treatment. *In:* British Columbia Cancer Agency (ed.), Cancer Management Guidelines Head and Neck. Vancouver, 2001a. Access date: 2004/11/06. http://www.bccancer.bc.ca/HPI/CancerManagementGuidelines/Headneck/Management/GeneralPrinciples.htm

BCCA, Oropharynx. *In:* British Columbia Cancer Agency (ed.), Cancer Management Guidelines Head and Neck. Vancouver, 2001b. Access date: 2004/11/06. http://www.bccancer.bc.ca/HPI/CancerManagementGuidelines/HeadnNeck/Management/Oropharynx.htm

BCCA, Radiation Reactions. *In:* British Columbia Cancer Agency (ed.), Cancer Management Guidelines Head and Neck. Vancouver, 2001c. Access date 2004/11/06. http://www.bccancer.bc.ca/HPI/CancerManagementGuidelines/HeadnNeck/Management/RadiationReactions.htm

BCCA, 1. Tumour Site/Type Demographics. *In:* British Columbia Cancer Agency (ed.), Cancer Management Guidelines Head and Neck. Vancouver, 2003. Access date 2004/11/06. http://www.bccancer.bc.ca/HPI/CancerManagementGuidelines/HeadnNeck/start.htm

Bedi, G., Westra, W., Gabrielson, E., Koch, W., and Sidransky, D. Multiple Head and Neck Tumors: Evidence for a Common Clonal Origin. Cancer Research, *56:* 2484-2487, 1996.

Bettendorf, O., Piffko, J, Bankfalvi, A Prognostic and predictive factors in oral squamous cell cancer: important tools for planning individual therapy? Oral Oncology, *40:* 110-119, 2004.

Blot, W., McLaughlin, J., Winn, D., Austin, D., Greenberg, R., Preston-Martin, S., Bernstein, L., Schoenberg, J., Stemhagen, A., and Fraumeni, J. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Research, *48:* 3282-3287, 1988.

Bokor-Bratic, M. The prevalence of precancerous oral lesions. Oral leukoplakia. Archives of Oncology, *8:* 169-110, 2000.

Bouquot, J. E. and Gorlin, R. Leukoplakia, lichen planus, and other oral keratoses in 23,616 white Americans over the age of 35 years. Oral Surgery, Oral Medicine, Oral Pathology, *61:* 373-381, 1986.

Bouquot, J. E. and Whitaker, S. B. Oral Leukoplakia--Rationale for diagnosis and prognosis of its clinical subtypes or "phases". Quintessence International, *25:* 133-140, 1994.

Boysen, M., Natvig, K., Winther, F., and Tausjö, J. Value of routine follow-up in patients treated for squamous cell carcinoma of the head and neck. The Journal of Otolaryngology, *14:* 211-214, 1985.

Braakhius, B. J. M., Tabor, M., Leemans, C., van der Waal, I., Snow, G. B., and Brakenhoff, R. Second Primary Tumors and Field Cancerization in Oral and Oropharyngeal Cancer: Molecular Techniques Provide New Insights and Definitions. Head and Neck, *February:* 198-206, 2002.

Braakhius, B. J. M., Tabor, M., Kummer, J., Leemans, C., and Brakenhoff, R. A genetic explanation of Slaughter's concept of field cancerization: Evidence and clinical implications. Cancer Research, *63:* 1727-1730, 2003.

Brennan, J. A., Mao, L., Hruban, R. H., Boyle, J. O., Eby, Y. J., Koch, W. M., Goodman, S. N., and Sidransky, D. Molecular Assessment of Histopathological Staging in Squamous-Cell Carcinoma of the Head and Neck. The New England Journal of Medicine, *332:* 429-435, 1995.

Brodland, D. The life of a skin cancer. Mayo Clinic Proceedings, *72:* 475-478, 1997.

Brunette, D. Critical Thinking Understanding and Evaluating Dental Research. Carol Stream: Quintessence, 1995.

Burkett, D. Zila to own unique method for detecting cancer; now holds 10 US patents for Zila tolonium chloride and/or OraTest. Vol. 2004. Phoenix, 2003. Access date: 2004/10/30. http://ir.shareholder.com/zila/ReleaseDetail.cfm?ReleaseID=118083

Burkhardt, A. Advanced methods in the evaluation of premalignant lesions and carcinomas of the oral mucosa. Journal of Oral Pathology, *14:* 751-778, 1985.

Cairns, P. and Sidransky, D. Molecular methods for the diagnosis of cancer. Biochimica et Biophysica Acta, *1423:* C11-C18, 1999.

Califano, J., van der Riet, P., Westra, W., Nawroz, H., Clayman, G., Piantadosi, S., Corio, R., Lee, D., Greenberg, B., Koch, W. M., and Sidransky, D. Genetic Progression Model for Head and Neck Cancer: Implications for Field Cancerization. Cancer Research, *56:* 2488-2492, 1996.

Califano, J., Westra, W., Menininger, G., Corio, R., Koch, W. M., and Sidransky, D. Genetic Progression and Clonal Relationship of Recurrent Premalignant Head and Neck Lesions. Clinical Cancer Research, *6:* 347-352, 2000.

Canto, M., Devesa, SS Oral cavity and pharynx cancer incidence rates in the United States, 1975-1998. Oral Oncology, *38:* 610-617, 2002.

Carvalho, A., Magrin, J., and Kowalski, L. Sites of recurrence in oral and oropharyngeal cancers according to the treatment approach. Oral Diseases, *9:* 112-118, 2003.

Chiesa, F., Boracchi, P., Tradati, N., Rossi, N., Costa, L., Giardini, R., Marazza, M., and Zurrida, S. Risk of preneoplastic and neoplastic events in operated oral leukoplakias. Oral Oncology, *29B:* 23-28, 1993.

Cusumano, R. and Persky, M. Squamous cell carcinoma of the oral cavity and oropharynx in young adults. Head and Neck Surgery, *10:* 229-234, 1988.

Damm, D. Dysplastic Leukoplakia (letter). The New England Journal of Medicine, *350:* 2718-2719, 2004.

Das, B. and Nagpal, J. Understanding the biology of oral cancer. Medical Science Monitor, *8:* RA258-267, 2002.

Day, G. and Blot, W. Second primary tumors in patients with oral cancer. Cancer, *70:* 14-19, 1992.

Day, G., Blot, W., Shore, R., McLaughlin, J., Austin, D., Greenberg, R., Liff, J., Preston-Martin, S., Sarkar, S., Schoenberg, J., and Fraumeni, J. Second cancers following oral and pharyngeal cancers: Role of tobacco and alcohol. Journal of the National Cancer Institute, *86:* 131-137, 1994a.

Day, G., Blot, W., Shore, R., Schoenberg, J., Kohler, B., Greenberg, R., Liff, J., Preston-Martin, S., Austin, D., McLaughlin, J., and Fraumeni, J. Second cancers following oral and pharyngeal cancer: patients' characteristics and survival patterns. Oral Oncology, *30B:* 381-386, 1994b.

De Stefani, E., Oreggia, F., Boffetta, P., Deneo-Pellegrini, H., Ronco, A., and Mendilaharsu, M. Tomatoes, tomato-rich foods, lycopene and cancer of the upper aerodigestive tract: a case-control in Uruguay. Oral Oncology, *36:* 47-53, 2000.

de Visscher, J., Gooris, PJJ, Vermey, A, Roodenburg, JLN Surgical margins for resection of squamous cell carcinoma of the lower lip. International Journal of Oral and Maxillofacial Surgery, *31:* 154-157, 2002.

Dunipace, A., Beaven, R., Noblitt, T., Li, Y., Zunt, S., and Stookey, G. Mutagenic potential of toluidine blue evaluated in the Ames test. Mutation Research, *279:* 255-259, 1992.

Eckardt, A., Barth, E., Kokemueller, H., and Wegener, G. Recurrent carcinoma of the head and neck: treatment strategies and survival analysis in a 20-year period. Oral Oncology, *40:* 427-432, 2004.

El-Naggar, Hurr, Batesakis, Luna, Goepfert, and Huff Sequential loss of heterozygosity at microsatellite motifs in preinvasive and invasive head and neck squamous cell carcinoma. Cancer Research, *55:* 2656-2659, 1995.

Emilion, G., Langdon, J., Spreight, P., and Partridge, M. Frequent Gene Deletions in Potentially Malignant Oral Lesions. British Journal of Cancer, *73:* 809-813, 1996.

Ephros, H. Leukoplakia and malignant transformation. Oral Surgery, Oral Medicine, Oral Pathology, *83:* 187, 1997.

Epstein, J., Scully, C., and Spinnelli, J. Toluidine blue and Lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy. Journal of Oral Pathology and Medicine, *21:* 160-163, 1992.

Epstein, J., Oakley, C., Millner, A., Emerton, S., van der Meij, E., and Le, N. D. The utility of toluidine blue application as a diagnostic aid in patients previously treatd for upper oropharyngeal carcinoma. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics, *83:* 537-547, 1997.

Epstein, J., Zhang, L, Poh, C, Nakamura, H, Berean, K, Rosin, MP Increased allelic loss in toluidine blue--positive oral premalignant lesions. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics, *95:* 45-50, 2003.

Epstein, J., Feldman, R., Dolor, R., and Porter, S. The Utility of Tolonium Chloride Rinse in the Diagnosis of Recurrent or Second Primary Cancers in Patients with Prior Upper Aerodigestive Tract Cancer. Head and Neck, *25:* 911-921, 2003.

Fioretti, F., Bosetti, C., Tavani, A., Franceschi, S., and La Vecchia, C. Risk factors for oral and pharyngeal cancer in never smokers. Oral Oncology, *35:* 375-378, 1999.

Franceschi, S., Levi, F., Dal Maso, L., Talamini, R., Conti, E., Negri, E., and La Vecchia, C. Cessation of alcohol drinking and risk of cancer of the oral cavity and pharynx. International Journal of Cancer, *85:* 787-790, 2000.

Franceschi, S., Bidoli, E., Herrero, R., and Munoz, N. Comparison of cancers of the oral cavity and pharynx worldwide: etiological clues. Oral Oncology, *36:* 106-115, 2000.

Franco, E., Kowalski, L., and Kanda, J. Risk factors for second cancers of the upper respiratory and digestive systems: A case-control study. Journal of Clinical Epidemiology, *44:* 615-625, 1991.

Gath, H. and Brakenhoff, R. Minimal residual disease in head and neck cancer. Cancer and Metastasis Reviews, *18:* 109-126, 1999.

Glover, E. and Glover, P. The smokeless tobacco problem: Risk groups in North America. National Cancer Institute Monograph, *2:* 3-10, 1992.

Gonzalez-Moles, M., Esteban, F, Rodriguez-Archilla, A, Ruiz-Avila, I, Gonzalez-Moles, S Importance of tumour thickness measurement in prognosis of tongue cancer. Oral Oncology, *38:* 394-397, 2002.

Greene, J., Ernster, V., Grady, D., Robertson, P., Walsh, M., and Stillman, L. Oral mucosal lesions: Clinical findings in relation to smokeless tobacco use among US baseball players. National Cancer Institute Monograph, *2:* 41-50, 1992. Access date: 2003/04/30. http://cancercontrol.cancer.gov/tcrb/monographs/2/m2_2.pdf

Greenspan, D. and Jordan, R. The White Lesion That Kills--Aneuploid Dysplastic Oral Leukoplakia. The New England Journal of Medicine, *350:* 1382-1384, 2004.

Guo, Z., Yamaguchi, K., Sanchez-Cespedes, M., Westra, W., Koch, W., and Sidransky, D. Allelic Lossed in OroTest-directed Biopsies of Patients with Prior Upper Aerodigestive Tract Malignancy. Clinical Cancer Research, *7:* 1963-1968, 2001.

Gupta, P., Mehta, F., Daftary, D., Pindborg, J., Bhonsle, R., Jalnawalla, P., Sinor, P., Pitkar, V., Murti, P., Irani, R., Shah, H., Kadam, P., Iyer, S., Iyer, H., Hegde, A., Chandrashekar, G., Shroff, B., Sahiar, B., and Mehta, M. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. Community Dentistry and Oral Epidemiology, *8:* 287-333, 1980.

Gupta, P. A study of dose-response relationship between tobacco habits and oral leukoplakia. British Journal of Cancer, *50:* 527-531, 1984.

Gupta, P. Smokeless tobacco use in India. National Cancer Institute Monograph, *2:* 19-25, 1992. Access date: 2003/04/30. http://cancercontro.cancer.gov/tcrb/monographs/2/m2_1.pdf

Ha, P. and Califano, J. The role of human papillomavirus in oral carcinogenesis. Critical Review of Oral Biology and Medicine, *15:* 188-196, 2004.

Hashibe, M., Jacob, B., Thomas, G., Ramadas, K., Mathew, B., Sankaranarayanan, R., and Zhang, Z. Socioeconomic status, lifestyle factors and oral premalignant lesions. Oral Oncology, *39:* 664-671, 2003.

Herlin, P., Marnay, J., Jacob, J., Ollivier, J., and Mandard, A. A study of the mechanism of the toluidine blue dye test. Endoscopy, *15:* 4-7, 1983.

Hicks, W., Loree, T., Garcia, R., Maamoun, S., Marshall, D., Orner, J., Bakamjian, V., and Shedd, D. Squamous cell carcinoma of the floor of the mouth: A 20-year review. Head and Neck, *19:* 400-405, 1997.

Hirata, R., Jaques, D., Chambers, R., Tuttle, J., and Mahoney, W. Carcinoma of the oral cavity. Annals of Surgery, *182:* 98-103, 1975.

Hittelman, W. N. Genetic instability in epithelial tissues at risk for cancer. Annals of the New York Academy of Sciences, *952:* 1-12, 2001.

Hoffman, H., Karnell, L., Funk, G., Robinson, R., and Menck, H. The National Cancer Data Base report on cancer of the head and neck. Archives of Otolaryngology - Head and Neck Surgery, *124:* 951-962, 1998.

Hoffman, M., Lohrey, C., Hunziker, A., Kahn, T., and Schwarz, E. Human papillomavirus type 16 E6 and E7 genotypes in head-and-neck carcinoma. Oral Oncology, *40:* 520-524, 2004.

Hogewind, W., van der Kwast, W., and van der Waal, I. Oral leukoplakia, with emphasis on malignant transformation. Journal of Cranio-Maxillo-Facial Surgery, *17:* 128-133, 1989.

Hong, W., Bromer, R., Amato, D., Shapsay, S., Vincent, M., Vaughan, C., Willet, B., Katz, A., Welch, J., Fofonoff, S., and Strong, M. Patterns of relapse in locally advanced head and neck cancer patients who achieved complete remission after combined modality therapy. Cancer, *56:* 1242-1245, 1985.

Hong, W. K., Lippman, S. M., Itri, L., Karp, D., Lee, J., Byers, R., Schantz, S., Kramer, A., Lotan, R., Peters, L., Dimery, I., Brown, B., and Goepfert, H. Prevention of second primary tumors with isotretinoin in squamous cell carcinoma of the head and neck. New England Journal of Medicine, *323:* 795-801, 1990.

IARC-WHO GLOBOCAN 2002 database: Cancer Incidence, Mortality and Prevalence Worldwide. Lyon: IARCPress, 2002. Access date: 2004/11/04. http://www-dep.iarc.fr/

Irish, J., Kamel-Reid, S., Gullane, P., and O-charoenat, P. Chapter 2: Molecular biology. *In:* P. Evans, P. Montgomery, and P. Gullane (eds.), Principles and practice of head and neck oncology. United Kingdom: Martin Dunitz, 2003.

Ishii, J., Fujita, K., and Komori, T. Laser surgery as a treatment for oral leukoplakia. Oral Oncology, *39:* 759-769, 2003.

Ivkic, M., Bedekovic, V., Kalogjera, L., Cupic, H., and Ferencic, Z. Invasive cell grading -- an overview. Acta Clinica Croatica, *41*, 2002.

Jaber, M., Porter, S., Gilthorpe, M., Bedi, G., and Scully, C. Risk factors for oral epithelial dysplasia--the role of smoking and alcohol. Oral Oncology, *35:* 151-156, 1999.

Jaber, M., Porter, SR, Speight, P, Eveson, JW, Scully, C Oral epithelial dysplasia: clinical characteristics of western European residents. Oral Oncology, *39:* 589-596, 2003.

Jacob, B., Thomas, G., Ramadas, K., Mathew, B., Zhang, Z., Sankaranarayanan, R., and Hashibe, M. Betel quid without tobacco as a risk factor for oral precancers. Oral Oncology, *40:* 697-704, 2004.

Jemal, A., Thomas, A., Murray, T., and Thun, M. Cancer Statistics, 2002. Cancer A Cancer Journal for Clinicians, *52:* 23-47, 2002.

Jin, Y., Myers, J., Tsai, S., Goepfert, H., Batsakis, J. G., and El-Naggar, A. Genetic alterations in oral squamous cell carcinoma of young adults. Oral Oncology, *35:* 251-256, 1999.

Johnson, N. Diagnosis Oral Cancer: can Toluidine blue mouthwash help? FDI World, *8:* 22-26, 1998.

Jones, K., Lodge-Rigal, R., Reddick, R., Tudor, G., and Shockley, W. Prognostic factors in the recurrence of stage I and II squamous cell cancer of the oral cavity. Archives of Otolaryngology - Head and Neck Surgery, *118:* 483-485, 1992.

Jordan, R. and Daley, T. Oral Squamous Cell Carcinoma: New Insights. Journal of the Canadian Dental Association, *63:* 517-525, 1997.

Jorde, L., Carey, J., Bamshad, M., and White, R. Medical Genetics, 2nd edition, p. 372. St Louis: Mosby, 2000.

Jovanovic, A., van der Tol, I., Schulten, E., Kostense, P., De Vries, N., Snow, G. B., and van der Waal, I. Risk of multiple primary tumours following oral squamous cell carcinoma. International Journal of Cancer, *56:* 320-323, 1994.

Kaugars, G. E., Riley, W., Brandt, R., Burns, J., and Svirsky, J. A. The prevalence of oral lesions in smokeless tobacco users and an evaluation of risk factors. Cancer, *70:* 2579-2585, 1992.

Kerawala, C., Beale, V., Reed, M., and Martin, I. The role of vital tissue staining in the marginal control of oral squamous cell carcinoma. International Journal of Oral and Maxillofacial Surgery, *29:* 32-35, 2000.

Khuri, F., Kim, ES, Lee, JJ, Winn, RJ, Benner, SE, Lippman, SM, Fu, KK, Cooper, JS, Vokes, EE, Chamberlain, RM, Williams, B, Pajak, TF, Goepfert, H, Hong, WK The Impact of Smoking Status, Disease Stage, and Index Tumor Site on Second Primary Tumor Incidence and Tumor Recurrence in the Head and Neck Retinoid chemoprevention Trial. Cancer Epidemiology, Biomarkers and Prevention, *10:* 823-829, 2001.

Kuffer, R. and Lombardi, T. Premalignant lesions of the oral mucosa. A discussion about the place of oral intraepithelial neoplasia (OIN). Oral Oncology, *38:* 125-130, 2002.

La Vecchia, C., Tavani, A., Franceschi, S., Levi, F., Corrao, G., and Negri, E. Epidemiology and prevention of oral cancer. Oral Oncology, *33:* 302-312, 1997.

La Vecchia, C., Franceschi, S., Bosetti, C., Levi, F., Talamini, R., and Negri, E. Time Since Stopping Smoking and the Risk of Oral and Pharyngeal Cancers. Journal of the National Cancer Institute, *91:* 726-728, 1999.

La Vecchia, C., Lucchini, F., Negri, E., and Levi, F. Trends in oral cancer mortality in Europe. Oral Oncology, *40:* 433-439, 2004.

Lacy, P., Spitznagel, E., and Piccirillo, J. Development of a new staging system for recurrent oral cavity and oropharyngeal squamous cell carcinoma. Cancer, *86:* 1387-1395, 1999.

Larsson, A. and Warfvinge, G. Malignant transformation of oral lichen planus. Oral Oncology, *39:* 630-631, 2003.

Lazar, A., Winter, M., Nogueira, C., Larson, P., Finnemore, E., Dolan, R., Fuleihan, N., Chakravarti, A., Zietman, A., and Rosenberg, C. Loss of heterozygosity at 11q23 in squamous cell carcinoma of the head and neck is associated with recurrent disease. Clinical Cancer Research, *4:* 2787-2793, 1998.

Lee, J. J., Hong, W. K., Hittelmen, W. N., Mao, L., Lotan, R., Shin, D. M., Benner, S. E., Xu, X.-C., Lee, J. S., Papadimitrakopoulou, V. M., Geyer, C., Perez, C., Martin, J. W., El-Niggar, A. K., and Lippman, S. M. Predicting Cancer

Development in Oral leukoplakia: Ten Years of Translational Research. Clinical Cancer Research, *6:* 1702-1710, 2000.

Leemans, C., Tiwari, R., Nauta, J., van der Waal, I., and Snow, G. B. Recurrence at the Primary Site in Head and Neck Cancer and the Significance of Neck Lymph Node Metastases as a Prognostic Factor. Cancer, *73:* 187-190, 1994.

Lenth, R.V. 2004. Java applets for power and sample size. Access date: 2004/11/15: http://www.stat.uiowa.edu/~rlenth/Power/index.html.

Li, X., Lee, N. K., Ye, Y.-W., Waber, P. G., Schweitzer, C., Cheng, Q.-C., and Nisen, P. D. Allelic Loss at Chromosomes 3p, 8p, 13q, and 17p Associated with Poor Prognosis in Head and Neck Cancer. Journal of the National Cancer Institute, *86:* 1524-1529, 1994.

Licciardello, J., Spitz, M., and Hong, W. K. Multiple primary cancer in patients with cancer of the head and neck: second cancer of the head and neck, esophagus, and lung. International Journal of Radiation Oncology, Biology and Physics, *17:* 467-476, 1989.

Lind, P. Malignant transformation in oral leukoplakia. Scandinavian Journal of Dental Research, 449-455, 1987.

Lippman, S. and Hong, W. K. Second malignant tumors in head and neck squamous cell carcinoma: the overshadowing threat for patients with early-stage disease. International Journal of Radiation Oncology, Biology and Physics, *17:* 691-694, 1989.

Lippman, S. M. and Hong, W. K. Molecular Markers of the Risk of Oral Cancer. The New England Journal of Medicine, *344:* 1323-1326, 2001.

Lissowska, J., Pilarska, A., Pilarska, P., Samolczyk-Wanyura, D., Piekarczyk, J., Bardin-Mikolajczak, A., Zatonski, W., Herrero, R., Munoz, N., and Franceschi, S. Smoking, alcohol, diet, dentition and sexual practices in the epidemiology of oral cancer in Poland. European Journal of Cancer Prevention, *12:* 25-33, 2003.

Liu, S. C. and Klein-Szanto, A. J. P. Markers of proliferation in normal and leukoplakic oral epithelia. Oral Oncology, *36:* 145-151, 2000.

Loree, T. and Strong, E. Significance of positive margins in oral cavity squamous carcinoma. The American Journal of Surgery, *160:* 410-414, 1990.

Lummerman, H., Freedman, P., and Kerpel, S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surgery, Oral Medicine, Oral Pathology, *79:* 321-329, 1995.

Lund, A., editorial coordinator Can tomatoes fight oral cancer? Journal of the American Dental Association, *132:* 154-156, 2001.

Mao, L. and Sidransky, D. Cancer Screening Based on Genetic Alterations in Human Tumors. Cancer Research (Suppl.), *54:* 1939s-1940s, 1994.

Mao, L., Lee, J. S., Fan, Y. H., Ro, J. Y., Batsakis, J. G., Lippman, S. M., Hittelmen, W. N., and Hong, W. K. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nature Medicine, *2:* 682-685, 1996.

Mao, L. Leukoplakia: molecular understanding of pre-malignant lesions and implications for clinical management. Molecular Medicine Today, *3:* 442-448, 1997.

Mao, L. Can Molecular Assessment Improve Classification of Head and Neck Premalignancy? Clinical Cancer Research, *6:* 321-322, 2000.

Mao, L. A New Marker Determining Clonal Outgrowth. Clinical Cancer Research, *8:* 2021-2023, 2002.

Martin, I., Kerawala, C., and Reed, M. The application of toluidine blue as a diagnostic adjunct in the detection of epithelial dysplasia. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics, *85:* 444-446, 1998.

Mashberg, A., Morrissey, J., and Garfinkel, L. A study of the appearance of early asymptomatic oral squamous cell carcinoma. Cancer, *32:* 1436-1445, 1973.

Mashberg, A. Reevaluation of toluidine blue application as a diagnostic adjunt in the detection of asymptomatic oral squamous carcinoma: A continuing prospective study of oral cancer III. Cancer, *46:* 758-763, 1980.

Mashberg, A. Tolonium (Toluidine blue) rinse--A screening method for recognition of squamous carcinoma. Journal of the American Medical Association, *245:* 2408-2410, 1981.

Mashberg, A. Final evaluation of tolonium chloride rinse for screening of high-risk patients with asymptomatic squamous carcinoma. Journal of the American Dental Association, *106:* 319-323, 1983.

Mashberg, A. Diagnosis of early oral and oropharyngeal squamous carcinoma: obstacles and their amelioration. Oral Oncology, *36:* 253-255, 2000.

Mincer, H., Coleman, S., and Hopkins, K. Observations on the clinical characteristics of oral lesions showing histologic epithelial dysplasia. Oral Surgery, *33:* 389-399, 1972.

Mork, J., Møller, B., and Glattre, E. Familial risk in head and neck squamous cell carcinoma diagnosed before the age of 45: a population-based study. Oral Oncology, *35:* 360-367, 1999.

Moyer, G., Taybos, G., and Pelleu, G. J. Toluidine blue rinse: Potential for benign lesions in early detection of oral neoplasms. Journal of Oral Medicine, *41:* 111-113, 1986.

Myers, E. The toluidine blue test in lesions of the oral cavity. Cancer: a cancer journal for clinicians, *20:* 134-139, 1970.

National Cancer Institute of Canada Canadian Cancer Statistics 2003. Toronto, Canada, 2003. Access date: 2004/01/30. http://www.ncic.cancer.ca

Neville, B. and Day, T. Oral cancer and precancerous lesions. Cancer A Cancer Journal for Clinicians, *52:* 195-215, 2002.

Nguyen, T. V., Yueh, B. Weight Loss Predicts Mortality after Recurrent Oral Cavity and Oropharyngeal Carcinomas. Cancer, *95:* 553-562, 2002.

Niebel, H. and Chomet, B. In vivo staining test for delineation of oral intraepithelial neoplastic change preliminary report. Journal of the American Dental Association, *68:* 801-806, 1964.

Onofre, M., Sposto, M., Navarro, C., and Scully, C. Assessment of blue toluidine stain in oral lesions with suspicious of malignancy. Journal of Dental Research, *74:* 782, 1995.

Onofre, M., Sposto, M., and Navarro, C. Reliability of toluidine blue application in the detection of oral epithelial dysplasia and in situ and invasive squamous cell carcinomas. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, *91:* 535-540, 2001.

Pandey, M., Thomas, G., Somanathan, T., Sankaranarayanan, R., Abraham, E., Jacob, B., and Mathew, B. Evaluation of surgical excision of non-homogeneous oral leukoplakia in a screening trial, Kerala, India. Oral Oncology, *37:* 103-109, 2001.

Partridge, M., Li, S., Pateromichelakis, S., Francis, R., Phillips, E., Huang, X., Tesfa-Selase, F., and Langdon, J. Detection of Minimal Residual Cancer to Investigate Why Oral Tumors Recur Despite Seemingly Adequate Treatment. Clinical Cancer Research, *6:* 2718-2725, 2000.

Partridge, M., Pateromichelakis, S., Phillips, E., Emilion, G., and Langdon, J. Profiling Clonality and Progression in Multiple Premalignant and Malignant Oral Lesions Identifies a Subgroup of Cases with a Distinct Presentation of Squamous Cell Carcinoma. Clinical Cancer Research, *7:* 1860-1866, 2001.

Patton, L. The effectiveness of community-based visual screening and utility of adjunctive diagnostic aids in the early detection of oral cancer. Oral Oncology, *39:* 708-723, 2003.

Petti, S. Pooled estimate of world leukoplakia prevalence: a systematic review. Oral Oncology, *39:* 770-780, 2003.

Pindborg, J., Renstrup, G., Jølst, O., and Roed-Petersen, B. Studies in oral leukoplakia A preliminary report on the period prevalence of malignant transformation in leukoplakia based on a follow-up study of 248 patients. Journal of the American Dental Association, *76:* 767-771, 1968.

Pizer, M. and Dubois, D. An assessment of Toluidine Blue for the diagnosis of lip lesions. Virginia Medical Journal, *106:* 860-862, 1979.

Portugal, L., Wilson, K., Biddinger, P., and Gluckman, J. The role of toluidine blue in assessing margin status after resection of SCC of the upperaerodigestive tract. Archives of Otolaryngology - Head and Neck Surgery, *122:* 517-519, 1996.

Poulson, T., Lindenmuth, J., and Greer, R. A comparison of the use of smokeless tobacco in rural and urban teenagers. Cancer: a cancer journal for clinicians, *34:* 248-261, 1984.

Rapidis, A., Langdon, J., Patel, M., and Harvey, P. STNMP: a new system for the clinico-pathological classification and identification of intra-oral carcinomata. Cancer, *39:* 204-209, 1977.

Reddy, C., Ramulu, C., Sundareshwar, B., Raju, M., Gopal, R., and Sarma, R. Toluidine blue staining of oral cancer and precancerous lesions. Indian Journal of Medicine, *61:* 1161-1164, 1973.

Reichart, P. Identification of risk groups fro oral precancer and cancer and preventive measures. Clinical Oral Investigations, *5:* 207-213, 2001.

Reid, B., Winn, D., Morse, D., and Pendrys, D. Head and neck *in situ* carcinoma: incidence, trends, and survival. Oral Oncology, *36:* 414-420, 2000.

Richart, R. A clinical staining test for the in vivo delineation of dysplasia and carcinoma in situ. American journal of Obstetrics and Gynecology, *86:* 703-712, 1963.

Rodriguez, T., Altieri, A., Chatenoud, L., Gallus, S., Bosetti, C., Negri, E., Franceschi, S., Levi, F., Talamini, R., and La Vecchia, C. Risk factors for oral and pharyngeal cancer in young adults. Oral Oncology, *40:* 207-213, 2004.

Rosen, I., Cornish, M., and Edelson, J. Detection of early oral cancer by toluidine blue. Journal of the Canadian Dental Association, *9:* 347-349, 1971.

Rosenberg, D. and Cretin, S. Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. Oral Surgery, Oral Medicine, Oral Pathology, *67:* 621-627, 1989.

Rosin, M. P., Cheng, X., Poh, C., Lam, W. L., Huang, Y., Lovas, J., Berean, K., Epstein, J. B., Priddy, R., Le, N. D., and Zhang, L. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clinical Cancer Research, *6:* 357-362, 2000.

Rosin, M. P., Lam, W. L., Poh, C., Le, N. D., Li, R., Zeng, T., Priddy, R., and Zhang, L. 3p14 and 9p21 loss is a simple tool for prediction second oral malignancy at previously treated oral cancer sites. Cancer Research, *62:* 6447-6450, 2002.

Rosin, M. P., Lam, W. L., and Zhang, L. Reply. Cancer Research, *63:* 5168-5169, 2003.

Rosin, M. P., Zhang, L., and Poh, C. Chapter 17: Molecular Markers of Oral Premalignant Lesion Risk. *In:* J. Ensley, J. Gutkind, J. Jacobs, and S. Lippman (eds.), Head and Neck Cancers, pp. 245-259. San Diego: Academic Press, 2003.

Roz, Wu, Porter, Scully, C., Speight, Read, Sloan, and Thakker Allelic imbalance on chromosome 3p in oral dysplastic lesions: An early event in oral carcinogenesis. Cancer Research, *56:* 1228-1231, 1996.

Saikawa, M., Ebihara, S., Yoshizumi, T., and Ohyama, W. Multiple primary cancers in patients with squamous cell carcinoma of the oral cavity. Japanese Journal of Cancer Research, *82:* 40-45, 1991.

Sapp, J., Eversole, L., and Wysocki, G. Contemporary Oral and Maxillofacial Pathology. St. Louis: Mosby, 1997.

Scheifele, C., Reichart, P., and Dietrich, T. Low prevalence of oral leukoplakia ina representative sample of the US population. Oral Oncology, *39:* 619-625, 2003.

Schepman, K. and van der Waal, I. A Proposal for a Classification and Staging System for Oral Leukoplakia: a Preliminary Study. Oral Oncology, *31B:* 396-398, 1995.

Schepman, K., van der Meij, E., Smeele, L., and van der Waal, I. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncology, *34:* 270-275, 1998.

Scholl, P., Byers, R., Batsakis, J. G., Wolf, P., and Santini, H. Microscopic cut-through of cancer in the surgical treatment of squamous carcinoma of the tongue. The American Journal of Surgery, *152:* 354-360, 1986.

Scully, C. Oral Precancer: Preventive and Medical Approaches to Management. Oral Oncology, *31B:* 16-26, 1995.

Shah, J., Cendon, R., Farr, H., and Strong, E. Carcinoma of the oral cavity. The American Journal of Surgery, *132:* 504-507, 1976.

Shah, K. Do human papillomavirus infections cause oral cancer? Journal of the National Cancer Institute, *90:* 1585-1586, 1998.

Shah, J., Johnson, N., and Batsakis, J. (eds.) Oral Cancer: Martin Dunitz, 2003.

Shedd, D., Hukill, P., and Bahn, S. In vivo staining properties of oral cancer. American Journal of Surgery, *110:* 631-634, 1965.

Shedd, D., Hukill, P., Bahn, S., and Ferraro, R. Further appraisal of in vivo staining properties of oral cancer. Archives of Surgery, *95:* 16-22, 1967.

Shedd, D. and Gaeta, J. In vivo staining of pharyngeal and laryngeal cancer. Archives of Surgery, *102:* 442-446, 1971.

Shin, D. M., Lee, J., Lippman, S. M., Lee, J. J., Tu, Z., Choi, G., Heyne, K., Shin, H., Ro, J. Y., Goepfert, H., Hong, W. K., and Hittelman, W. N. p53 Expression: Predicting recurrence and second primary tumors in head and neck squamous cell carcinoma. Journal of the National Cancer Institute, *88:* 519-529, 1996.

Shiu, M., Chen, T., Chang, S., and Hahn, L. Risk factors for leukoplakia and malignant transformation to oral carcinoma: a leukoplakia cohort in Taiwan. British Journal of Cancer, *82:* 1871-1874, 2000.

Sidransky, D. Nucleic acid-based methods for the detection of cancer. Science, *278:* 1054-1058, 1997.

Silverman, S. J. and Rozen, R. Observations on the clinical characteristics and natural history of oral leukoplakia. Journal of the American Dental Association, *76:* 772-777, 1968.

Silverman, S. J., Bhargava, K., Mani, N., Smith, L., and Malaowalla, A. Malignant transformation and natural history of oral leukoplakia in 57,518 industrial workers of Gujarat, India. Cancer, *38:* 1790-1795, 1976.

Silverman, S. J., Gorsky, M., and Lozada, F. Oral leukoplakia and Malignant Transformation. Cancer, *53:* 563-568, 1984.

Silverman, S. J., Migliorati, C., and Barbosa, J. Toluidine blue staining in the detection of oral precancerous and malignant lesions. Oral Surgery, *57:* 379-382, 1984.

Silverman, S. J. and Sugerman, P. Oral premalignancies and squamous cell carcinoma. Clinics in Dermatology, *18:* 563-568, 2000.

Silverman, S. J. Chapter 1: Epidemiology. *In:* S. J. Silverman (ed.), Oral Cancer, 5th edition. Hamilton: BC Decker, Inc, 2003.

Singh, M., Krishanappa, R., Bagewadi, A., and Keluskar, V. Efficacy of oral lycopene in the treatment of oral leukoplakia. Oral Oncology, *40:* 591-596, 2004.

Slaughter, D., Southwick, HW, Smejkal, W "Field Cancerization" oral stratified squamous epithelium: clinical implications of multicentric origin. Cancer, *6:* 963-968, 1953.

Smith, E., Ritchie, J., Summersgill, K., Hoffman, H., Wang, D., Haugen, T., and Turek, L. Human Papillomavirus in Oral Exfoliated Cells and Risk of Head and Neck Cancer. Journal of the National Cancer Institute, *96:* 449-455, 2004.

Sollecito, T. and Alawi, F. Dysplastic Leukoplakia (letter). The New England Journal of Medicine, *350:* 2719, 2004.

Spiro, R., Huvos, A., Wong, G., Spiro, J., Gnecco, C., and Strong, E. Predictive value of tumor thickness in squamous carcinoma confined to the tongue and floor of the mouth. The American Journal of Surgery, *152:* 345-350, 1986.

Squier, C. Smokeless tobacco and oral cancer: A cause for concern? Cancer: a cancer journal for clinicians, *34:* 242-247, 1984.

Sudbø, J., Kildal, W., Risberg, B., Koppang, H., Danielson, H., and Reith, A. DNA Content as a Prognostic Marker in Patients with Oral Leukoplakia. The New England Journal of Medicine, *344:* 1270-1278, 2001.

Sudbø, J., Bryne, M., Mao, L., Lotan, R., Reith, A., Kildal, W., Davidson, B., Soland, T., and Lippman, S. M. Molecular based treatment of oral cancer. Oral Oncology, *39:* 749-758, 2003.

Sudbø, J., Lippman, S. M., Lee, J. J., Mao, L., Kildal, W., Sudbo, A., Sagen, S., Bryne, M., El-Naggar, A., Risberg, B., Evensen, J., and Reith, A. The Influence of Resection and Aneuploidy on Mortality in Oral Leukoplakia. The New England Journal of Medicine, *350:* 1405-1413, 2004.

Tabor, M., Brakenhoff, R., Ruijter-Schippers, H., van der Waal, J., Snow, G. B., Leemans, C., and Braakhius, B. J. M. Multiple head and neck tumors frequently originate from a single preneoplastic lesion. American Journal of Pathology, *161:* 1051-1060, 2002.

Talamini, R., La Vecchia, C., Levi, F., Conti, E., Favero, A., and Franceschi, S. Cancer of the Oral Cavity and Pharynx in Nonsmokers who Drink Alcohol and in Nondrinkers who Smoke Tobacco. Journal of the National Cancer Institute, *90:* 1901-1903, 1998.

Thomson, P. Field change and oral cancer: new evidence for widespread carcinogenesis? International Journal of Oral and Maxillofacial Surgery, *31:* 262-266, 2002.

Tradati, N., Grigolat, R., Calabrese, L., Costa, L., Giugliano, G., Morelli, F., Scully, C., Boyle, P., and Chiesa, F. Oral Leukoplakias: to Treat or Not? Oral Oncology, *33:* 317-321, 1997.

Vahidy, N., Zaidi, S., and Jafarey, N. Toluidine blue test for detection of carcinoma of the oral cavity: an evaluation. Journal of Surgical Oncology 434-438, 1972.

van der Riet, P., Nawroz, H., Hruban, R., Corio, R., Tokino, K., Koch, W., and Sidransky, D. Frequent loss of chromosome 9p21 early in head and neck cancer progression. Cancer Research, *54:* 1156-1158, 1994.

van der Tol, I., de Visscher, JGAM, Jovanovic, A, van der Waal, I Risk of second primary cancer following treatment of squamous cell carcinoma of the lower lip. Oral Oncology, *35:* 571-574, 1999.

van der Toorn, P., Veltman, J., Bot, F., de Jong, J., Manni, J., Ramaekers, F., and Hopman, A. Mapping of resection margins of oral cancer for p53 overexpression and chromosome instability to detect (pre)malignant cells. Journal of Pathology, *193:* 66-72, 2001.

van der Waal, I., Schepman, K., van der Meij, E., and Smeele, L. Oral Leukoplakia: a Clinicopathological Review. Oral Oncology, *33:* 291-301, 1997.

van der Waal, I., Schepman, K., and van der Meij, E. A modified classification and staging system for oral leukoplakia. Oral Oncology, *36:* 264-266, 2000.

van der Waal, I. and Axéll, T. Oral leukoplakia: a proposal for uniform reporting. Oral Oncology, *38:* 521-526, 2002.

van Es, R., van Niew Amerongen, N., Slootweg, P., and Egyedi, P. Resection margin as a predictor of recurrence at the primary site for T1 and T2 oral cancer. Archives of Otolaryngology - Head and Neck Surgery, *122:* 521-525, 1996.

van Houten, V., Leemans, C., Kummer, J., Dijkstra, J., Kuik, D., van den Brekel, M., Snow, G. B., and Brakenhoff, R. Molecular diagnosis of surgical

margins and local recurrence in head and neck cancer patients: A prospective study. Clinical Cancer Research, *10:* 3614-3620, 2004.

Velly, A., Franco, E., Schlecht, N., Pintos, J., Kowalski, L., Oliveira, B., and Curado, M. Relationship between dental factors and risk of upper aerodigestive tract cancer. Oral Oncology, *34:* 284-291, 1998.

Vikram, B., Strong, E., Shah, J., and Spiro, R. Failure at the primary site following multimodality treatment in advanced head and neck cancer. Head and Neck Surgery, *6:* 720-723, 1984.

Vokes, E., Weichselbaum, RR, Lippman, SM, Hong, WK Head and Neck Cancer. New England Journal of Medicine, *328:* 184-194, 1993.

Waldron, C. and Shafer, W. Leukoplakia revisited. Cancer, *36:* 1386-1392, 1975.

Warnakulasuriya, K. and Johnson, N. Sensitivity and specificity of OroScan toluidine blue mouthrinse in the detection of oral cancer and precancer. Journal of Oral Pathology and Medicine, *25:* 97-103, 1996.

Warnakulasuriya, S. Lack of molecular markers to predict malignant potential of oral precancer. Journal of Pathology, *190:* 407-409, 2000.

Warnakulasuriya, S. Chapter 51: Cancers of the oral cavity and pharynx. *In:* B. Vogelstein and K. W. Kinzler (eds.), The genetic basis of human cancer. New York: McGraw-Hill, 2002.

Warren, S. and Gates, O. Multiple primary malignant tumors: A survey of the literature and a statistical study. American Journal of Cancer, *16:* 1358-1414, 1936.

Weijers, M., Snow, G. B., Bezemer, P., van der Wal, J., and van der Waal, I. The clinical relevance of epithelial dysplasia in the surgical margins of tongue and floor of mouth squamous cell carcinoma: an analysis of 37 patients. Journal of Oral and Maxillofacial Surgery, *31:* 11-15, 2002.

Whitehurst, J. and Droulias, C. Surgical treatment of squamous cell carcinoma of the oral tongue. Archives of Otolaryngology, *103:* 212-215, 1977.

WHO Collaborating Centre for Oral Precancerous Lesions Definition of leukoplakia and related lesions: An aid to studies on oral precancer. Oral Surgery, *46:* 518-539, 1978.

Wight, A., Ogden, GR Possible mechanisms by which alcohol may influence the development of oral cancer-a review. Oral Oncology, *34:* 441-447, 1998.

Wong, L., Wei, W., Lam, L., and Yuen, A. Salvage of recurrent head and neck squamous cell carcinoma after primary curative surgery. Head and Neck, *25:* 953-959, 2003.

Woolgar, J., Brown, J., Scott, J., West, C., Vaughan, E., and Rogers, S. Survival, metastasis and recurrence of oral cancer in relation to pathological features. Annals Royal College of Surgery England, *77:* 325-331, 1995.

Woolgar, J., Rogers, S, West, CR, Errington, RD, Brown, JS, Vaughan, ED Survival and patterns of recurrence in 200 oral cancer patients treated by radical surgery and neck dissection. Oral Oncology, *35:* 257-265, 1999.

Yueh, B., Feinstein, A., Weaver, E., Sasaki, C., and Concato, J. Prognostic staging system for recurrent, persistent, and second primary cancers of the oral cavity and oropharynx. Archives of Otolaryngology - Head and Neck Surgery, *124:* 975-981, 1998.

Zhang, L., Michelsen, Cheng, X., Zeng, Priddy, R., and Rosin, M. P. Molecular Analysis of oral lichen planus. American Journal of Pathology, *151:* 323-327, 1997.

Zhang, L., Cheng, X., Yong-hua, L., Poh, C., Zeng, T., Priddy, R., Lovas, J., Freedman, P., Daley, T., and Rosin, M. P. High Frequency of Allelic Loss in Dysplastic Lichenoid Lesions. Laboratory Investigations, *80:* 233-237, 2000.

Zhang, L., Cheung, K.-J., Lam, W. L., Cheng, X., Poh, C., Priddy, R., Epstein, J. B., Le, N. D., and Rosin, M. P. Increased genetic damage on oral leukoplakia from high-risk sites: potential impact on staging and clinical management. Cancer, *91:* 2148-2155, 2001.

Zhang, L. and Rosin, M. P. Loss of heterozygosity: A potential tool in management of oral premalignant lesions? Journal of Oral Pathology and Medicine, *30:* 513-520, 2001.

Zhang, L., Epstein, J., Poh, C., Berean, K., Lam, W. L., Zhang, X., and Rosin, M. P. Comparison of HPV infection, *p53* mutation and allelic losses in posttransplant and non-posttransplant oral squamous cell carcinomas. Journal of Oral Pathology and Medicine, *31:* 134-141, 2002.