IMPROVING THE DETECTION AND TRIAGE OF ORAL PREMALIGNANT LESIONS IN HIGH-RISK CLINICS AND COMMUNITY DENTAL PRACTICES

by

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ABSTRACT

Oral cancer occurs at a cancer site that is easily examined; yet more than 40% of oral cancers are diagnosed at a late stage when the chance of death is high and treatment can be devastating. Although oral cancer screening is part of every oral health professional’s (OHP) training, it is often difficult for OHPs to differentiate high-risk oral premalignant lesions (OPLs) from benign reactive lesions. A primary goal of this thesis was to evaluate two approaches to enhancing visualization of clinical lesions: the application of toluidine blue (TB) stain, used to improve contrast of suspicious mucosal areas in normal tissue (Project 1), and fluorescence visualization (FV), used to identify an alteration to tissue optics that is associated with morphological and biochemical change seen in cancers and premalignant disease (Projects 2 and 4). A second goal was to develop and evaluate an educational strategy for oral cancer screening in community dental clinics aimed at strengthening conventional screening activities and providing a framework for integration and assessment of visualization techniques in community settings (Projects 3 and 4). Studies were conducted on patients within two settings: referral clinics of the BC Oral Cancer Prevention Program (Projects 1 and 2) and community dental clinics in the Vancouver lower mainland (Projects 3 and 4). Use of two settings is important: technology developed within high-risk referral settings needs to be re-evaluated in community clinics where the spectrum of disease is different and expertise is variable. Among key results of these studies were: a strong association between TB positive staining and increased (6-fold) cancer risk for OPLs; an association of FV and high-risk clinical, histological and molecular change; and; identification of barriers and facilitators for oral cancer screening in OHPs with evaluation of a triaging framework to support key decision points in community practices. In summary, this thesis data supports the use of TB and FV visualization approaches in high-risk clinics to improve detection of OPLs. In addition, the community studies have produced a framework for transfer of new technology into general dental practice building upon an enhanced triage and referral system.
Keywords:
Oral cancer; oral premalignant lesion; opportunistic screening; fluorescence visualization; toluidine blue; adjunctive screening tools; focus groups; oral health professionals; dentists; dental hygienists
DEDICATION

To my family.
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Abbreviations

BCCA  British Columbia Cancer Agency
CIHR  Canadian Institutes of Health Research
CIS  carcinoma-in-situ
CTFPHE  Canadian Task Force for Periodic Health Examinations
FV  fluorescence visualization
FVE  fluorescence visualization equivocal
FVL  fluorescence visualization loss
FVR  fluorescence visualization retention
HNCA  head and neck cancer
KT  knowledge translation
LOH  loss of heterozygosity
LP  lichen planus
OHS  Oral Health Study
OPL  oral premalignant lesion
OR  Odds Ratio
RCT  randomized controlled trial
RR  relative risk
SCC  squamous cell carcinoma
SD  standard deviation
SFU  Simon Fraser University
TB  Toluidine blue
TSG  tumour suppressor gene
UADT  upper aerodigestive tract
UBC  University of British Columbia
USPSTF  U.S. Preventive Services Task Force
VC  verrucous carcinoma
WHO  World Health Organization
CHAPTER 1
GENERAL INTRODUCTION
AND LITERATURE REVIEW

1.1 Overview

Oral cancer is a substantial, though often unrecognized issue globally, with close to 300,000 new cases reported annually. The disease represents a management conundrum: this is a cancer site that is easily examined; yet more that 40% of oral cancers are diagnosed at a late stage when the chance of death is high and treatment can be disfiguring and devastating. Diagnosing the disease while it is still in the premalignant stage could have a great impact on survival, allowing for early intervention.

Oral cancer screening is part of every oral health professional’s (OHP) education and training. Unfortunately, it is often difficult for OHPs to differentiate high-risk oral premalignant lesions (OPLs) from benign lesions or normal tissue variations. This can be frustrating to the clinician, reducing adherence to screening behaviour and resulting in both a delay in identification of clinical lesions and in the decision to biopsy. There is a need for development of visualization approaches that could aid detection of worrisome lesions and for the validation of such approaches in different settings.

The overall goal of this thesis was to develop strategies for improving the detection of high-risk OPLs through enhanced visualization of clinical lesions. The thesis evaluated two approaches to visualization: staining with a vital dye, toluidine blue (TB), used to improve contrast of suspicious mucosal areas in normal tissue, and fluorescence visualization (FV), used to identify an alteration to tissue optics that is associated with morphological and biochemical change seen in cancers and premalignant disease. Studies were conducted in patients within two settings: referral clinics of the BC Oral Cancer Prevention Program (Projects 1 and 2 below) and community dental clinics in the Vancouver lower mainland (Projects 3 and 4). The use of two settings was important. The development and evaluation of new technology for cancer management frequently takes place within secondary care referral settings since more disease is
present; however, the spectrum of disease is quite different from that seen in community clinics where a greater proportion of oral anomalies are benign or reactive lesions as compared OPLs and cancers and confounding can be much greater. The training and experience of the health providers in making clinical judgment is also crucial and is quite different between the two settings. An additional objective of this study was the development, implementation and evaluation of an oral cancer educational module that would strengthen conventional screening activities and lay a baseline for comparing new technology as it is developed.

This thesis is comprised of 4 subprojects:

Chapter 2: **Utility of Toluidine Blue Stain for Identification of High-Risk OPLs in High-Risk Clinics.**

Although TB staining has a long history of use in detection of oral cancers, its utility for detection of OPLs is very controversial, with only a portion of such lesions positive for the endpoint. The question is, are positive lesions more likely to be those that are at high risk for cancer development? This project used data collected prospectively from 100 dysplasia patients enrolled in an ongoing longitudinal study to determine whether TB staining was associated with risk of progression to cancer, looking for associations with clinical, histological and molecular high-risk attributes. TB was significantly associated with the larger size and nonhomogeneous appearance of lesions, as well as the presence of high-risk molecular patterns. Overall, there was a six-fold elevation (4-fold for low-grade dysplasia only) in cancer risk for positive lesions, with 12 of 15 progressing lesions staining positive for the dye. These data suggest that TB stain can be used in referral clinic settings to triage those early lesions that need intervention.

Chapter 3: **Fluorescence visualization as an adjunctive tool for detection of high-risk oral premalignant lesions in High-Risk Clinics.**

Loss of autofluorescence is documented as being present in cancer and premalignant lesions at several sites. However, until recently there has been little information available on associations of FV loss with oral cancer and premalignant lesions and no information collected with direct fluorescence visualization. Early studies used computer algorithms and spectroscopy and did not allow the clinician to directly observe regions of change. Direct FV was made possible only recently with the development in BC of a hand-held direct visualization (FV) device. This device has since been on the market globally with rapid uptake. There is a need to
establish its efficacy. The objective of this study was to evaluate direct FV, within the constraints of the ongoing longitudinal study in BC in a high-risk clinic, to determine its ability to detect high-risk oral premalignant disease and cancer. This study compared FV loss in lesions that had low vs. high-risk clinical, histological progression and molecular features. Loss of FV was significantly associated with high-risk clinical features and molecular risk patterns. Virtually all cancers and high-grade dysplasias were correctly identified by the disease

Chapter 4: **Opportunistic screening in community dental practices has been proposed as a cost effective method for early detection of oral cancers and premalignant lesions.**

However, a wide variation in both the frequency and quality of such activity exists in community settings. The long-term goal of the BC OCPP is to begin the process of establishing a high-quality screening network in BC, with defined strategies for improving both the frequency and quality of screens. In this first phase, the objective was to collect information on knowledge of oral cancer risk and self-reported screening activity in a cohort of OHPs from 10 Vancouver practices. A 1-day workshop was given to train on conventional and FV-guided screening activities. Screening practices were followed for 3 months and assessment made of types of lesions identified and problems in diagnosis. A chart audit of screening practices prior to the intervention was completed. Barriers and facilitators to screening activity were determined at study end in a focus group. Attitudes of patients towards device usage and problems with the technology were ascertained. Barriers to screening included concern on how it would impact their existing workload, an uncertainty in how to talk to their patients about screening, concern regarding how their patients would react and a lack of screening guidelines. Facilitators to screening was chairside information sheets, scripts on how to talk to their patients, and patients viewing the office as progressive for screening. Participants also stressed the need for increased professional and public awareness of the disease and screening, review of biopsy procedures, information on how to integrate screening into practice and increased access to courses.

Chapter 5: **Decision-making on Triage of Oral Mucosa Lesions in Community Dental Practices.**

This project used lessons learned on screening activities in Project 3 to develop a study aimed at better characterizing the types and frequencies of oral mucosal lesions identified in an independent group of 15 dental practices using conventional and FV-targeted screening. A chief
objective was to evaluate the ability of clinicians to differentiate high- from low-risk lesions and to make decisions for appropriate and timely follow-up according to recommendations established in Project 3. Identification of key decision points in oral cancer screening, the value of a 3 week reassessment period and areas of knowledge which need to be reinforced with further education.

Chapter 6: Discussion. Integrates and summarizes the finding of this thesis and includes the limitations of this research as well as future directions.

1.2 Literature Review

1.2.1 Overview

Oral cancer is the 6th most common cancer in the world [1]. In some South Asian countries such as India, Pakistan, Taiwan and Sri Lanka oral cancer is one of the most common types of cancer. There have been increases in the incidence rate in some European countries as well including Denmark, France, Germany, Scotland and Hungary [2]. In Canada, 3350 Canadians are expected to be diagnosed with oral cancer in 2009, 420 in British Columbia (BC) [3]. This is more than the number of expected cases of cervical, ovarian, stomach and other more commonly known cancers [3]. The project incidence rate for oral cancer in Canada is 11 per 100,000 males and 5 per 100,000 females. With the aging of the baby boomers, more cases of oral cancers are expected. In British Columbia, the BC Cancer Agency has predicted a 21% increase in the number of oral cancer cases by 2023 (from 2009) and a 52% increase in the number of deaths. These predictions were based on oral cancer trends in the province over the last 10 years[4].

Despite advances in treatment and technology there has been only a modest improvement in the 5 year survival rate over the last few decades. Worldwide 1 in 2 people with oral cancer will die as a result of their disease (5-year survival rate about 50%). Even in developed countries, the prognosis is still poor. The 5-year survival rate for oral cancer in Canada is 63% [3]. This prognosis is even worse in India at 30%[5]. More Canadians are expected to die from oral cancer in 2009 than from cancers of the cervix, melanoma, liver and Hodgkin’s Lymphoma[3].
The main problem for the poor prognosis is late diagnosis. When oral cancer is detected early (stage I) the survival rate in the United States is 83% and 28% if detected after it has metastasized distantly [6]. Although the oral cavity is easily accessible approximately 67% of oral cancers in the United States are diagnosed after the disease has metastasized regionally or distantly [6] and require more aggressive treatment. Research is needed to address the problems of late diagnosis.

1.3 Oral cancer etiology

No single factor causes oral cancer, it is a combination of extrinsic and intrinsic factors over time and is dependent on each person’s unique response to both known and unknown risk factors. The most modifiable risk factors are tobacco and alcohol use, followed by betel quid and poor diet. Other risk factors that can be ascertained in a dental office include age, a previous history of oral cancer or precancer, and immunosuppression. Awareness of human papilloma virus (HPV) status and exposure to ultraviolet light may not be easily identifiable on a patient health history.

1.3.1 Tobacco

Tobacco is the most significant and modifiable risk factor for cancer. It is available in many forms both smoked and smokeless. While nicotine is the addictive element of tobacco, the primary carcinogens are in the tar. Tobacco cessation is the most valuable form of primary prevention. The highest percentage of smokers in Canada is in the 20-24 year age group [7]. Within oral cancer in the western world, tobacco is most associated with cancers of the floor of the mouth.

The risk of oral cancer and premalignant lesions increases both with the amount and the length of time tobacco has been used [8]. A meta-analysis found current smokers had a relative risk of oral cancer of 3.43 versus non-smokers [9]. Patients who continue to smoke after an oral cancer diagnosis are at a greater risk for a second oral cancer. Fortunately, the risk for premalignant lesions, oral cancer and second oral cancers decreases once a smoker has quit and continues to decrease. After 10 years of not smoking a former smoker's risk of oral cancer is on par with that of a never smoker [10].
There has been an increase in the use of smokeless tobacco in young men. Tobacco companies are advertising it as a safer alternative to smoking yet more than 30 carcinogens are found in smokeless tobacco [11]. As with smoked tobacco there is dose-response relationship with smokeless tobacco. In the United States, the use of smokeless tobacco more than doubles the risk of oral cancer according to a recent meta-analysis [11]. In India smokeless tobacco products accounts for almost 50% of oral cancer [11]. Smokeless tobacco has been associated with a greater risk of premalignant lesions such as erythroplakia and leukoplakia [12]. One study found that almost 15% of smokeless tobacco users had an intraoral lesion at the site where they placed their tobacco [13].

Betel quid is a popular product chewed primarily by people of South Asian decent. Betel quid is made up of a betel leaf, areca nut, lime (calcium hydroxide) and flavouring agents. The lime, used to hold the ingredients together, is an irritant and will erode the tissue in the oral cavity. While betel quid can be chewed without tobacco it is more common to have tobacco included. Betel quid without tobacco is also known to be a carcinogen. As with tobacco, risk is associated with dose and duration of use. In India, where betel quid chewing is widespread, the most common site for oral cancer is the buccal mucosa.

There are conflicting results for oral cancer risk and marijuana smoking [14-17]. However, marijuana has 4 times the tar burden that tobacco has and is typically inhaled deeper and held longer.[18]

Despite the obvious cause and effect relationship between oral cancer and tobacco use, the history of tobacco use is not commonly recorded in the dental chart thereby preventing an opportunity for oral health professionals to discuss tobacco cessation and identify patients who may be at high-risk for oral cancer.

### 1.3.2 Alcohol

The heavy use of alcohol is also a major risk factor for oral cancer. While there is no reported minimal threshold for alcohol risk [19], men who drank more than 21 units per week and women who drank more than 14 units of alcohol per week were found to have an increased risk of oral cancer [20-23]. (One unit of alcohol is equal to 1 - 4 ounce glass of wine, 1 - 8 ounce beer or 1 - 1 ounce shot of spirits, as per BCCA standards and [24]). The total amount of ethanol consumed is more important than the type of alcohol. Alcohol also has a dose-response
relationship, risk of oral cancer increases both with the duration of alcohol consumption and with the amount consumed. There are some geographical differences found with risk as the processing of alcohol can vary by country. In the past, the risk from alcohol alone has been difficult to study as subjects who drank but did not smoke were not common. More recent studies have identified alcohol as a significant risk factor on its own. An increase in oral cancer in people in their 40s in the United Kingdom is believed to be due to alcohol use [25]. Unlike tobacco, the risk for oral cancer in former heavy drinkers can persist for more than 20 years after a person quits drinking [26].

How alcohol acts as a carcinogen is not fully known. Some theories include acetaldehyde, a known carcinogen and metabolite of alcohol, and ethanol causing DNA damage; nutritional deficiencies associated with heavy drinking; the carcinogenic effect of chemicals other than ethanol in alcoholic beverages; the induction of microsomal enzymes that enhance the metabolic activation of other carcinogens; or the capacity of alcohol to enhance the penetration of carcinogens (tobacco) in the tissue [26].

Together tobacco and alcohol have a synergistic effect and account for approximately 75% of oral cancers. Heavy smoking and drinking in men can increase the risk of oral and oropharyngeal cancer by 38 times, and in women a 100 fold increased risk has been found [27]. The greatest risk for developing an oral cancer or premalignant lesions is for patients who both smoke and drink, with decreasing risk for non-drinking smokers and non-smoking drinkers, respectively. The interaction of tobacco and alcohol was analyzed using pooled data from 17 studies [28]. Results found tobacco and alcohol contributed to 72% of head and neck cancers and 64% of oral cancers. Of the head and neck cancers, 4% was due to alcohol alone, 33% was due to tobacco alone and 35% was due to the 2 combined. These risk habits were found to have a greater effect on men and older subjects (≥45 years). The Odds Ration (OR) for oral cancer in people who drink 3 or more drinks per day and smoke less than a pack/day is almost 10 and drinkers who smoke more than a pack/day the OR is greater than 15. Smokers who drink a moderate amount (1-2 drinks/day) had an OR of 2.7 for a pack/day or less and 3.2 for more than a pack/day.

In an earlier study by the same authors, smokers who did not drink alcohol had a greater than 2 times risk for head and neck cancers, a group that accounted for 24% of head and neck
cancer cases in the study population[29]. Similarly non-smoking, heavy drinkers had twice the increased risk of head and neck cancer and attributed for 7% of the cancer cases.

Alcohol use is very rarely documented by the oral health professional, again preventing an opportunity to educate patients about their oral cancer risk and the use of alcohol in moderation.

1.3.3 Age

The majority of oral cancers are diagnosed in the 7th decade of life. This is a result of the accumulated lifelong exposure to carcinogens and damage to DNA. The risk for men 40 – 59 years of age is 11 times that of men under the age of 40. This risk increases to 21 times once men reach the age of 60. Women over the age of 40 years have 5 times the risk and over 60 have 12 times the risk of women under the age of 40 [30](Figure 1-1). While oral SCC is predominantly a disease of the elderly, there appears to be an increase in the incidence of oral cancer in young adults under 40 years of age [31-33]. The gender difference observed in older oral cancer patients is not evident in this group and females appear to be more prominent in the young cancer sufferers [34].

Figure 1-1. Probability of Developing Invasive Cancer over Selected Age Intervals, by gender, US, 2004-2006 [30]

Blue columns are male and purple columns are females. If risk of oral cancer is 1 for people under 40 years of age then risk for 40 – 59 year olds is 11 and 5 fold respectively for men and women. Risk for people over the age of 60 years is 21 and 12 times that of those under 40 (men and women respectively).
For younger oral cancer patients, under the age of 40 years, there appears to be 2 variations. One group is similar to older oral cancer patients with similar risk habits and disease progression [35-37]. The second group develops oral cancer without a known etiological cause and their cancer seems to progress quicker than most oral cancers[33, 38, 39]. This group is often composed of young females who present at a more advanced stage possible due to diagnostic delays attributed to their lack of known risk factors [33]. This group may be at an increased risk due to inherited risk factors or altered immunity.

Another challenge to controlling oral cancer is the aging baby boomer generation which is expected to increase the number of oral cancer cases as noted previously [4]. Oral health professionals must also be aware of the increase in oral cancer in younger patients so that oral cancer screening is not limited to older patients or that clinical lesions are not ignored due to the younger age of a patient.

1.3.4 Diet

A person with a diet low in fruits and vegetables is at an increased risk for oral cancer. The combination of poor nutrition with tobacco and alcohol use is believed to account for 85% of oral cancers [40]. A multiplicative effect was also found for oropharyngeal cancer between a diet low in fruits and vegetables and heavy smoking or alcohol use [41].

A diet which includes the recommended daily allowance of fruit and vegetables can reduce the risk of oral cancer by half. Citrus fruits, orange and yellow vegetables have been found to be particularly protective. In a meta-analysis of 16 studies regarding nutrition and oral cancer, a 49% reduced risk for each portion of fruit eaten and a 50% reduced risk for vegetables consumed was discovered [42]. Citrus fruits had a greater effect than total fruits consumed and were also found to reduce the risk of oropharyngeal cancer, particularly when consumed for a long duration. The protective effect of fruits, citrus fruits and vegetables against oral cancer and second oral cancers, has been supported by other research [43-45].

The role of diet in oral cancer is important information for oral health professionals both for educating their patients about prevention but also in the follow-up of patients with oral premalignant lesions (OPL) and cancer.
1.3.5 HPV

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 of the cervix, anogenital area and the oropharynx. HPV is divided into low-risk and high-risk subtypes. The high-risk subtypes are associated with cancer. The two subtypes of HPV associated with oral and oropharyngeal cancers are HPV-16 and -18 with HPV-16 being the most common. HPV-16 is believed to activate oncogenes whose proteins alter the function of tumour suppressor genes p53 and Rb, cell cycle regulators [46] leading to cell proliferation [47]. The risk of oral cancer related to HPV increases with the number of sexual partners, oral sex partners and younger age of first sexual encounter [48, 49]. In one study, more than 50% of non-smoking non-drinkers with oropharyngeal cancer were positive for HPV-16 [50].

HPV is mainly associated with oropharyngeal cancers – tonsils, soft palate, and base of tongue. HPV related cancers have a lower recurrence rate than other oral cancers [51].

Oral health professionals should be aware of the role HPV plays in the etiology of oral cancer so that they do not ignore clinical lesions in patients who do not have other more obvious risk factors.

1.3.6 Susceptibility and genetics

Patients who have had a previous oral cancer are at an increased risk of a second oral malignancy and should be monitored closely. The risk of a second oral malignancy is approximately 30%. Former oral cancer patients who develop a lesion at a former cancer site, regardless of histology, are more likely to have a recurrence [52].

1.4 Oral cancer staging

Oral cancer is believed to develop from oral premalignant lesions (OPL). If undiagnosed or untreated, oral SCC will spread by local invasion and increase in size, by lymph node metastasis and rarely distant blood metastasis. 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 [53]. The tumour (T1-T3) aspect refers to the increasing size of the invasive tumour. T1 is a tumour less
than 2 cm, T₂ is 2 – 4 cm and T₃ is greater than 4 cm in size. The label T₄ is given to a tumour that has invaded an adjoining structure. Carcinoma in situ, the highest grade of dysplasia, is labelled T₀. N reflects the absence or presence of local lymph nodes as well as their number, size and site [54, 55] and M refers to the absence or presence of distant metastasis. The TNM code is then used to stage the tumours. Early stage tumours, stage 0 (T₀), stage I (T₁N₀M₀) and stage II (T₂N₀M₀) have no local lymph node or distant metastasis involvement and are less than 4cm in size. Stage III (T₃N₀M₀ or T₁–3N₁M₀) and stage IV tumours (T₁–3N₂–3M₀) are called late stage tumours [56]. Regardless of T or N stage patients who have distant metastases are ranked as stage IV disease [56, 57]. The system reflects prognosis; as clinical stage increases the prognosis decreases.

Almost 90% of oral cancer originates from the lining epithelium of the oral mucosa and is known as squamous cell carcinoma (SCC) [58] and hence, the term oral cancer generally refers to oral SCC. Oral SCC may occur anywhere in the mouth with the areas at highest risk being the floor of mouth, the lateroventral border of the tongue and the soft palate. The sites in decreasing order of frequency are the lateroventral tongue, floor of mouth, hard palate, gingiva and buccal mucosa (no geographical parameters listed) [47]. In the United States, the most frequent site for oral cancer was the lateroventral tongue and floor of the mouth [59]. 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% [58, 60]. The more posterior and inferior the tumour site is within the oral cavity the worse the patient’s prognosis [58].

With late stage oral cancer, clinical decision-making is not difficult. They often present as non-healing ulcers, frequently indurated, or become a fungating lesion. However, early oral cancers can be a problem as they could clinically resemble benign reactive lesions or OPLs such as leukoplakia and erythroplakia which will be discussed in the following sections.

Oral health professionals require ongoing education to maintain or increase their awareness of oral cancer, its clinical presentation and the benign lesions it may resemble clinically.
1.5 Clinical risk factors for Oral Premalignant Lesions (OPLs)

Oral squamous cell carcinomas (SCC) are often preceded by clinically visible premalignant changes [61, 62]. A premalignant lesion is defined by the WHO as “a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart” [63]. Oral premalignant lesions present most frequently as a leukoplakia (white patch) that cannot be scraped off, and erythroplakia (red patch), or a combination of both, known as erythroleukoplakia. Many authors consider the terms leukoplakia and OPL to be interchangeable.

1.5.1 Clinical appearance and cancer risk

1.5.1.1 Leukoplakia

Leukoplakia accounts for 85% of oral premalignant lesions [64, 65]. Leukoplakia is a definition of exclusion, defined by the WHO as a “white patch or plaque that cannot be characterized clinically or pathologically as any other disease” [63]. This definition was later revised 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” [66]. The term, leukoplakia, is most commonly used as a clinical term with no reference to histological factors. The white appearance of the lesion is due to the thickening of the stratum corneum and/or acanthosis, a thickening of the intermediate layer of the epithelium.

The prevalence of leukoplakia varies from study to study, from 0.7% to 24.8% [67] dependent on the definition used, geographical location of the study and whether biopsies were taken to support the clinical diagnosis. In a review of 23 studies, the true global prevalence of leukoplakia was estimated to be approximately 1.7% - 2.7% [68]. From this the authors 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 which found leukoplakia slightly more common in males and occurred mainly in the 5th, 6th and 7th decades of life [69]. The most common site for leukoplakia is the buccal mucosa [64, 67, 70-72]. The buccal mucosa is not a common site for dysplasia or cancer in the west and many of these lesions are reactive hyperplasia.
1.5.1.2 Erythroplakia

Erythroplakia, similarly, is a red patch that “cannot be characterized clinically or pathologically as being due to any other condition” [63] 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.

Erythroplakia generally has a higher risk of malignant transformation and a more severe histological diagnosis. 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 has been found to have the most cellular change [73].

1.5.1.3 Variation of clinical presentation and cancer risk

Cancer risk can also be estimated by variations in clinical presentation other than colour. Variations in appearance, texture, size, site and margin presentation have all been associated with an increased risk of cancer progression.

OPL risk can be classified by their appearance. Non-homogeneous lesions (not uniform in colour or texture) have a greater risk of cancer than homogeneous lesions (white with a predominantly smooth texture) [74-76]. Lesions with a smooth texture are less likely to progress than fissured, nodular, velvety/grainy, ulcerated or verrucous lesions. Non-homogeneous lesions presenting with a red colour and/or an erosive texture had a four fold increased risk of progressing to cancer [77]. Lesions can progress from an homogeneous to non-homogeneous appearance [65].

The site of the lesion can also be associated with risk of progression. Lesions found on the ventrolateral tongue, floor of the mouth and soft palate are high-risk in the western world. [69, 77-80] OPLs at high-risk sites have been found to be more likely to have high-risk molecular patterns than similar lesions from low-risk sites [81]. These sites may be at greater risk because they normally have either no or a thin keratinized layer and hence less barrier to carcinogens. The floor of the mouth and the ventrolateral tongue are also in the lower half of the oral cavity and carcinogens in the saliva may pool on or near this tissue increasing its exposure to the carcinogens. It is also more unlikely for these sites to have traumatic or reactive lesions.
Another clinical predictor of progression is the size of the lesion. Larger OPLs have a higher risk of cancer, including cumulative size of multiple OPLs. While the exact size at which risk increases is unknown, it is believed that lesions greater than 2 cm have an increased risk and lesions greater than 4 cm have a high risk of progression. [66, 74, 82, 83] Lesion margins are also associated with risk as well-defined, discrete margins are believed to be at less risk of becoming cancer than a lesion with diffuse margins.

The duration of the lesion is also a clinical risk factor. It is also believed that the longer a lesion is present the greater the risk of progression. This may be a result of longer contact with carcinogens. Lesions that regress over time or after the removal of a high-risk habit have a smaller risk than lesions that persist, grow or become increasingly non-homogeneous in appearance. [69]

Lesions in patients with a history of oral cancer or precancer are also at an increased risk of progressing to cancer, possible due to an undetected outgrowth of malignant cells or as a result of field cancerization. Unfortunately due to the sequelae of oral cancer treatment reactive lesions may be difficult to differentiate from potentially serious lesions. Adjunctive tools may aid clinicians in their biopsy decision making. [84, 85]

Persistent lesions in patients with no history of a high-risk habit (non-smoker and non-drinker) have all been found to be associated with an increased cancer risk [65, 67, 70, 75, 86, 87]. An increased rate of progression for lesions found in females, particularly non-smoking females has been found [75, 88].

When a patient presents with an OPL 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. It is important to note that not all lesions with high-risk clinical signs progress to cancer and conversely, lesions with no high-risk clinical signs may progress to cancer.

1.5.2 Problems of diagnosing leukoplakia and erythroplakia

1.5.2.1 Reactive benign white and red lesions

There are many benign lesions or variations of normal in the oral cavity that are far more common than oral cancer or precancer. The difficulty in distinguishing them is that their
Clinical appearance is very similar to those lesions at risk. Even expert clinicians can have difficulty determining the risk of a lesion by visual inspection alone. Often a biopsy is done to rule out dysplasia or SCC and to confirm a benign diagnosis. It is unknown how many oral SCC derive from subclinical OPLs and adjunctive tools (see chapter 1.7) may help clinicians visualize these lesions through ‘new eyes’ as well as differentiate between lesions at risk and benign conditions [89].

White or red patches (leukoplakia and erythroplakia), non-healing ulcers, lichen planus, discoid lupus erythematosus, submucosal fibrosis and obvious SCC are considered by most to be “positive” lesions because their clinical presentation can be very similar [21]. Biopsy is generally necessary to confirm diagnosis or rule out risk. Since many benign conditions can be the result of trauma or infection it is generally recognized that lesions be revisited in 2-3 weeks to allow time for healing. Lesions that persist without obvious cause should be investigated further.

Common benign conditions and variations of normal include candidiasis, linea alba and other trauma induced lesions and ulcers, benign rhomboid glossitis, nicotine stomatitis, fibroepithelial polyp, geographic tongue, aphthous lesions, herpetic lesions, papilloma, leukoedema, and hemangioma amongst others.

1.5.2.2 Occult OPLs

Many OPLs and early oral cancers may not be clinically apparent. This presents a great problem in the clinical diagnosis. Visual tools are needed.

1.6 Histological risk factors for Oral Premalignant Lesions (OPLs)

1.6.1 Dysplasia

Currently a lesion is considered premalignant when it exhibits changes in the epithelium known as dysplasia [63]. Dysplasia, a term meaning disordered or abnormal growth, is graded on cellular and architectural changes that can occur in the structure of the epithelium or in the individual cells themselves. Depending on the amount and severity of these changes the pathologist grades the dysplasia as mild, moderate, severe or carcinoma in-situ (CIS). 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 [67].

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 CIS. Once the changes break through the basement membrane the lesion is considered SCC, an invasive cancer.

1.6.2 Histological progression model

Currently, the prediction of the malignant transformation rate of OPLs is based primarily on histopathological factors. The current gold standard to determine risk is to determine the presence and degree of dysplasia found in a histological sample. Simply, the risk of malignant transformation increases with the severity of the grade of dysplasia (see Figure 1-2). 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 than low-grade dysplastic lesions. Studies have found a range of progression from 16% (in less than 3 years) [86] to more than one third of dysplastic lesions progressing to cancer [77]. In another paper, moderate and severe dysplasias were found to progress twice as often as mild dysplasia or hyperplasia [90]. The WHO [63] 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 1-2. **Histological progression model of oral premalignant and malignant lesions.**
As the level of dysplasia increase so does the risk of malignancy. Inversely, as the level of dysplasia increases the chance of regression decreases. Currently in BC, lesions with a diagnosis of severe dysplasia or higher are treated, while mild and moderate dysplasias are closely monitored.

### 1.6.3 Problems with the histological gold standard

As mentioned earlier the risk of progression to oral SCC increases with the severity of the histological diagnosis. 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, the grade of dysplasia is considered by some to be an unreliable prognostic indicator of cancer development [91]. The majority of OPLs in an aforementioned study [86] were diagnosed with mild dysplasia and 16% still progressed to SCC.

New clinical tools and biomarkers are needed to help predict the risk of progression of low-grade OPLs.
1.7 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 [77]. The primary reason to treat OPLs is the belief that their early recognition and treatment may well thwart the lesions progression to SCC [92] particularly those lesions at a high-risk site with a diagnosis of dysplasia. Conventional surgery is the current treatments of choice for high-grade dysplasias. Low-grade dysplasia is generally monitored closely as surgery on these lesions may be over treatment due to only a small percentage progressing to SCC.

While only a portion of premalignant lesions will progress to cancer it is important to identify and refer them for proper management and treatment, if necessary.

1.8 Visual Tools

The area of adjunctive cancer screening devices is controversial. While it is believed that the diagnosis of cancer at an early stage will increase the chance the disease will be cured there are very few prospective trials which validate screening tools. It is important that the majority of the evidence establishes that the benefits of early diagnosis outweigh the risks of testing such as over diagnosis, injury due to testing, patient anxiety and the question of what to do with false positives. The introduction of adjunctive screening tools such as the Pap smear and mammography have led to the earlier detection of cervical and breast cancers, respectively [93]. The best evidence is in breast cancer. While breast self examination has been found to have no benefit, mammography has had multiple randomized control trials (RCT) studies which provided evidence that it can reduce mortality due to breast cancer, especially for high risk individuals [94].

For most organ sites, cancer screening tools imply an invasive examination that may subject the patient to injury or discomfort, for example, a Pap smear, a colonoscopy or a mammogram. Since accessing the oral cavity is easy and the clinical exam is non-invasive and painless, the adjunctive tools also pose less of threat for injury than other organ screening tools. The majority of oral lesions will be benign but many present in a clinically similar appearance to
malignant or premalignant lesions. Two tools that can be used in clinic with minimal risk are toluidine blue (TB) and fluorescence visualization (FV). These tools may help the clinician to discriminate premalignant lesions from benign conditions and identify clinically occult lesions as well as encouraging health care professionals to perform screening.

1.8.1 Toluidine Blue as an adjunctive screening tool

One of the more common adjunctive tools available for the clinical detection of oral SCC and OPLs is toluidine blue (TB). TB also known as Tolonium Chloride, is a metachromatic, acidophilic vital dye that is soluble in water and alcohol [95] and is used by clinicians to help in the identification of primary SCC and dysplasias.

TB was first used approximately 50 years ago as an anti-heparin agent for certain types of bleeding disorders. During the 1960s TB began to be studied as an aid in the diagnosis of cervical [96] and oral cancer.

1.8.1.1 The Mechanism of Staining

The purported mechanisms of action for TB include:

1. Binding to the phosphate groups of the nucleic acids (found in high concentrations in neoplastic tissue, [95]).

2. Penetrating down between the cells due to a defective intercellular barrier in neoplastic tissue [97]

A stained lesion is considered positive when it maintains the intense dark blue colour after completion of the test. If the lesion stains weakly it is called ‘equivocal’, and if no stain remains on the lesion it is considered negative. Equivocal results should be “considered positive unless proven otherwise” [98]. No reported reaction or side effects have been reported with TB use however one study determined that TB had a mutagenic effect via the Ames test [95].

1.8.1.2 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 lesions or areas of field cancerization [84, 99] by highlighting those areas with more cellular change. Clinician desire to biopsy has been found to increase when TB positive results are found, which can lead to faster diagnosis and treatment [100].

TB has many uses as a clinical tool. It has been found to help delineate diffuse and faint lesions, aid in the visualization of lesions that appear clinically normal, help find second oral malignancies (primary or recurrences), satellite lesions, and at the time of surgery to help visualize tumour margins, extensions and satellites [101-106].

1.8.1.3 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 detection of early oral cancer that may not be distinguishable clinically from benign lesions. 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 [107]. Table 1-1 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. One study [108] found a 100% sensitivity rate for the rinse form of TB (OroScan ®) 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 (second application), allowing for the healing of traumatic or inflammatory lesions [104]. 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 the type of application [93]. One concern is false positives may lead inexperienced clinicians to cause undo risk, physical and emotional, to their patients via unnecessary procedures, hence a certain level of proficiency is necessary [109]. The interpretation of TB is subjective and improves with experience. 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 also be retained by tumours other than SCC such as malignant melanoma, fibrosarcoma and lymphosarcoma (all ulcerated) as long as they involve mucosal change. Patients with deeper tumours and no mucosal change will not pick up the stain. Benign ulcers can pick up stain but with much less intensity than malignant tissue [103].
## Table 1-1. Toluidine blue efficacy in the detection of oral SCC

<table>
<thead>
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<th>Author(s)</th>
<th>Year</th>
<th>Number of subjects</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<td>Epstein et al. [118]</td>
<td>2003</td>
<td>81</td>
<td>96.7</td>
<td>NR</td>
</tr>
</tbody>
</table>

*Patients with a previously treated SCC

* Patients returned 14 days later for second application of stain
1.8.1.4 Sensitivity and Specificity of TB in detection of OPL

TB has been found to be highly sensitive in the detection of oral CIS or SCC but has mixed results in the detection of OPLs [84, 111]. TB has been found previously to be on little help in the detection of low-grade dysplasias [85, 116] and in some cases, no different than a clinical exam alone [85]. This may be a result of dysplasia not staining as intensely as SCC resulting in an equivocal result. Variation in TB uptake across dysplasia may be due to molecular differences in TB positive, TB negative, and equivocally stained dysplasias [118]. Interestingly, when TB was found to be highly sensitive in the detection of dysplasia (as well as CIS and SCC) there was also a high rate of false positives among ulcerated and erythemic benign tissue [100].

Patients with homogeneous leukoplakia, non-homogeneous leukoplakia, erythroplakia, reticular and erosive lichen planus and suspicious ulcerations were stained twice with TB, fourteen days apart, to eliminate lesions that were the result of mechanical trauma, inflammation and potential false-positives [117]. All lesions that remained positive after the second staining were biopsied along with a portion of TB negative lesions that the clinicians felt were warranted based on their judgment. While 100% of the lesions diagnosed as SCC or CIS were TB positive, only 50% of the dysplasias (mild or moderate) were TB positive. The remaining lesions (including some false-positives) were confirmed to be benign keratosis, lichen planus or other benign lesions. In the dysplastic tissue surrounding oral cancers the sensitivity of TB was poor with only 42.5% moderate and severe dysplasias found to be TB positive [119].

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, in an early study nine TB false positive lesions (negative histologically) eventually were diagnosed as SCC after the second or third biopsy [98].
### Table 1-2. Toluidine blue efficacy in the detection of dysplasia

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of subjects</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single Application of TB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mashberg [116]</td>
<td>1983</td>
<td>98</td>
<td>33</td>
</tr>
<tr>
<td>Silverman et al [100]</td>
<td>1984</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Warnakulasuriya and Johnson [108]</td>
<td>1996</td>
<td>102</td>
<td>79.5</td>
</tr>
<tr>
<td>Epstein et al [85]</td>
<td>1997</td>
<td>45</td>
<td>53</td>
</tr>
<tr>
<td>Martin et al [119]</td>
<td>1998</td>
<td>11 (14 lesions)</td>
<td>58</td>
</tr>
<tr>
<td><strong>Second Application of TB at reassessment appointment</strong> b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onofre et al [117]</td>
<td>2001</td>
<td>43</td>
<td>50</td>
</tr>
</tbody>
</table>

a NR – not reported  
b Patients returned 10 – 14 days after first appointment for second application of TB stain.

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 [93, 112, 120].

There is a need for a simple, easy to use, patient acceptable tool to help clinicians determine the risk of a lesion and to decide where to biopsy should it be necessary.

#### 1.8.2 Fluorescence visualization (FV) as an adjunctive screening tool

##### 1.8.2.1 Autofluorescence and fluorescent visualization

Research into fluorescence visualization began in the 1950s and its use has been studied on cervical, lung, skin and oral tissues. Early papers studied fluorescence spectroscopy which helped to define the excitation and emission wavelengths for different tissues and the
determination of various fluorophores. Spectroscopy is a point measurement technique which uses a probe to collect detailed information [121]. More recent research has been into fluorescence imaging which allows the direct comparison of both abnormal and normal tissue within the same field of view, allowing the clinician to subjectively evaluate changes in regards to other tissue [122] and to capture the result via camera.

One form of electromagnetic radiation is light, emitted in the form of waves and particles. Visible light is the form of radiation we can see and covers the spectrum from red to violet (ROYGBV). Photons (particles of energy) of electromagnetic radiation (EMR) travel in waves at the speed of light and contain energy which varies with the wavelength. The shorter the wavelength, the higher the energy, hence red light with a longer wavelength has less energy than violet which has a short wavelength.

Light is scattered as a result of its interaction with matter, which for the purposes of this paper is tissue. Information on how the light interacts with the tissues is determined from reflected light. Cell nuclei and other tissue structures scatter the light, causing it to deviate from a straight pathway within the field it is passing through [123]. Light that is reflected back out of the tissue is referred to as back scatter. Forward scatter refers to light that is scattered forward further into the tissue and isotropic scatter refers to light that is scattered in directions that is neither back nor forward. [122]

Energy from the light can be absorbed by components of the tissue. These absorbers do not fluoresce. Light can also be reflected diffusely from the tissue surface. How light is absorbed, reflected or scattered depends on the structural and biochemical composition of the tissue [124]. Fluorophores, tissue components which fluoresce, absorb light at particular excitation wavelengths, becoming unstable and then quickly release the energy (emission) as it stabilizes in the form of fluorescence. Fluorescence is very sensitive to the biochemical and environmental changes within cells and tissue. Endogenous fluorophores include: nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), aromatic amino acids, collagen and elastin [125].

NADH and FAD are coenzymes involved in cellular metabolism. NADH and FAD fluoresce with a wavelength of ~340nm and 450nm, respectively. Both NADH and FAD are sensitive to metabolic rate and their fluorescence intensity is altered in tumour tissue.
Increased metabolism causes NADH to fluoresce more intensely while FAD fluorescence fades [125].

Collagen and elastin, structural proteins in the connective tissue, also fluoresce. Collagen has a broad excitation wavelength (~250 – 450nm). The intensity of both collagen and elastin’s fluorescence decreases during carcinogenesis as the collagen cross-links are broken down by matrix metalloproteinases (MMPs) [126].

Hemoglobin and melanin are chromophores, molecules which absorb light. Hemoglobin, with absorption strongest at 420nm, is increased in carcinogenesis due to increased microvascularization. However, hemoglobin is also the most common confounder for FV due to increased vascularity associated with trauma or inflammation. [126]

To summarize, the 5 properties which influence fluorescence visualization during carcinogenesis are:

- Increased breakdown of collagen cross-links and the basement membrane by MMPs, including collagenase, causing less collagen fluorescence.
- Increased nuclear scattering due to changes to the cell nuclei resulting in less back scatter.
- Increased metabolism alters FAD and hence less fluorescence intensity.
- Increased microvascularity leads to more absorption by hemoglobin.
- Increased thickening of the epithelium leads to less reflectance and back scatter. [122]

One study found fluorescence spectroscopy to have a high rate of sensitivity differentiating normal from abnormal cervical tissue but was less sensitive differentiating neoplastic tissue from non-neoplastic abnormal tissue [127] while a second found a much lower sensitivity for differentiating high risk cervical lesions [128]. In the lung, fluorescence was 50% more sensitive than white light bronchoscopy in the detection of both cancer and dysplasia in the lung [129]. The authors found that FV was able to detect dysplastic lesions not visible with white light in the bronchi.
1.8.2.2  FV and oral tissues

Fluorescence imaging for oral tissues is performed with a handheld device with a light source, dichromatic mirror and filters [122]. Excitation is within the blue light spectra (400 – 460nm) while the emission wavelength is greater than 475nm. The primary fluorophores at this excitation wavelength are collagen and FAD while the primary absorber is hemoglobin. At this excitation wavelength normal tissue will fluoresce apple green while abnormal tissue does not fluoresce and appears dark in comparison. This device allows real-time visualization granting the clinician the opportunity to compare other tissue to the area in question.

Light scattering may be affected by site. There can also be variation in the intensity of fluorescence due to variation in the tissues and between patients. The thickness of buccal mucosa epithelium makes it harder for the light to reach the collagen and hence there is less scattering while the floor of the mouth, nonkeratinized tissue, has greater scattering. Trauma, infection, inflammation or any condition that increases tissue vascularization and pigmented tissue may confound results [130].

In oral tissues FV was able to differentiate normal from malignant tissue with 94% sensitivity [131], however it was more difficult to differentiate normal from premalignant tissue [132]. In a study from the BCCA, high-grade dysplasia and SCC were distinguishable from normal tissue with a sensitivity of 98% and a specificity of 100% among patients in a high-risk clinic [133]. FV was also found to be an important tool in the operating room where FV helped define the surgical margin, in some cases up to 25mm from the clinical lesion border [134]. FV has also been helpful in the detection of occult lesion in a high-risk clinic [135].

There is a need for a simple, handheld device to aid clinicians in clinical decision making and to aid in the early detection of oral cancer and OPLs.

1.9  Molecular markers

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 [136] 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 [137].

1.9.1 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 2 types: the oncogene and the tumour suppressor gene [138]. 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 [53].

Tumour suppressor genes (TSGs) prevent abnormal proliferation by acting like a brake in regulating the cell cycle [139]. The loss of a TSG results in uncontrolled cell proliferation [53]. These mutations result in a “loss of genetic material from one region of a pair of chromosomes that are inherited from both parents” [138] 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 [140-143]. Other common genes studied for their early role in the development of oral cancer are the FHIT gene found on 3p21 [144] 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 [145]. Other TSG thought to be involved in oral SCC are found on 4q, 5q, 6p, 8p 11q, 13q, 14q and 18q [138, 146, 147]. 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 [141, 148].

1.9.2 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. DNA analysis is 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 [149]. Using these PCR techniques, microsatellite analysis can search for LOH and microsatellite instability (a change in the length of the nucleotide repeat) [150]. LOH and other molecular tools can be used 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 [151].

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, tumours with significant LOH were more apt to be aneuploid, at an advanced stage and were poorly differentiated [142].

### 1.9.3 Molecular Research and Oral Cancer

As mentioned earlier, the current ‘gold standard’ to determine which OPL will progress to SCC is the histopathological diagnosis. While the majority of mild and moderate (low-grade) dysplasias do not become SCC [152], it is not possible to differentiate which low-grade dysplasias will progress to SCC from histology alone. Studies published over the last ten years have found that specific genetic changes or patterns may help identify lesions at risk of progression [152].

### 1.9.4 LOH and Oral Cancer

Early research attempted to find which genetic events were linked to oral SCC. The LOH of normal and tumour DNA tissue samples was studied on all 22 q limbs and 17 of the p limbs and 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 [153]. LOH at more than 2 loci was also significantly associated with a poor prognosis [154].
1.9.5 LOH and OPL

1.9.5.1 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. A genetic model of progression for head and neck cancer was designed by searching for LOH at 10 loci in benign and premalignant lesions [141]. 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. Similar patterns of loss, albeit less, were also found in tissues adjacent to the lesions leading to the conclusion 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 period of time between exposure to a carcinogen and the appearance of a HNSCC may be as long as 25 years and genetic changes may significantly precede the histologic and morphologic changes [145].

Leukoplakia, with or without dysplasia, exhibiting LOH at 9p21 and 3p14 have been found to progress to SCC more often 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 [155].

In a retrospective study, tissue from progressing oral premalignant lesions was compared to non-progressing cases for changes in LOH [152]. 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 to 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. It was proposed that screening for a loss at 3p and or 9p might be a good initial screening to assess risk of progression.
1.9.5.2 LOH, OPL and other issues

High-risk sites such as the FOM, ventral and lateral tongue and soft palate, were found to have significantly higher LOH frequency at 3p, 9p and 17p than low risk sites [81]. 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.

TB staining and LOH in the oral cavity has been studied. TB positive areas in 46 patients with a history of treated head and neck cancer were biopsied [156]. Of the TB-positive biopsies 13 were SCC, 11 were CIS, and 22 were histopathologically normal. LOH at one or more of 3p21, 9p21 and 17p13 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.

A significantly higher proportion of LOH was found in TB positive OPL biopsy samples than TB negative OPL samples [118]. 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. TB staining may help identify OPLs with increased risk of progression associated with LOH regardless of histopathological diagnosis.
1.10 Oral health professionals and screening

1.10.1 Oral Cancer Prevention

Increased command over oral cancer and precancer must involve both prevention and early detection. The Crete Declaration [157], co-sponsored by the WHO, encourages researchers, national and international health authorities, and nongovernmental organizations from 57 countries to improve “their efforts for the effective control and prevention of oral cancer.” Specifically the document issued an appeal for “active involvement of oral health professionals in oral cancer prevention through control of risk factors such as tobacco, alcohol and diet” and to train primary health workers in screening [158].

1.10.1.1 Primary prevention

Tobacco cessation and drinking alcohol in moderation could prevent up to 75% of oral cancers. Oral health professionals could play a large part in this by asking patients about their tobacco and alcohol consumption and educating patients about these risk habits. In a systematic review, good evidence for health professionals to provide tobacco cessation counselling to reduce oral cancer and precancer was found [159]. Although counselling for alcohol moderation was found to reduce alcohol intake there have been no randomized controlled trials (RCTs) to determine if it has an effect on oral cancer and precancer. Campaigns to raise public awareness about oral cancer may also have an impact on oral cancer mortality. At the minimum, the identification of people at increased risk may benefit from preventive intervention [160].

1.10.1.2 Secondary prevention - Screening

“Screening is the early detection of cancer by testing or checking for disease in a group of people who don’t show any symptoms of the disease” [161, 162]. Its purpose is to reduce the burden of cancer. Tests for screening are evaluated by sensitivity and specificity. Sensitivity is the proportion of patients with the disease who are correctly identified while specificity is the proportion of patients without the disease who are correctly identified [162]. Ideally, a screening test will have high sensitivity and specificity but for screening high sensitivity is important. The next level of testing can improve specificity (evaluation by a specialist for
example). The positive and negative predictive value (PPV, NPV) are an important element of screening. PPV is the proportion of people with a positive test who actually have the disease while the NPV is the proportion of the people with a negative test who actually do not have the disease [162].

Oral cancer screening meets many of the conditions required to justify screening. Currently, there is an inconsistency between the global burden of oral cancer and the potential ease of detecting visible changes in the oral cavity [62]. Screening occurs in an easily accessible area and is painless, quick, inexpensive, minimally invasive, can be repeated regularly and at its most basic requires no sophisticated equipment. Oral cancer is believed to have a long premalignant stage and the disease has a high mortality rate if detected late. Screening for oral cancer and OPLs may lead to increased lesions diagnosed at an early stage with higher survival rates, reduced late stage diagnosis, decreased treatment costs and reduced mortality and morbidity than late stage disease. Negative outcomes associated with any screening program include false positives, overtreatment of precursor lesions which may not have progressed, false negatives, anxiety and the financial costs of screening and diagnostic workup [163]. Oral cancer is an important health issue but the incidence in developed countries is not high enough to justify population screening. The rate of oral premalignant lesions (dysplasia) in the general public is unknown. While oral cancer has accepted treatment methods, oral precancers do not have accepted criteria for treatment. There are no universal guidelines or referral pathways for oral cancer screening or agreement on the start age or frequency required.

The main method to screen for oral cancer and precancer is a short (30 – 120 seconds) visual exam consisting of an extraoral and intraoral exam. Extraoral tissues are examined for symmetry and the lymph nodes of the head and neck are palpated. The intraoral exam consists of a thorough inspection and palpation of all tissues of the oral cavity with a dental mirror including the floor of the mouth, the dorsal, ventral and lateral tongue (retracted with gauze), the gingiva, buccal mucosa, hard and soft palate, tonsils, and uvula. Screening also allows the opportunity to provide education and counselling to alter high-risk behaviours.

The Cochrane Review [164], the UK working group on screening for oral cancer and precancer [165], the Canadian Task Force for Periodic Health Examinations (CTFPHE) [166], the U.S. Preventive Services Task Force (USPSTF) [167] and a systematic review by Downer and colleagues [168], do not recommend population-based screening for oral cancer or have found
insufficient evidence to support it. It should be noted that the lack of evidence regarding the benefit of screening does not mean there is no benefit but that there may be a lack of well-designed studies which prevents us from seeing the benefit [169]. A workable alternative is to screen opportunistically at either routine dental or medical visits, particularly for high-risk individuals (age, tobacco or alcohol).

Screening at dental recall examinations provides an excellent opportunity to detect asymptomatic early SCCs and OPLs and to refer patients for biopsies and follow-up with specialists [170]. According to Statistics Canada [171], in 2005, 65% of British Columbians over the age of 12 had seen an oral health professional (dentist, dental hygienist or orthodontist) within the last 12 months. The same report stated 68% and 54% of people 45 - 64 and 65 years and older, respectively had visited an oral health professional within 12 months. Depending on the health authority the percent of 45 – 64 year olds in the Greater Vancouver area who had accessed dental care within the last year ranged from 62 – 73%. Dentists and dental hygienists can lower the mortality and morbidity due to oral cancer by incorporating an oral cancer screening program within their practice [172]. In fact, in one study, oral cancers detected by dentists and hygienists during a non-symptom driven exam at a dental office were found to be of significantly lower stage at time of diagnosis than symptom driven exams or those lesions detected by physicians [173]. While opportunistic screening presents the likelihood of selection bias, individuals attending dental offices may be more health aware than those who do not attend and older individuals who are more likely to be edentulous, may not visit the dental office, it still provides a chance to screen a large proportion of the population.

The Cochrane Review [164] states “systematic examination of the oral cavity by general dental practitioners or physicians should remain an integral part of their routine daily work. Particular attention should be paid to high-risk individuals.” The American Cancer Society (ACS) recommends including oral cancer screening in annual exams for patients over 40 [174] and both the CTFPHE and the UK working group recommend opportunistic screening of high-risk individuals. A pamphlet published by the National Institute of Dental and Craniofacial Research stipulated that “a thorough head and neck examination should be a routine part of each patient’s dental visit. Clinicians should be particularly vigilant in checking those who use tobacco or excessive amounts of alcohol.” [175] The Surgeon General’s Report, Oral Health in America [176], also calls for health professionals to perform routine soft tissue exams for early detection,
to counsel patients about high-risk behaviours and to pursue further education on the subject. Opportunistic screening of high-risk groups is an effective alternative for health professionals’ particularly oral health professionals such as dentists, dental hygienists, assistants and dental mechanics [93]. Knowledge about oral cancer and OPLs amongst oral health professionals and ways to encourage oral cancer screening must be developed.

Different strategies have been used to study oral cancer screening including screening in the workplace, screening by invitation, and screening by trained health care workers (Table 1-3). For many of the studies the gold standard is evaluation by a specialist, confirmed in some cases by biopsy. The best evidence to support oral cancer screening comes from India, where in the only randomized control trial (RCT) to date, trained health care workers performed 3 rounds of oral cancer screening over a 9 year period. There was a 32% reduction in mortality amongst screened high-risk patients versus those not screened. Almost half of participants completed 2 rounds of screening. Sensitivity and specificity to detect lesions by visual inspection were over 80% [177]. Health care workers were also used to screen for oral cancer in Sri Lanka where 4% of more than 29,000 people had lesions [178]. In a follow-up study [179] the authors found that delivering a health education program improved the referral compliance. Two studies from Japan and the aforementioned India study provide support for annual oral cancer and precancer screening. In the first Japanese study, invitations were mailed to people aged 40 years and older asking them to participate in free annual general health screening and oral examinations. More than 19,000 people were examined by dentists over 3 years. Oral lesions were found in 783 (4.1%) of the subjects and 217 were cancer or precancer. Two hundred were referred forward for follow-up, 137 complied (68%). Thirty-nine were confirmed with oral cancer (2) or precancer (37 leukoplakias) and 40 lichen planus were documented. Of those individuals referred forward to a specialist, sensitivity was 92% and specificity was 64% [180].

The second Japanese study followed the screening program over a 3 year period. During this period, of 9536 participants screened over 3 years, two-thirds returned for a second annual screening and more than 40% returned a third time. One case of oral cancer was detected in an individual who had no clinical lesion the previous year. The lesion negative group was followed for 3 years with almost 80% compliance. In all 18 leukoplakias, 24 lichen planus and 343 benign lesions were found. The authors concluded that the interval between
screenings should not be greater than 12 months for this population to allow time to detect new lesions. [181]

An oral cancer screening program for individuals 15 years of age and older has been in place in Cuba since 1984 [182]. This program identified 16% of the more than 4400 oral cancers diagnosed from 1984 - 1990. Fewer than 30% of referred patients complied with follow-up. By 1990 the proportion of stage 1 cancers had doubled to 49% of all oral cancers but there had been no difference in mortality during this time.

Oral cancer screening within the workplace has also been studied in the UK. Patients in an industrial dental clinic were invited to participate in an oral cancer exam as part of their routine dental exam [24]. All but 2 of 1949 patients agreed to participate by filling out a habit questionnaire and undergoing a screening exam. Including the exam as part of routine dental care was found to be an important issue as it added no inconvenience to the patient. Eight percent of the patients had some form of lesion, 151 determined to be benign by the dental practitioners, the majority due to trauma. Four patients were found to have a positive screening exam (leukoplakia, lichen planus and one SCC).

An invitational screening program in the UK had a poor response rate with poor compliance for referred lesions [183]. Of interest in this study, the group who did not have an oral cancer leaflet with the invitation had better attendance than those who did. In a second invitational study, more than 53% of 553 employees over the age of 40 responded to an invitation for oral cancer screening arranged by their company [184]. The initial dentists’ screening results were followed up by an oral medicine specialist (a soft gold standard). 17 positive lesions were found. Sensitivity was 71% and specificity was 99%.

In an opportunistic study in the UK, the prevalence of mucosal lesions in a population attending typical general dental practices was 14.1%, with 4.2% of lesions regarded as malignant or potentially malignant [21]. The authors concluded that this study of opportunistic screening in a UK dental practice revealed that opportunistic screening in a dental practice was effective. Another UK study looking at the economic aspect of opportunistic screening found screening patients aged 40 – 70 years in the dental office is the most cost effective, saving up to US$21,000/ healthy year in health care costs. [185]. Dentists and dental hygienists can lower the mortality and morbidity due to oral cancer by incorporating an oral cancer screening
program within their practice [172]. Early detection could contribute to financial savings for the health care system and all oral health professionals should educate themselves about oral cancer and make the time to screen high-risk patients [172].
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Type of screening</th>
<th>Inclusion criteria</th>
<th>Number eligible</th>
<th>Number screened</th>
<th>Screeners</th>
<th>Diagnostic criteria</th>
<th>Number positive</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mehta et al[185]</td>
<td>1986</td>
<td>Workplace</td>
<td>&gt;35 years; industrial worker</td>
<td>NA</td>
<td>57,518</td>
<td>calibrated dentists</td>
<td>Oral cancer</td>
<td>29</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bouquot and Gorlin[64]</td>
<td>1986</td>
<td>Multiple mass screenings</td>
<td>&gt;20 years</td>
<td>87,277</td>
<td>29,295</td>
<td>oral pathologist and oral surgeon</td>
<td>White or red lesion which cannot be scraped off; ulcers</td>
<td>660 referred; 384 cancer or precancer</td>
<td>95%</td>
<td>81%</td>
</tr>
<tr>
<td>Warnakulasuriya and Nanyakkara[177]</td>
<td>1991</td>
<td>Population</td>
<td>≥20 years</td>
<td>72,867</td>
<td>57,124</td>
<td>primary health care workers</td>
<td></td>
<td>3559 referred; 2193 complied; 20 cancers 1716 precancers</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fernandez Garrote et al[180]</td>
<td>1995</td>
<td>Opportunistic</td>
<td>&gt;15 years at dental exam</td>
<td>Entire population of Cuba &gt;15 years</td>
<td>12,990,667</td>
<td>dentist</td>
<td>Leukoplakia, erythroplakia, SCC, LP</td>
<td>30,244 referred; 8703 compiled; 705 cancer, 2367 leukoplakia, 852 other precancer, 4779 benign lesions and normal variations</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Jullien et al[181]</td>
<td>1995</td>
<td>Invitational</td>
<td>≥ 40 years from medical practice</td>
<td>4348</td>
<td>985</td>
<td>dental professionals; all reviewed by specialist</td>
<td>white or red patch or ulcer ≥2 weeks</td>
<td>12</td>
<td>no SCC</td>
<td>NA</td>
</tr>
<tr>
<td>Downer et al[182]</td>
<td>1995</td>
<td>Workplace</td>
<td>≥ 40 years, employees</td>
<td>570</td>
<td>309</td>
<td>dentists</td>
<td>red or white patch or ulcer ≥2 weeks; confirmed by Oral medicine specialist</td>
<td>17</td>
<td>no SCC</td>
<td>71</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Type of screening</td>
<td>Inclusion criteria</td>
<td>Number eligible</td>
<td>Number screened</td>
<td>Screeners</td>
<td>Diagnostic criteria</td>
<td>Number positive</td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td>-----------------------------</td>
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<td>-----------------</td>
</tr>
<tr>
<td>Mathew et al[186]</td>
<td>1997</td>
<td>Population</td>
<td>Home visits</td>
<td>NA</td>
<td>2069</td>
<td>Trained health workers</td>
<td>leukoplakia, erythroplakia, submucous fibrosis, ulcers and growths; confirmed by dentists (gold standard)</td>
<td>1 cancer</td>
<td>94</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>144 leukoplakia, 36 erythroplakia, 33 submucous fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nagao et al[178]</td>
<td>2000</td>
<td>Invitational</td>
<td>&gt;40 years</td>
<td>26,856</td>
<td>19,056</td>
<td>trained dentists</td>
<td>Clinical appearance of SCC, leukoplakia, erythroplakia and LP</td>
<td>39 SCC or precancer</td>
<td>92</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40 LP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lim et al[21]</td>
<td>2003</td>
<td>Opportunistic</td>
<td>&gt;35 years</td>
<td>NA</td>
<td>2,265</td>
<td>trained dentists</td>
<td>white or red patch or ulcer &gt;2 weeks, LP actinic keratosis, submucous fibrosis</td>
<td>319 lesions; 94 malignant or premalignant</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nagao et al[179]</td>
<td>2003</td>
<td>Invitational annual screening over 4 years</td>
<td>&gt;40 years and screened negative previous year or new</td>
<td>NA</td>
<td>9,536</td>
<td>trained dentists</td>
<td>Clinical appearance of SCC, leukoplakia, erythroplakia and LP</td>
<td>1 cancer in previously negative screen; 18 leukoplakias, 24 LP, 343 benign lesions</td>
<td>NA</td>
<td>NA</td>
</tr>
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<td></td>
</tr>
<tr>
<td>Sankaranarayana et al[175]</td>
<td>2005</td>
<td>Population 3 rounds over 9 years</td>
<td>≥35</td>
<td>96,517</td>
<td>87,655</td>
<td>Trained health workers</td>
<td>LP, leukoplakia, submucous fibrosis, oral cancer</td>
<td>5145 referable lesions, 3218 complied; 205 oral cancers 2252 precancer lesions</td>
<td>64</td>
<td>PPV = 74</td>
</tr>
</tbody>
</table>

Adapted from Patton, 2003.[93]
1.10.2 Knowledge, practice and opinions of oral health professionals

An important aspect of oral cancer screening is the ability of the oral health professional to carry out the exam. Health care professionals may miss or ignore some lesions which may be due to a lack of awareness that small asymptomatic lesions can become malignant [188]. Unfortunately, surveys of the public found only 14-30% respondents reported ever having had an oral cancer examination [189, 190]. In the latter study, the majority of those screened were female and less than 40 years of age.

Surveys of dentists, dental hygienists and other health care professionals in Canada and the United States have been completed to determine the practices, opinions and knowledge of oral cancer and screening [191-198]. Oral health professionals were found lacking in oral cancer and early detection knowledge (Table 1-4). While the majority of respondents in these studies claim to provide regular oral cancer screening many lesions are not detected until they are at a late stage. This may be due to clinicians not suspecting lesions to be malignant, inadequate screening [170], providing a cursory exam [199] or that many of these professionals overstate their oral cancer screening in surveys and are “remiss in the provision of oral examinations and in the detection of early oral cancers” [170] and very few document prior tobacco and alcohol use [170]. Oral health professionals were found lacking in oral cancer and early detection knowledge and require systematic education updates in oral cancer prevention and detection and to improve participation in control [191, 194-196].

Table 1-4. Gaps in Diagnostic and Risk Factor Knowledge
(Percent of oral health professionals that identified the following as risk factors)

<table>
<thead>
<tr>
<th>Risks</th>
<th>Myths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco (98%)</td>
<td>Poorly fitting dentures (40-82%)</td>
</tr>
<tr>
<td>Alcohol (90%)</td>
<td>Poor oral hygiene (40-60%)</td>
</tr>
<tr>
<td>HPV (60-80%)</td>
<td>Hot food and beverages (20%)</td>
</tr>
<tr>
<td>Mostly older age (50 - 90%)</td>
<td>Spicy foods (23-43%)</td>
</tr>
<tr>
<td>(&gt;60 years 18-35%)</td>
<td>Obesity (20-40%)</td>
</tr>
<tr>
<td>Most early cancers are asymptomatic (~75%)</td>
<td></td>
</tr>
<tr>
<td>Most oral cancers diagnosed at late stage</td>
<td></td>
</tr>
<tr>
<td>(50%)</td>
<td></td>
</tr>
</tbody>
</table>

[193, 198, 200-202]
Approximately 90% of dentists and dental hygienists in survey-type studies from the US and Canada reported that they ask their patients about current tobacco use while 50-80% collect information on past use, amount and duration of use [191, 192, 195-197, 200]. Similarly about 60% of these same respondents claim to ask about current alcohol use, significantly fewer (24-50%) ask about past use, amount and duration. Within these samples 80-90% of respondents could identify the complete exam of the tongue, 75% were aware that most early cancers are asymptomatic, 50% agreed that most oral cancers are diagnosed at a late stage, only a third were aware that leukoplakia and erythroplakia were associated with oral cancer and only about half knew the tongue and the floor of the mouth were the most common sites of oral cancer. The surveys also questioned screening behaviour, dentists in BC and Nova Scotia [192] and the United States [191] reported screening 71 and 81% at new patient exams, respectively. The Canadian dentists reported screening only about half their patients at recall appointments while the American dentists claimed to screen 78% of their recall patients. Lymph nodes were palpated in roughly one third of the patients in both surveys. The Canadian dentists did report a much higher rate of screening edentulous patients than their American counterparts (73% versus 14%).

There is an urgent need for oral cancer education and health promotion interventions for both health professionals and the public to increase their awareness and knowledge of this disease [203]. Few oral health professionals feel adequately trained to provide tobacco (28-32%) or alcohol cessation (11-13%) [191, 201]. The majority of dentists felt their dental training did not put enough emphasis on oral cancer screening [191].

A survey of general medical practitioners in Scotland found that more than 70% of these respondents felt they lacked training in oral cancer examination and almost 40% had never received any education on oral cancer screening. A major barrier for almost half was lack of time to perform the exam. More than 40% of general dentists in this study felt that lack of time and financial reimbursement were the greatest barriers to screening. Many felt that causing patient anxiety was also a barrier. Less than 20% routinely asked about tobacco use and very few inquired about alcohol use due to practitioner discomfort [204].

In a similar survey of oral cancer knowledge and practice among doctors and dentists in Massachusetts, more dentists reported screening for oral cancer and were more aware of risk factors than doctors [196]. However, doctors were much more likely to ask and counsel their
patients about tobacco and alcohol use and felt more adequately trained to do then their dental counterparts. Both groups did poorly in identifying the 2 most common sites and the most common symptom of oral cancer. A US survey of health care professionals discovered that dentists and hygienists felt more current and more able to provide an intraoral cancer exam than physicians and nurse practitioners [205]. But the nurse practitioners and physicians felt more adequately trained to palpate for lymph nodes and to provide tobacco and alcohol cessation education as well they inquired about their patients’ use of these risk factors more often than the dental personnel.

In 2 separate studies by Horowitz, dentists [206] and hygienists [207] from Maryland took part in separate focus groups on oral cancer. Themes that emerged were a lack of or incorrect knowledge about the oral cancer problem, the statistics and risk factors. Barriers to screening included lack of time, discomfort performing extraoral exams, employer expectations, and level of formal training particularly in those who had graduated years earlier. Another important barrier is the clinician’s concern over discussing oral cancer with their patients. It was noted that they did not want to cause any unnecessary anxiety by mentioning cancer nor did they know how to discuss the need for biopsy and referral. [206, 207]

The public is not aware of many of the risk factors for oral cancer and in some cases the disease itself. In a survey of the public in the US approximately 15% had never heard of oral cancer [189]. Tobacco users believed themselves to be at an increased risk, however alcohol and older age were not perceived as risk factors [208]. In a telephone poll of over 1200 people in the United States, 89% agreed with the statement “Knowing that my dentist performs a routine oral cancer examination at every visit and uses a painless oral cancer early detection test on spots that are identified, makes me feel that I getting the best possible care from my dentist?” [203]

Oral health professionals also need some recommendations for documentation and follow-up of oral lesions [209]. Proper documentation may potentially aid patients but may also limit the liability of the clinician. Questions such as who makes the appointment with the specialist, which type of specialist to use, how to follow-up to ensure appointment compliance, were to send pathology samples, when is a biopsy indicated, and whether a lesion requires a second biopsy if the clinician remains suspicious. In a review of the literature and discussions with specialists, the authors confirmed there is agreement for follow-up of 14 days. When in
doubt it is better to perform a biopsy than not to determine a definitive diagnosis, unless the procedure endangers the health of the patient. Lesion documentation should include the site, size, colour and photographs and radiographs when possible. Any referrals should be documented as well as phone calls between clinicians.

The early detection of the disease and managing or treating it effectively is paramount for the success of a screening program. The examiner’s screening ability and knowledge builds the confidence which is key to the early detection of disease and discerning it from more common oral pathology. In theory, the ability of the screener should increase with their level of knowledge [210].

Oral health professionals play an important role in the prevention and detection of oral precancer and cancer. Oral cancer screening is within the scope of practice of both dentists and dental hygienists. Within BC 65% of the population attend their dental office at least yearly [171]. Unfortunately, a large percentage of oral cancers are diagnosed at a late stage, well after symptoms have appeared. Annual oral cancer screening may increase the number of lesions detected at the premalignant or early stage of cancer and decrease the number of late stage diagnoses. By performing a routine, thorough extraoral and intraoral examination, early signs of disease may be found.

The key risk factors for oral cancer are well known. Prevention of the disease by eliminating modifiable risk factors is the preferred approach. If health care personnel and the public are aware of oral cancer symptoms and act early, the survival rate will improve. The oral health professional can play a key role in the prevention of oral cancer and premalignancy via education and screening. Major risk factors that can be modified include the cessation of tobacco and betel quid, moderate alcohol use, and maintaining a health diet with fruits and vegetables.

1.11 Knowledge Translation

Positive practice changes are required for health care delivery to reflect recent research findings and to improve health outcomes. It is critical to not only create new research but to ensure its transfer to the appropriate setting and application to practice. By sharing new oral cancer knowledge around early detection of premalignant lesions, while being aware of
clinicians’ needs, knowledge translation may facilitate an improvement in the health of the dental patient by decreasing the number of lesions diagnosed at a late stage.

The transfer of research findings into practice is slow and unpredictable often because it is not known what influences clinicians behaviour or how to help them effectively use new information [211]. Within the dental profession, continuing education (CE) is commonplace. It is paramount that CE be based on the most current and accurate knowledge available. Integrated into CE should be effective transfer strategies for the benefit of the patient and the most effective use of health care costs [212].

1.11.1 Knowledge translation defined

Knowledge translation (KT) is defined by the CIHR as:

“...a dynamic and iterative process that includes synthesis, dissemination, exchange and ethically-sound application of knowledge to improve the health of Canadians, provide more effective health services and products and strengthen the health care system.” [213]

Simply, it is the study of the knowledge to action gap from the creation of new knowledge to its application and how this knowledge is used by clinicians, policymakers, patients and the public [212]. KT is the bidirectional (or multidirectional) transfer of information between researchers, health care providers, and other key stakeholders [214]. Key stakeholders include the researchers, clinicians, the public and policymakers. These individuals should be included in all levels of research from the development to the implementation and evaluation of the strategy [215]. The intention of KT is by addressing this gap between knowledge and its implementation health outcomes will improve. It is of paramount importance to the uptake of new knowledge that the barriers and facilitators are analysed and that the methods utilize this information.

KT was initiated to deal with the inability of health professionals to keep up with the exponential growth of information [216]. Clinicians have only limited time and abilities to access information, they may not be aware of evidence based practice (EBP) methods, they may not be aware of a potential problem, they may show a lack of interest or there may be a slow circulation of new techniques. Researchers may not be aware of clinical problems that need
solutions. The best available evidence must be available and accessible for clinicians to practice at their best [217].

KT is set in the practice setting, produces tools to target all participants and involve all relevant disciplines [214].

1.11.2 What is knowledge?

The knowledge aspect of KT can be defined in a variety of ways from scientific research to all forms of knowing [218]. The most common source for knowledge is systematic reviews, a standardized method to identify and assess a collection of knowledge related to the issue. Knowledge can include more than scientific research, knowledge synthesis can extend broader to include expert opinion, practical experience, cultural wisdom and public opinion, all of which can be used to determine best practices and to provide current, understandable, accurate and relevant knowledge for decision makers [216, 219], if they are aware of it, acknowledge it and can apply it. “A shift from “focusing” on the evidence to solving problems is overdue” [219]. This is particularly true for community level programs where RCT are difficult and uncommon.

Knowledge synthesis is the collection and analysis of information relevant to the research question via systematic reviews and meta-analyses. Knowledge synthesis and tools help the clinician by filtering the research down to clear tailored recommendations in user-friendly formats thus saving them time and effort [220]. This synthesis of information helps solve one of the biggest barriers clinicians face – the size and complexity of research [221]. Dissemination is the identification of the audience and tailoring the message for its users as well as the development of user-friendly knowledge tools such as clinical practice guidelines, pathways and decision aids to help facilitate change. Exchange relates to the interaction between the knowledge user and the researcher. This interaction is bidirectional, the user learning from the researcher and the researcher learning from the user. The ethically sound application of knowledge means it complies with legal, moral and social values. [213]

1.11.3 Evidence based practice

Evidence based practice (EBP) is the use of best evidence in making health care decisions regarding patient care by integrating scientific evidence with clinical experience [217]. The ability to make good clinical decisions is perhaps the best trait clinicians bring to their
patients [222]. EBP is a dynamic process, evidence will change over time, which includes KT [223]. Due to the complexity of patient care and making good clinical decisions’ the evidence may come from a variety of sources dependent on the context and focusing on both research results and practice knowledge. Research is only one part of the evidence that leads to decision-making [224], others include clinical experience, patient experience and the context [225]. While RCTs present some of most convincing evidence they are not always feasible (due to need for an extremely large sample size and time constraints) or unethical [169]. When there is limited research to guide practice or when a study is not feasible other types of evidence may be included [217, 223].

The hierarchy of best evidence is:

- Systematic reviews and meta-analyses of RCTs
- Individual RCTs
- Less controlled and descriptive studies
- Expert opinion and consensus conferences
- Conventional wisdom and common sense [217]

### 1.11.4 Implementation

Implementation research is “the scientific study of methods to promote the systematic uptake of clinical research findings and other evidence-based practices into routine practice and, hence, to improve the quality and effectiveness of health care” [212]. Its role is to discover what influences clinicians beliefs, decision making and the influence of policy makers and other stakeholders have to help determine which intervention would be more effective to cause and sustain a change in behaviour [212, 218]. The most successful implementations occur when there is a high level of evidence to support the change, when the context or setting is receptive (strong leadership, valued staff and group open to change) and strong facilitation from appropriate sources [226]
1.11.4.1 Barriers

The barriers and facilitators to changing clinical practice influence the uptake of knowledge [220]. Prior to creating an intervention it is important to assess those factors which may aid or hinder the outcome.

A barrier is anything that limits or prevents health care professionals from adhering to guidelines or implementing new research into practice [227]. Barriers to change can occur at any stage of the health-care system: the patient, the professional, the team, the health-care organization or the wider environment [220]. Barriers can come from many sources including cognitive, attitudinal, professional, patient resources and system barriers [227]. Patient barriers include social and cultural norms, belief of the likelihood of getting the disease, pain, cost, fear and repercussions should disease be found. Barriers at the professional level affect knowledge, attitudes or behaviour and include lack of knowledge, lack of confidence or training, lack of agreement, a lack of financial reimbursement, lack of role models, lack of time (for counselling or changing habits), the feeling that change won’t result in a positive effect, risk of liability, habit, employer and patient’s expectations [228] and resistance to external interventions [226]. Current standards of practice, lack of support from opinion leaders, training and obsolete knowledge, poor self-efficacy in the area, lack of confidence in their skills, and information overload may also act as barriers to change. Lack of support of the health care team will also negatively effect behavioural changes [220]. Interventions are designed to address the barriers to change.

1.11.4.2 Facilitators

Facilitators help in the adoption of desired change of behaviour. Different facilitators which have been found to be helpful include a full understanding of the clinicians who will use this behaviour, the issue, the available evidence and research and its relevancy to the user group, the relationship between the researcher and the user group and an awareness of dissemination strategies [229]. Working with opinion leaders to overcome cultural norms, gaining acceptance through the social system and to aid in the education of the patient would facilitate patient uptake. Facilitators to overcome professional barriers include consensus of clinical recommendations, financial reimbursement, education to confirm necessity of the
behaviour change (attitude change), and in office planning and organization for the entire health care team.

1.11.5 Interventions

To plan an intervention it is necessary to identify a problem, review the relevant knowledge and adapt it to the setting, access the barriers to implementing the change, design and implement interventions, and monitor and evaluate how the knowledge was used [212]. It is essential to verify what the audience wants and to tailor the message to what they think they need to know [230, 231]. A well-prepared strategy for change will include all the relevant stakeholders, be evidence based, include multiple interventions and be tailored to the desired audience and setting [220, 232]. Strategies that include multiple interventions have been found to be more successful than single intervention strategies [233]. The desired change will be more easily embraced if it is simple to implement, requires few new skills, can be related to practice and is compatible with the values of the clinicians [220, 223]. The message delivered must be simple, clear, consistent and compelling. The use of stories, emotion and anecdotes without an overwhelming amount of information increases the chance of success [234]. KT is a slow, continuous process and it is invaluable to keep informing and listening to the audience [234].

Many varieties of interventions have been designed. Interactive interventions have been found to be effective at evoking clinical change. The two most common types of CE for health professionals are educational meetings and printed educational materials [235]. Education alone does not appear to change practice but interactive education, including a practice session with didactic information is more successful [223, 236]. In a Cochrane Review of a systematic review of the effects of CE meetings and workshops effect on professional practice and health care outcomes, it was determined that interactive workshops can result in moderately large changes in professional practice [235]. Educational outreach (academic detailing), where an expert provides information and feedback to a clinician in their practice setting has been found to be helpful [223]. Other effective strategies include multiprofessional collaborations, expanding professional roles, audit and feedback, computer reminders, using the mass media and offering financial incentives [223, 237].

People can also play important roles to alter health care behaviour by increasing the availability and impact of knowledge. These roles include opinion leaders, knowledge brokers,
facilitators, champions, linking agents and change agents. Personal contact with prominent individuals has been rather effective in changing behaviour [237]. Opinion leaders are individuals from the target peer group who are respected, and trusted and hence capable of influencing their peers [223].

Once published research is often unseen by practitioners and the public [238]. This passive dissemination of information has not been found to be an effective method of changing behaviour as the research may not reach the end user [235-237]. Interventions designed to aid the clinician in implementing new knowledge are required.

1.11.6 Theory of Planned Behaviour

A theory that describes reasons for why a problem exists is known as an explanatory theory. Theory helps identify factors which influence health behaviours, such as barriers and facilitators, and leads to more useful interventions by addressing these factors [239].

The Theory of Planned Behaviour (TPB) as proposed by Ajzen is an explanatory theory, a theory that describes reasons why a problem exists. TPB believes that a behaviour change is driven by a person’s intention to change. TPB helps identify factors which explain health behaviours and has been useful in altering physician behaviour [239] Intentions are based on the key constructs of attitude (behavioural beliefs), subjective norms (opinions of people who are important) and self-efficacy (personal agency, perceived behavioural control). If the person has a strong intention to change then the intervention should focus on skills training otherwise interventions will be designed to focus on attitudes, societal norms and self-efficacy. Attitudes which may be a barrier to change include not believing there is a need to change, not being aware of the problem or the depth of the problem. Behaviour can also be influenced by what other practitioners are doing, lack of policy or leadership, fear of patient response, and poor staff support. Issues with self-efficacy include not being able to do the behaviour, how to follow-up, and an uncertainty in how to talk to patients. Multiple interventions can then focus on all these issues.

An educational component can update knowledge to focus on attitudes, opinion leaders can be used to face societal norms, and clinical sessions can review clinical skills or role play. Interventions can then be developed that focus on changing beliefs, on consequences, the
expectations of others and on the ability to perform the behaviour under varying circumstances.[240]

TPB has been found to alter physicians’ behaviour regarding mammography screening, STD and HIV counselling, etc, and has been found to be adaptable to different specialities and settings [239]. The use of theory can be used to aid in the identification and design of interventions that can target those factors which influence clinician behaviour.

1.11.7 Dissemination of Information

The dissemination of information is moving research into practice by tailoring the message to the targeted audience [212].

The Diffusion of Innovations (DoI) model, as proposed by Rogers, is a change theory, in that it guides the development of health interventions. DoI describes how ideas are spread throughout society. Diffusion is the process by which an innovation is communicated through certain channels over time among members of a social system [218]. There are four key concepts in this theory:

- Innovation (the idea that is new to the community),
- Communication channels (how messages get from one individual to another, mass media or interpersonal),
- Social system (the group who adopts the innovation) and
- Time (how long it takes to adopt the innovation).

This model can take place in diverse settings including the individual level, the organizational level and at the community level. Individual decision making follows the process of knowledge (aware), persuasion (forms an attitude), decision (rejects or adopts idea), implementation and confirmation (evaluates results).

Adoption (and speed of adoption) of the innovation is affected by the user’s belief of whether it will enhance their effectiveness, which will be associated with the following five attributes:
• Relative advantage (is it better than its predecessor),
• Compatibility (how well does it fit in with target group),
• Complexity (how easy is it?),
• Triability (can it be used before making a decision to adopt it?),
• Observability (are there tangible results?) [218]

A variety of communication channels can be used. The decision to change is strongly affected by the decisions of others within their social system. Opinion leaders play a large role by sharing their opinion of the change and have had variable effectiveness in leading to change [236]. People’s personal characteristics will also play a role in the speed of innovation uptake. Some people take more risks than others. The rate of how quickly a person adopts an innovation depends on whether the individual is an:

• innovator (on the cutting edge),
• early adopter (opinion leaders),
• early majority,
• late majority or
• laggards (traditionalists).

The uptake of the innovation depends on the other members of the system. The majority of people are early or late majority (~34% each). Opinion leaders tend to fall into the category of early adopter. Since the majority of the social system does not keep up with recent information about new innovations they rely on the decisions of opinion leaders. If opinion leaders accept the new innovation then it is drives the next wave of adopters. [240]

Interventions based on DoI will focus on the use of opinion leaders, ways to integrate the new idea into current practice, how the innovation will benefit the practice of an adopter and the use of both interpersonal and mass media forms of communication.

Oral health professionals are not screening for oral cancer consistently. The 5 year survival rate is much better when the disease is diagnosed early. It is important to identify the
barriers and facilitators to oral cancer screening in the general dental practice to design interventions that will lead to a change in this behaviour.

1.12 Oral Health Study (OHS)

The OHS study is one of the first cohort studies of patients with oral premalignant 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 OHS 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.
1.13 References


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CHAPTER 2 PROJECT I: TOLUIDINE BLUE STAINING IDENTIFIES
HIGH-RISK PRIMARY ORAL PREMALIGNANT LESIONS WITH
POOR OUTCOME

Lewei Zhang, Michele Williams, Catherine F. Poh, Denise Laronde, Joel B. Epstein, Scott Durham, Hisae Nakamura, Ken Berean, Alan Hovan, Nhu D. Le, Greg Hislop, Robert Priddy, John Hay, Wan L. Lam and Miriam P. Rosin

Research within the Oral Health Study of the BC Oral Cancer Prevention Program is multi-disciplinary and multi-institutional. My role in this research project was in clinical data collection, statistics, Kaplan Meier curves, results discussion and the editing of the publication.


2.1 Abstract

There is a pressing need for the development of visual aids that will facilitate the detection of oral premalignant lesions (OPLs) with a high-risk of progression. Preliminary data suggest that toluidine blue (TB) stain may be preferentially retained by OPLs with high-risk molecular clones. In this study we monitored OPLs from 100 patients without any history of oral cancer for an average of 44 months in order to evaluate the association of TB status with clinicopathological risk factors, molecular patterns (microsatellite analysis on 7 chromosome arms: 3p, 9p, 4q, 8p, 11q, 13q and 17p) and outcome. TB-positive staining correlated with clinicopathological risk factors and high-risk molecular risk patterns. Significantly, a greater than 6-fold elevation in cancer risk was observed for TB-positive lesions, with positive retention of the dye present in 12 of the 15 lesions that later progressed to cancer ($P = 0.0008$). This association of TB status with risk factors and outcome was evident even when the analysis was restricted to OPLs with low-grade or no dysplasia. Our results suggest the potential use of TB in identifying high-risk OPLs.
2.2 Introduction

Despite refinement of surgical techniques and adjuvant therapies, the prognosis for patients with oral squamous cell carcinoma (SCC) remains poor with a 5-year survival rate (40-50%) that has not changed significantly for several decades (1). Early detection of oral premalignant lesions (OPLs) is central to the improvement of this prognosis. However, this detection relies heavily on the clinician’s ability to differentiate such lesions from reactive and inflammatory conditions. Even when OPLs are identified, our ability to predict outcome is a challenge since the majority of OPLs will not progress. The presence of dysplasia, the current gold standard, is a good predictor of high grade lesions but has only a limited capacity to predict outcome for lesions with minimal or no dysplasia, which constitute the majority of OPLs.

Toluidine blue (TB) staining is considered to be a sensitive adjunct tool for identifying early oral SCC and high-grade dysplasias (2-5). However, the detection of low-grade (mild/moderate) oral dysplasia has been less consistent, with a significant portion of such lesions not staining with TB (3, 5). Recent reports have associated TB retention in oral lesions with the presence of high-risk molecular clones, even in lesions with minimal or no dysplasia (6,7), raising the possibility that TB could identify those low-grade lesions that are more likely to progress. In this study, we monitored 100 patients with primary OPLs to relate their TB status to outcome as well as to conventional histopathological features and molecular risk patterns.

2.3 Materials and Methods

Patients. The study group was chosen from 162 consented patients in longitudinal follow-up at the Oral Dysplasia Clinic, British Columbia Cancer Agency (BCCA) between 1996 and 2004. This is a referral centre for oral dysplasia in the Greater Vancouver area. Criteria for eligibility included: a history of histologically confirmed oral dysplasia but no history of head and neck cancer, the availability of a biopsy since enrolment with concurrent assessment of TB status for that biopsy (called the index lesion), and finally at least 6 months follow-up after the latter biopsy. Of these cases, 62 had less than 6 months of follow-up.

Of the 100 patients remaining, 47% were male, 69% had a smoking history, and 78% were Caucasian, with the rest being Asian and others. The average age was 59 (34 - 93 years). Index lesions were assessed for TB status using a topical application of 1% TB (OraTest) and de-
staining with acetic acid (1%) as previously described (4). Lesions were biopsied and histological diagnosis confirmed by 3 head and neck pathologists (RP, KB and LZ). Subsequently, TB status, lesion appearance and size were evaluated at six-month intervals. The mean follow-up time for the 100 OPLs was 44 months, with more than half followed for ≥ 3 years (56%), and 27% ≥ 5 years. Of the 100 cases, 4 were lost to follow-up (2 TB-positive and 2 TB-negative).

Assessment of molecular risk pattern

Areas of hyperplasia and dysplasia were microdissected from the index biopsies for microsatellite analysis. The underlying stroma was also collected as a source of matched control DNA. All samples were coded so that LOH analysis was performed without knowledge of diagnosis. The microsatellite markers mapped to the following regions: 3p14.2 (D3S1234, D3S1228, D3S1300); 4q26 (FABP2); 4q31.1 (D4S243); 8p21.3 (D8S261); 8p23.3 (D8S262); 8p23.3 (D8S264); 9p21 (IFNA, D9S171, D9S1748, D9S1751); 11q13.3 (INT2); 11q22.3 (D11S1778); 13q12.3-13 (D13S170); 13q14.3 (D13S133); 17p11.2 (CHRNB1) and 17p13.1 (tp53 and D17S786). These were markers used in previous studies to predict cancer risk of OPLs (8-13). The protocol for digestion and extraction of samples, LOH analysis and scoring is described in Zhang et al. (14).

Statistical analysis

Differences and associations between groups (e.g., TB-positive vs. TB-negative) were examined using either Fisher’s exact test for categorical variables or t-test for continuous variables. We used event charts to evaluate the history of the patients (15). Time-to-progression curves were estimated by the Kaplan-Meier method, and the resulting curves were compared using the log-rank test. Relative risks and the corresponding 95% confidence intervals (95% CI) were determined using Cox regression analysis. All tests were two sided. $P < 0.05$ was considered to be statistically significant.

To test the hypothesis that TB-positive OPLs with low-grade dysplasia or no dysplasia have higher cancer risk than those histologically similar but TB-negative lesions, these lesions were also examined independently using the above statistical methods.

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1 The molecular study was done by another graduate student.
2.4 Results and Discussion

We monitored 100 patients with primary OPLs to test for associations between TB status and outcome as well as to conventional clinicopathological features and molecular risk patterns. Based on TB staining of the index lesion, the cases were classified as TB-positive (n = 36) and TB-negative (n = 64) (Table 2-1). Patients with TB positive and negative OPLs were similar for gender (42% vs. 50% male), ethnicity (83% vs. 81% Caucasian), and smoking history (64% vs. 72%; all comparisons showed $P > 0.05$). The average age of patients with TB-positive lesions was 64 years while the TB-negative lesions averaged 57 years ($P = 0.005$)

**TB preferentially stains lesions with clinical features associated with risk.** A number of retrospective studies suggest that 3 clinical features of primary OPLs are predictive of progression for OPLs: location on the floor of mouth, and ventrolateral tongue (termed high-risk sites); large size; and a nonhomogeneous clinical appearance (16-18). We examined the 100 cases for association of TB stain with these features (Table 2-1).

A higher proportion of TB-positive lesions were located at high-risk sites (69% vs. 53%); however, the difference was not significant ($P = 0.14$). TB-positive lesions tended to be larger than TB-negative lesions. This size difference was not significant at the beginning of the study (mean dimension 19 mm ± 15 vs. 14 ± 10, $P = 0.16$); however, during follow-up, more TB-positive lesions grew in size compared to negative lesions, and the size difference became significant (27 ± 19 vs. 18 ± 13, $P = 0.0049$). Finally, TB staining was significantly associated with a nonhomogeneous clinical appearance: a higher number of nonhomogeneous OPLs were TB-positive both at the beginning of the study (59% vs. 24% in TB-negative lesions, $P = 0.0015$) and during follow-up (83% vs. 41%, $P < 0.0001$). These results suggest that TB preferentially stains high risk OPLs as judged by the traditional clinical risk parameters.

**TB-positive OPLs increase in frequency with histological progression.** TB uptake was associated significantly with degree of dysplasia, consistent with the literature. The stain was positive in 26% (5/19) of non-dysplastic OPLs, 23% (15/64) of lesions with low-grade (mild/moderate) dysplasia, and 94% (16/17) with high-grade dysplasia ($P < 0.0001$; Table 2-1). These results suggest that TB preferentially stains high risk OPLs as judged by histological parameters. It should be noted that the 19 non-dysplastic OPLs were judged clinically as non-reactive.
**TB recognizes lesions with high-risk molecular patterns.** TB-positive OPLs showed a consistently higher frequency of LOH for all 7 chromosome arms, and 4 of these were significant: 3p, 9p, 11q, and 17p (all \( P < 0.05 \); supplemental Table 2-1). In the context of previously established LOH patterns indicative of risk (8-13), TB staining was strongly associated with those LOH patterns with considerably increased cancer risk: multiple LOH (\( P < 0.0002 \)), and LOH at 3p and/or 9p plus losses at any other arm (\( P < 0.0001 \), Table 2-1). (The later pattern has been associated with a 33-fold increase in risk of progression in a previous retrospective study of primary OPL (10, 11)). These data support the ability of TB staining to delineate areas with high molecular risk.

**TB staining correlates with outcome.** The mean follow-up time for the 100 OPLs was 44 ± 26 months. Within this follow-up period, 15 of the 100 OPLs progressed to oral SCC (from 4/19 hyperplasias, 4/64 low-grade dysplasias and 7/17 high-grade dysplasias). The majority (60%) of the cancers developed at the same site as the index biopsy. The remaining 40% were adjacent to the index biopsy (within 2 cm). The average time for the OPLs to develop into SCC was 30 ± 22 months (29 ± 20 for TB-positive lesions and 34 ± 32 for TB-negative lesions). Only three (5%) of the 64 TB-negative OPLs progressed into SCC, whereas 12 (33%) of 36 TB-positive lesions developed into SCC (\( P = 0.0002 \)).

Time-to-development of SCC was significantly decreased for TB-positive when compared to the TB-negative lesions (\( P < 0.01 \), Fig. 1A). The hazard ratio based on the Cox regression for SCC development was more than 6-fold higher for TB-positive cases compared to the TB-negative cases (6.67; 95% CI: 1.87 – 23.70).

**TB predicts risk and outcome for OPLs with minimal or no dysplasia.** To test whether TB staining had value in identifying high-risk OPLs with little or no dysplasia, these lesions were examined independently. Of the 100 lesions, 83 belonged to the histologically low-risk lesions (19 hyperplasias and 64 low-grade dysplasias). TB status and clinical and molecular features in this subgroup showed similar associations to those reported above (Table 2-2). Lesion size was not significantly different in the two groups at the beginning of the study (mean dimensions 18 mm ± 16 vs. 14 ± 10, \( P = 0.74 \)). However, during follow-up, TB positive lesions became significantly larger than the negative lesions (27 ± 19 vs. 18 ± 13, \( P = 0.028 \); Table 2-2). In contrast, a nonhomogeneous high-risk clinical appearance was more often apparent among TB-
positive lesions, both at the beginning of the study (53% vs. 22%, \(P = 0.02\)) and during follow-up (75% vs. 40%, \(P = 0.0097\)).

TB was retained at sites of high molecular risk lesions even among OPLs with little or no dysplasia. Again, an increase was observed for all chromosome arms in this sub-group, although differences in frequency for TB-positive and -negative lesions were not statistically significant, (Supplementary Table 2-1). However, when the high-risk molecular patterns were compared, TB staining again was found to be associated with the high-risk molecular pattern: LOH at 3p &/or 9p plus any other arm (40% vs. 14%, \(P = 0.023\), supplementary Table 2-1).

Finally, TB staining and outcome for this sub-group was examined. Of the 83 OPLs with low-grade dysplasia or no dysplasia, 8 (10%) progressed into oral SCC (4 hyperplasias, 2 mild dysplasias and 2 moderate dysplasias). Only three of the 63 (5%) TB-negative OPLs progressed into SCC compared with 5/20 (25%) TB-positive lesions \(P = 0.0177\). Time-to-development of SCC was shorter for TB-positive lesions but the estimate was based on the small number of events and was only close to significance \(P = 0.06\), Fig. 1B). The relative risk for cancer progression was almost 4-fold higher for TB-positive OPLs (RR=3.92; 95% CI: 0.92-16.80).

**Temporal analysis of TB status.** Figure 2-2 displays an event chart that summarizes the clinical time course of the OPLs in this study with respect to TB status and outcome. The date of the first TB assessment was set as time 0, and the time to treatment (either with excision or topical bleomycin), to subsequent cancers, or the last follow-up visit are shown. Also shown are dates at which an alteration in TB status occurred. When the TB status did not change during follow-up, the time event bar for that case remained unmarked.

For those lesions that were TB-negative at study entry, the majority (52/64, 81%) remained negative. Of interest, the 3 TB-negative cases that developed into cancer were predicted by a change in TB status. In 2 cases this transition in status was detected 6 and 15 months prior to diagnosis. For the third case, the cancer developed quickly within 8 months of the initial evaluation, with a change to TB-positive leading to the diagnostic biopsy of SCC. Of the remaining 10 TB-negative lesions which showed a transition to positive staining, 2 returned to negative after treatment and 4 were found at the last visit. The remaining 4 cases showed a fluctuation of the staining between TB+ and TB- over time, possibly due to a biopsy effect.
In contrast, only 1 of 36 (3%) TB-positive lesions became negative without apparent intervention. Five TB-positive lesions (14%) became TB-negative either after treatment (2 lesions), or after incisional biopsy (3 lesions). The majority of TB-positive lesions (30/36, 83%) remained positive continuously (15 cases) or intermittently (15 cases) throughout follow-up. In the latter case, the transition to a TB-negative status followed an incisional biopsy (2/15, 13%) or, more frequently, treatment with an intention to cure (10/15, 67%; 8 surgery, and 2 bleomycin). These lesions later showed a reversion to TB-positive status and in 2 of these cases the later development of cancer. These data illustrate the difficulty in managing these lesions, with frequent re-emergence of the TB-positive phenotype with time after treatment. The results strongly suggest the necessity of continuous monitoring of dysplastic lesions, even post-treatment and especially those with a history of TB retention.

Cancer development is a complex process. Currently we have little understanding of factors affecting the speed of cancer transformation for high-risk lesions judged by either gold standard histology or molecular markers. For example, our previous study has shown that some OPLs with the high-risk LOH pattern (33-fold increase in cancer risk) took 8 years to become cancer while others in a short 6 months (10). Similarly, while TB staining showed a predictive value for cancer transformation, the speed of the transformation differed greatly as shown by the wide range of time interval between emergence of TB-positive lesions and tumor progression (Fig. 2). Nonetheless, like molecular markers, TB staining could provide clinicians with an additional tool in judging cancer risk of OPLs and guide the management of these lesions, including long-term follow up, before we understand factors that could trigger ‘when’ the transformation of these high-risk lesions happen.

**Clinical translation potential.** Our data support the potential value of using TB as an adjunct tool for clinical diagnosis of high-risk primary OPLs in a high-risk clinic (a referral centre for dysplasias). Not only did TB detect virtually all of the high-grade dysplasia (16 of 17 cases) in this study, but it also preferentially stained OPLs with minimal or no dysplasia with high-risk clinical and molecular attributes. Moreover, the staining status was strongly associated with outcome. Admittedly, the study benefited from the involvement of Oral Medicine and Pathology specialists who were experienced in both the use of the dye and in clinical assessment of OPLs, reducing confounding false positive staining of reactive or inflammatory lesions such as denture trauma. Given these promising results from this pilot study, the efficacy of this stain in
predicting outcome for primary OPLs needs to be further evaluated within a clinical trial scenario, next in a community setting and with a larger cohort. However, the significance of the study is that it points to a need to re-access TB stain not just with its association with histology, but also with molecular risk predictors and with outcome.

2.5 Acknowledgments

We gratefully acknowledge the assistance of Dr. Jack Lee for suggesting our use of event charts for assessment of data in the Dysplasia Clinic and of Ms. Kathryn Richardson, for making these charts.
2.6 References


### Table 2-1. Clinical, pathological and molecular features of TB + and TB - OPLs.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>TB -</th>
<th>TB +</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of lesions</strong></td>
<td>100</td>
<td>64</td>
<td>36</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% located at high-risk sites</td>
<td>59/100 (59%)</td>
<td>34/64 (53%)</td>
<td>25/36 (69%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Largest dimension (mean ± SD)</td>
<td>16 ± 12</td>
<td>14 ± 10</td>
<td>19 ± 15</td>
<td>0.16</td>
</tr>
<tr>
<td>Largest dimension (mean ± SD)</td>
<td>21 ± 16</td>
<td>18 ± 13</td>
<td>27 ± 19</td>
<td>0.0049</td>
</tr>
<tr>
<td>% of nonhomogeneous lesions at start of study</td>
<td>34/95 (36%)</td>
<td>14/59 (24%)</td>
<td>20/34 (59%)</td>
<td>0.0015</td>
</tr>
<tr>
<td>% of nonhomogeneous lesions during follow-up</td>
<td>56/99 (56%)</td>
<td>26/63 (41%)</td>
<td>30/36 (83%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Histological features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPLs without dysplasia</td>
<td>19</td>
<td>14/64 (22%)</td>
<td>5/36 (14%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>OPLs with low-grade dysplasia</td>
<td>64</td>
<td>49/64 (77%)</td>
<td>15/36 (42%)</td>
<td></td>
</tr>
<tr>
<td>OPLs with high-grade dysplasia</td>
<td>17</td>
<td>1/64 (2%)</td>
<td>16/36 (44%)</td>
<td></td>
</tr>
<tr>
<td><strong>Molecular features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPLs with LOH</td>
<td>79</td>
<td>47/64 (73%)</td>
<td>32/36 (89%)</td>
<td>0.077</td>
</tr>
<tr>
<td>OPLS with LOH &gt; 1 arm</td>
<td>41</td>
<td>17/64 (27%)</td>
<td>24/36 (67%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>OPLS with LOH &gt; 2 arms</td>
<td>27</td>
<td>7/64 (11%)</td>
<td>20/36 (56%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low risk pattern (c) (retention of 3p &amp; 9p)</td>
<td>43</td>
<td>35/64 (55%)</td>
<td>8/36 (22%)</td>
<td>0.0018</td>
</tr>
<tr>
<td>High risk pattern (d) (3p &amp;/or 9p LOH plus other arm)</td>
<td>33</td>
<td>9/64 (14%)</td>
<td>24/36 (67%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPLs progressing to cancer</td>
<td>15/100 (15%)</td>
<td>3/64 (5%)</td>
<td>12/36 (33%)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

\(a\) Site at high-risk for cancer progression: floor of mouth, ventrolateral tongue.

\(b\) These patients had a history of oral dysplasia that was excised, but now had a hyperplastic lesion. For all of these cases, the lesion was at the former dysplasia site.

\(c\) Based on Rosin et al (2000). RR for Low risk was 1, and high risk 33.4.

\(d\) Includes loss at evaluated loci on 4q, 8p, 11q, 13q, or 17p.
Table 2-2. Risk features in OPLs with minimal or no dysplasia.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>TB -</th>
<th>TB +</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lesions</td>
<td>83</td>
<td>63</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% located at high-risk sites$^a$</td>
<td>44/83 (53%)</td>
<td>33/63 (52%)</td>
<td>11/20 (55%)</td>
<td>1</td>
</tr>
<tr>
<td>Largest dimension (mean ± SD) at start of study</td>
<td>15 ± 12</td>
<td>14 ± 10</td>
<td>18 ± 16</td>
<td>0.74</td>
</tr>
<tr>
<td>Largest dimension (mean ± SD) during follow-up</td>
<td>20 ± 15</td>
<td>18 ± 13</td>
<td>27 ± 19</td>
<td><strong>0.028</strong></td>
</tr>
<tr>
<td>% of nonhomogeneous lesions at start of study</td>
<td>23/77 (30%)</td>
<td>13/58 (22%)</td>
<td>10/19 (53%)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>% of nonhomogeneous lesions during follow-up</td>
<td>40/82 (49%)</td>
<td>25/62 (40%)</td>
<td>12/20 (75%)</td>
<td><strong>0.0097</strong></td>
</tr>
<tr>
<td><strong>Molecular features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPLs with LOH</td>
<td>62</td>
<td>46/63 (73%)</td>
<td>16/20 (80%)</td>
<td>0.77</td>
</tr>
<tr>
<td>OPLs with LOH &gt; 1 arm</td>
<td>25</td>
<td>17/63 (27%)</td>
<td>8/20 (40%)</td>
<td><strong>0.28</strong></td>
</tr>
<tr>
<td>OPLs with LOH &gt; 2 arms</td>
<td>13</td>
<td>7/63 (11%)</td>
<td>6/20 (30%)</td>
<td><strong>0.072</strong></td>
</tr>
<tr>
<td>Low risk pattern$^b$ (retention of 3p &amp; 9p)</td>
<td>43</td>
<td>35/63 (56%)</td>
<td>8/20 (40%)</td>
<td>0.31</td>
</tr>
<tr>
<td>High risk pattern$^b$ (3p &amp;/or 9p LOH plus other arm)$^c$</td>
<td>17</td>
<td>9/63 (14%)</td>
<td>8/20 (40%)</td>
<td><strong>0.023</strong></td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPLs progressing to cancer</td>
<td>8/83 (10%)</td>
<td>3/63 (5%)</td>
<td>5/20 (25%)</td>
<td><strong>0.0177</strong></td>
</tr>
</tbody>
</table>

$^a$Site at high-risk for cancer progression: floor of mouth, ventrolateral tongue.
$^b$Based on Rosin et al (2000). RR for Low risk was 1, and high risk 33.4.
$^c$Includes loss at evaluated loci on 4q, 8p, 11q, 13q, or 17p.
Supplemental Table 2-1. Frequency of loss of heterozygosity (LOH) and TB staining

<table>
<thead>
<tr>
<th>LOH pattern&lt;sup&gt;a&lt;/sup&gt;</th>
<th>All</th>
<th>TB-negative</th>
<th>TB-positive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td># of lesions</td>
<td>100</td>
<td>64</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td># with LOH</td>
<td>79</td>
<td>47/64 (73%)</td>
<td>32/36 (89%)</td>
<td>0.077</td>
</tr>
<tr>
<td>&gt;1 arm</td>
<td>41</td>
<td>17/64 (27%)</td>
<td>24/36 (67%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>&gt;2 arms</td>
<td>27</td>
<td>7/64 (11%)</td>
<td>20/36 (56%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LOH on 3p</td>
<td>32</td>
<td>15/64 (23%)</td>
<td>17/33 (52%)</td>
<td>0.0068</td>
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<tr>
<td>LOH on 4q</td>
<td>11</td>
<td>5/60 (8%)</td>
<td>6/28 (21%)</td>
<td>0.097</td>
</tr>
<tr>
<td>LOH on 8p</td>
<td>23</td>
<td>11/62 (18%)</td>
<td>12/33 (36%)</td>
<td>0.077</td>
</tr>
<tr>
<td>LOH on 9p</td>
<td>47</td>
<td>23/64 (36%)</td>
<td>24/36 (67%)</td>
<td>0.0037</td>
</tr>
<tr>
<td>LOH on 11q</td>
<td>14</td>
<td>4/62 (6%)</td>
<td>10/34 (29%)</td>
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<td>LOH on 13q</td>
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<td>5/62 (8%)</td>
<td>8/35 (23%)</td>
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<tr>
<td>LOH on 17p</td>
<td>31</td>
<td>13/64 (20%)</td>
<td>18/36 (50%)</td>
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<td>3p &amp;/or 9p retention</td>
<td>43</td>
<td>35/64 (55%)</td>
<td>8/36 (22%)</td>
<td>0.0018</td>
</tr>
<tr>
<td>LOH on 3p &amp;/or 9p plus other arm</td>
<td>33</td>
<td>9/64 (14%)</td>
<td>24/36 (67%)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Lesions without dysplasia or with low-grade dysplasia (severe dysplasia excluded)

<table>
<thead>
<tr>
<th># of lesions</th>
<th>83</th>
<th>63</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td># with LOH</td>
<td>62</td>
<td>46/63 (73%)</td>
<td>16/20 (80%)</td>
</tr>
<tr>
<td>&gt;1 arm</td>
<td>25</td>
<td>17/63 (27%)</td>
<td>8/20 (40%)</td>
</tr>
<tr>
<td>&gt; 2 arm</td>
<td>13</td>
<td>7/63 (11%)</td>
<td>6/20 (30%)</td>
</tr>
<tr>
<td>LOH on 3p</td>
<td>22</td>
<td>15/63 (24%)</td>
<td>7/17 (41%)</td>
</tr>
<tr>
<td>LOH on 4q</td>
<td>6</td>
<td>5/59 (8%)</td>
<td>1/18 (6%)</td>
</tr>
<tr>
<td>LOH on 8p</td>
<td>18</td>
<td>11/61 (18%)</td>
<td>7/17 (41%)</td>
</tr>
<tr>
<td>LOH on 9p</td>
<td>31</td>
<td>22/63 (35%)</td>
<td>9/20 (45%)</td>
</tr>
<tr>
<td>LOH on 11q</td>
<td>5</td>
<td>4/61 (7%)</td>
<td>1/19 (5%)</td>
</tr>
<tr>
<td>LOH on 13q</td>
<td>9</td>
<td>5/61 (8%)</td>
<td>4/19 (21%)</td>
</tr>
<tr>
<td>LOH on 17p</td>
<td>18</td>
<td>13/63 (21%)</td>
<td>5/20 (25%)</td>
</tr>
<tr>
<td>3p &amp;/or 9p retention</td>
<td>43</td>
<td>35/63 (56%)</td>
<td>8/20 (40%)</td>
</tr>
<tr>
<td>LOH on 3p &amp;/or 9p plus other arm</td>
<td>17</td>
<td>9/63 (14%)</td>
<td>8/20 (40%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Loss/informative cases (% loss).
2.8 Figure legends.

Figure 2-1. Probability of development of cancer from primary OPLs, according to TB-staining pattern. A, Progression as a function of TB-staining capacity for all 100 OPLs (TB-positive = 36; TB-negative = 64). B, Progression as a function for TB staining for OPLs without dysplasia or with low-grade dysplasia (severe dysplasia excluded) (TB-positive = 20; TB-negative = 63).
Figure 2-2. The time event charts show (1) treatment, (2) changes in TB status during follow-up and (3) outcome. Each case was labelled for its TB status at the beginning of the study (orange for TB- and blue for TB+). Treatment: Excision = T; Topical bleomycin = B. Change in TB status during follow-up: Blue square = Change of TB staining from TB- to TB+; Orange square = Change of TB staining from TB+ to TB. When the TB status had not changed, e.g., started as TB- and remained negative during the whole follow-up time, the time event bar for that case would remain unmarked. Outcome: Empty square = Last follow-up time for lesions that had not progressed. This square was coloured blue or orange when there was a change in TB status on that date. Red diamond = Cancer development.
Supplemental Figure 1.

**A**

Supplemental figure 2-1. Clinical photos of (A) a TB-positive moderate dysplasia pre- and post-TB staining; and (B) a TB-negative moderate dysplasia pre- and post-TB staining. In each case, the lesion before staining is at the far left with that after staining on the right.
CHAPTER 3 PROJECT II: FLUORESCENCE VISUALIZATION AS A VISUAL ADJUNCT TOOL TO IDENTIFY HIGH-RISK ORAL PREMALIGNANT LESIONS

3.1 Introduction

Oral squamous cell carcinoma (SCC) ranks as the 8th most common malignancy in the world, annually accounting for 274,000 new cases and 127,000 deaths [1]. The disease represents a management conundrum: It occurs at a site that is easily accessible for examination, but is often diagnosed at an advanced stage when both functional and cosmetic impairments due to treatment and mortality rates are high. Later stages are associated with severe morbidity and high mortality, with 5-year survival rates ranging from 30 - 60%, depending on the global locale. Early detection of the disease has the potential to impact significantly on disease prognosis, providing the opportunity for intervention when treatment is most effective.

One of the main barriers to detection and management of early disease is that it relies heavily on the clinician’s ability to discriminate sometimes subtle alterations associated with oral premalignant lesions (OPLs) and cancers from benign reactive change in the oral mucosa. Several approaches have been suggested to improve such decision making including optical imaging, a procedure that enables the identification of disease as it develops in situ. Optical visualization techniques are comprised of two main approaches. One revolves around the development of exogenously applied contrast agents that can be “painted” on the tissue surface to enhance detection of change. The other utilizes optical devices that identify morphologic and biochemical alterations that affect optical properties of a tissue.

Toluidine blue (TB) is an example of an optical contrast agent that has been used for over 40 years as a method of detecting mucosal abnormalities in the oral cavity [2, 3]. This metachromatic dye has a fairly high sensitivity for the detection of oral cancers [4, 5]; its ability to detect OPLs has been controversial. A significant proportion of dysplasia do not stain with the dye [5-7]. However, results from an ongoing longitudinal study suggest that TB may
preferentially stain OPLs at elevated risk of malignant progression [8]. In that study, TB retention in tissue was shown to associate strongly with clinicopathological risk factors, high-risk molecular patterns and with outcome.

A more recent approach to visualization of the oral mucosa uses the alternate type of optical imaging, tissue optics, more specifically, autofluorescence imaging. Loss of autofluorescence is documented as being present in cancer and premalignant lesions at several sites including the cervix, lung, skin and oral cavity and devices have been developed that have already had a significant impact on disease management in several tissue sites [9-13]. Autofluorescence is produced when tissue is illuminated by ultraviolet (<400 nm) or short visible light (~400 to 550 nm) at the tissue surface. The resulting fluorescence emanates from fluorophores in the tissue, predominantly in the submucosa, mainly collagen and elastin, but also includes fluorescence due to two metabolic indicators, NADH and FAD, in the epithelial layer [14-16]. Alterations to fluorescence during carcinogenesis reflects a combination of change to these fluorophores with disease progression and alterations to tissue morphology that accompany cancer development and that affect the absorption and scattering of light, such as mucosal thickening, loss of tissue stratification, increased nuclear scattering and neovascularisation.

Recently, the development of a hand-held visualization device, marketed as the VELScope (LED Dental, Inc., White Rock, British Columbia, Canada) by our research group made it possible to directly assess fluorescence of the oral cavity. Since that time, direct fluorescence visualization (FV) has been reported in several papers to enhance visualization of neoplastic and premalignant areas in patients with OPLs and cancer [17-20]; however, the data to date has been limited. The need for such knowledge is particularly urgent in view of the fact that the tool is already being mass marketed to general dental practices in North America as an adjunct tool for detection of OPLs.

In this study we report our experience with the use of FV in 170 patients enrolled in an ongoing longitudinal study in British Columbia. Our primary goal in the present analysis was to evaluate the ability of direct FV to detect high-risk oral premalignant disease and cancer within patients in this study. In this paper, we show that FV loss in lesions is associated with high-risk clinical, histological and molecular features. We also show a correlation of FV with TB staining and present data that support the potential value of integrating wide-field assessment of the
oral mucosa with FV for the presence of oral anomalies and targeted “painting” with an optical contrast agent, in this case TB stain, to further define cancer risk.

3.2 Materials and Methods

Patients

The study group was chosen from patients accrued between 1999 and 2007 to an ongoing Oral Cancer Prediction Longitudinal study situated in the Oral Dysplasia Clinics of the British Columbia Oral Cancer Prevention Program. These clinics are referral centres for oral dysplasia for the province of BC and accept patients if they are 18 years of age or older and have precancerous or cancerous squamous lesions located in the oral cavity. Patients with precancerous lesions had no history of invasive oral squamous cell carcinoma (SCC). To be eligible for this analysis, patients required a FV examination and a subsequent biopsy. The clinical protocol for this study was reviewed and approved by the British Columbia Cancer Agency Research Ethics Board. All subjects enrolled in the study gave written informed consent.

A total of 170 patients were available. Of these, 54% were male, with the majority being Caucasian (82%). Patients were on average 60 years of age (22 - 87) and 70% had had smoked cigarettes, cigars or pipes more than once per week for one year or longer; of these, 42% were smoking at time of assessment (Table 3-1).
Table 3-1. Demographics and smoking history of study participants.

<table>
<thead>
<tr>
<th>ALL PATIENTS (n=170)</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>92 (54%)</td>
</tr>
<tr>
<td>Female</td>
<td>78 (46%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>140 (82%)</td>
</tr>
<tr>
<td>Other</td>
<td>30 (18%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Mean age (yrs) ± S.D.</td>
<td>60 ± 13</td>
</tr>
<tr>
<td>Median (years) (range)</td>
<td>60 (22 - 87)</td>
</tr>
<tr>
<td>Proportion &lt;40</td>
<td>9 (5%)</td>
</tr>
<tr>
<td>Smoking (n=168)(^1)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>118 (70%)</td>
</tr>
<tr>
<td>None</td>
<td>50 (30%)</td>
</tr>
<tr>
<td>Ever smoker (n=118)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>68 (58%)</td>
</tr>
<tr>
<td>Current</td>
<td>50 (42%)</td>
</tr>
</tbody>
</table>

\(^1\) Missing smoking data for 2 patients: 1 SCC and 1 nondysplasia

A total of 192 lesions were observed in these 170 patients: 34 with no evidence of dysplasia (termed “nondysplastic” and including 21 hyperplasias, 10 lichenoid mucositis, and 3 lichen planus); 66 low-grade (mild and moderate) dysplasia, 52 high-grade (severe and CIS) dysplasia and 40 with squamous cell carcinoma (SCC). Twenty-one patients had multiple lesions. Such lesions were defined as being present at different anatomical sites or if in the same site, separated by at least 2 cm of clinically normal mucosa. In 14 cases, the multiple lesions occurred in patients with either nondysplastic lesions or low-grade dysplasia (1 of these 14 patients had 3 distinct lesions, the rest had two). In addition, 7 of the 52 patients with high-grade dysplasia had 2 separate lesions.
Overview of study protocol.

The study protocol consisted of the clinical assessment of the oral mucosa of each individual using conventional examination, followed by FV examination and TB analysis. Biopsies were obtained from each lesion site and histological diagnoses determined by a board-certified oral pathologist (either LZ or CP) without knowledge of FV status of the lesion, using criteria from the World Health Organization (WHO, 1996). Molecular analysis was performed on tissue dissected from these biopsies using microsatellite analysis. In addition, information on demographics (age, gender) and tobacco history were obtained by questionnaire (see Chapter 3 Appendix 1).

Clinical Examination.

The clinical examination followed a standardized step-by-step protocol used by the British Columbia Oral Cancer Prevention Program, as described in Williams et al [21]. The exam consisted of both extraoral and intraoral components with both manual palpation of tissue and visual inspection. A notation was made of the presence of a lesion in any part of the oral cavity, including areas of leukoplakia, erythroplakia, cancer and ulcer. All lesions were characterized by location(s), size, colour and appearance with these characteristics described below:

Lesion location: Site identification included an indication of anatomic location of the lesions, which side of the site was involved, where applicable (e.g., right or left buccal mucosa or both) and a drawing out of the lesion on a map of the mucosa that was attached to each patient’s record (see Chapter 3 Appendix 2).

Lesion size: A Marquis colour coded periodontal probe (Henry Schein Ash Arcona, Canada) was used to measure the length, width and thickness of each lesion.

Lesion appearance: Lesion colour, texture and thickness were each described. Colour options were white, predominantly white, predominantly red, red and other. Texture of a lesion was described as smooth, verrucous, fissured, nodular, velvety/grainy, ulcerated or other. A “homogeneous” lesion was uniform in its color and appearance, white with a smooth, thin or slightly fissured texture. All other lesions were categorized as “nonhomogeneous”. Such lesions included those with a rough or speckled surface. [21]
Fluorescence visualization (FV) procedure.

FV was assessed using an autofluorescence imaging device, marketed as the Velscope™, (LED Dental, Inc., White Rock, British Columbia, Canada). This device uses a blue/violet light (400 – 460 nm wavelengths) to illuminate oral tissue with long-pass and notch filters to allow clinicians to directly view fluorescence.

The examination was performed under reduced room lighting and involved the inspection of the entire oral mucosa in the same manner as the conventional intraoral examination, with special attention to the lesion site. Lesions that retained the normal green autofluorescence under FV were classified as having FV retained (FVR). Tissue that showed a reduction in the normal pale green, appearing as dark patches were categorized as having FV loss (FVL). This distinction involved a comparison of the lesion site with adjacent tissue and with tissue without lesions on the contralateral side.

Toluidine blue (TB) examination.

To avoid potential confounding of FV, the TB examination was made after completion of FV. The lesions were first swabbed with 1% acetic acid to remove debris and excess mucous. 1% TB was then applied topically to the entire lesion using a cotton tip applicator. The stain was left for 45 seconds and then followed by a second application of acetic acid (1%) and a thorough rinse with water. TB status was recorded as positive (TB stain retained) or negative (no retention of stain). Equivocal staining was recorded, but lesions belonging to this category were subsequently transferred to the positive staining group since there was no significant difference between these two groupings with respect to association with clinical appearance, histology or outcome.

Photography of lesions.

Digital images were taken of every lesion under 3 conditions: under white incandescent light, with FV and after TB stain. White-light and TB images were captured with a digital Single Lens Reflex Camera (SLR, Fuji FinePix S2 or S3 Pro) that was equipped with a 105 mm f/2.8 macro lens (Nikkor-Micro, United States) and a ring flash (Nikon Macro Speedlight SB-29s). A long-pass filter (Schott GG475-3, Howard Glaas, Worcester, MA) was used to collect FV images.
Microsatellite analysis

All biopsies from non- and low grade dysplasias that had sufficient tissue (83 of 100 samples) were microdissected and assessed for loss of heterozygosity (LOH). As a positive control, an additional 18 biopsies from high-grade dysplasia or SCC were analyzed. The protocol for digestion and extraction of samples, LOH analysis and scoring are described in Zhang et al. [22], and the analysis was done without knowledge of FV status. Microsatellite markers were those used in previous studies to predict cancer risk of oral premalignant lesions [23-28] and mapped to the following regions: 3p14.2 (D3S1234, D3S1228, D3S1300); 4q26 (FABP2); 4q31.1 (D4S243); 8p21.3 (D8S261); 8p23.3 (D8S262); 8p23.3 (D8S264); 9p21 (IFNA, D9S171, D9S1748, D9S1751); 11q13.3 (INT2); 11q22.3 (D11S1778); 13q12.3-13 (D13S170); 13q14.3 (D13S133); 17p11.2 (CHRNB1) and 17p13.1 (tp53 and D17S786).

Analysis and statistical methods.

Differences and associations between groups (e.g., FVR vs. FVL) were examined using Fischer’s exact test or Pearson’s chi square test for categorical variables. Continuous variables were compared with either unpaired t-tests or a nonparametric Mann-Whitney test if the data failed to have a normal distribution. All tests were two sided. Time-to-progression curves were estimated by the Kaplan-Meier method, and the resulting curves were compared using the log-rank test. Relative risks and the corresponding 95% confidence intervals (95% CI) were determined using Cox regression analysis. Results were considered statistically significant when P < 0.05. Statistical analysis was performed with SPSS software, version 16.0 for Windows, 2007 (SPSS Inc., Chicago, Illinois).

2 The molecular study was done by another graduate student.
3.3 Results

We assessed a total of 192 lesions in 170 subjects for autofluorescence status, classifying them as either FVL (showing a loss of fluorescence) or FVR (retention of fluorescence). In the data analysis we looked for associations between FV status and demographics, smoking habit, conventional clinicopathological features, toluidine blue staining and outcome.

The data presented below is given first for an analysis using data from all lesions and examining demographics, smoking habit and clinicopathological features. This is followed by a parallel analysis that focuses only on premalignant lesions, involving a categorisation into the following histological groups: nondysplasia, low-grade and high-grade dysplasia. Final sections describe analysis of FV status in relation to toluidine blue (TB) stain retention and with respect to outcome.

Demographics and smoking habit of study group and FV status

We first looked for associations of FV status with demographics and smoking habit (Table 3-2). For this analysis, we classified patients as either FVL or FVR. For patients with multiple lesions, the presence of any FVL lesion resulted in classification into the FVL group. When patients with FVL and FVR oral lesions were compared, there was no association of FV status with gender, ethnicity or age. Similarly, smoking habit did not differ significantly between patients with FVL and FVR status (smoking data was available for 168 patients).
<table>
<thead>
<tr>
<th>Demographics</th>
<th>No. of patients (%)</th>
<th>FVR (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>FVL (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # of patients</td>
<td>170</td>
<td>27 (16%)</td>
<td>143 (84%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>92 (54%)</td>
<td>12 (44%)</td>
<td>80 (56%)</td>
<td>0.298</td>
</tr>
<tr>
<td>Female</td>
<td>78 (46%)</td>
<td>15 (56%)</td>
<td>63 (44%)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>140 (82%)</td>
<td>24 (89%)</td>
<td>116 (81%)</td>
<td>0.419</td>
</tr>
<tr>
<td>Other</td>
<td>30 (18%)</td>
<td>3 (11%)</td>
<td>27 (19%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (yrs) ± S.D.</td>
<td>61 ± 12</td>
<td>58 ± 11</td>
<td>63 ± 12</td>
<td>0.078</td>
</tr>
<tr>
<td>Median (range)</td>
<td>60 (39 - 84)</td>
<td>56 (42 - 83)</td>
<td>64 (39 - 84)</td>
<td></td>
</tr>
<tr>
<td>Proportion &lt;40</td>
<td>9 (5%)</td>
<td>0 (0%)</td>
<td>9 (6%)</td>
<td>0.357</td>
</tr>
<tr>
<td>Smoking (n=168)&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>50 (30%)</td>
<td>5 (19%)</td>
<td>45 (32%)</td>
<td>0.248</td>
</tr>
<tr>
<td>Ever</td>
<td>118 (70%)</td>
<td>21 (81%)</td>
<td>97 (68%)</td>
<td></td>
</tr>
<tr>
<td>Ever smoker (n=118)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>68 (58%)</td>
<td>11 (52%)</td>
<td>57 (59%)</td>
<td>0.632</td>
</tr>
<tr>
<td>Current</td>
<td>50 (42%)</td>
<td>10 (48%)</td>
<td>40 (41%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>% of FVR (or FVL) lesions that displayed indicated feature  
<sup>2</sup> Missing smoking data for 2 patients: 1 SCC and 1 nondysplasia

**Clinical Features and FV Status**

We next determined whether there was a relationship between FV status and the presence of clinical features that have been previously associated with risk of progression to cancer for premalignant lesions and with the presence of cancer itself. These included: the location on the lesion on the floor of mouth and ventrolateral tongue (termed high-risk sites, [29-32]), lesion size (> 20 mm, [33, 34]), and nonhomogeneous clinical appearance [29, 30, 32, 34, 35] (Table 3-3).
FVL lesions were more likely to be located at high-risk sites compared with FVR lesions (63% FVL group vs. 41% FVR group, \( P = 0.018 \)) and to have a nonhomogeneous clinical appearance (74% FVL group vs. 34% FVR group; \( P < 0.001 \)). The association with size however was not significant. When all 3 clinical risk factors were examined together, a higher proportion of FVL lesions showed the presence of multiple (2 or 3) clinical risk factors (64% vs. 36%, \( P = 0.002 \)).

### Table 3-3. Clinical features and FV status of all lesions.

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>No. of lesions (%)</th>
<th>FVR (%(^1)/%(^2))</th>
<th>FVL (%(^1)/%(^2))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk site(^3) (n=192)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>80 (42%)</td>
<td>23 (29%/59%)</td>
<td>57 (71%/37%)</td>
<td>0.018</td>
</tr>
<tr>
<td>High</td>
<td>112 (58%)</td>
<td>16 (14%/41%)</td>
<td>96 (86%/63%)</td>
<td></td>
</tr>
<tr>
<td>Appearance (n=176)(^4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogeneous</td>
<td>58 (33%)</td>
<td>21 (36%/66%)</td>
<td>37 (64%/26%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nonhomogeneous</td>
<td>118 (67%)</td>
<td>11 (9%/34%)</td>
<td>107 (91%/74%)</td>
<td></td>
</tr>
<tr>
<td>Size (n=181)(^4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20mm</td>
<td>99 (55%)</td>
<td>21 (21%/64%)</td>
<td>78 (79%/53%)</td>
<td>0.334</td>
</tr>
<tr>
<td>≥20mm</td>
<td>82 (45%)</td>
<td>12 (15%/36%)</td>
<td>70 (85%/47%)</td>
<td></td>
</tr>
<tr>
<td>Number of clinical risk factors (n=181)(^4,(^5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 features</td>
<td>76 (42%)</td>
<td>22 (29%/67%)</td>
<td>54 (71%/37%)</td>
<td>0.002</td>
</tr>
<tr>
<td>≥2 features</td>
<td>105 (58%)</td>
<td>11 (10%/33%)</td>
<td>94 (90%/64%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) % of lesions with indicated feature that were FVR or FVL

\(^2\) % of FVR (or FVL) lesions that displayed indicated feature

\(^3\) Lateral, ventral tongue and floor of mouth

\(^4\) Missing data: 16 appearance, 11 size, 11 total risk factors

\(^5\) Clinical risk factors: high risk site, large size, appearance
**Histological diagnosis and FV status**

Table 3-4 shows FV status in lesions belonging to different histological groups. Loss of fluorescence was strongly associated with histological progression. FVL was present in virtually all high-grade dysplasias and SCC (96% severe dysplasia, 100% CIS, 95% SCC), supporting its ability to detect high-risk histological lesions. In addition 74% of low grade dysplasia also showed FVL (67% of mild dysplasia, 82% of moderate dysplasia).

A striking number of nondysplastic lesions (44%) were also FVL, possibly reflecting the high-risk nature of these patients. When the history of these non-dysplastic lesions was examined, it was determined that half of the lesions had had a previous biopsy of the site that showed dysplasia. Lesions with a previous history of dysplasia were more likely to show FVL: 65% of such lesions were FVL compared to only 24% of nondysplastic lesions without dysplasia history ($P = 0.037$). This suggests that in these patients, FV status in non-dysplastic lesions was still tracking histological risk.
Table 3-4. Histological diagnosis and FV status in all lesions.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of lesions (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>FVR (%&lt;sup&gt;2&lt;/sup&gt;/%&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>FVL (%&lt;sup&gt;2&lt;/sup&gt;/%&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All lesions (N=192)</td>
<td>192</td>
<td>39 (20%)</td>
<td>153 (80%)</td>
<td></td>
</tr>
<tr>
<td>Nondysplastic</td>
<td>34 (18%)</td>
<td>19 (56%/49%)</td>
<td>15 (44%/10%)</td>
<td></td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>33 (17%)</td>
<td>11 (33%/28%)</td>
<td>22 (67%/14%)</td>
<td></td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>33 (17%)</td>
<td>6 (18%/15%)</td>
<td>27 (82%/18%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>28 (15%)</td>
<td>1 (4%/3%)</td>
<td>27 (96%/18%)</td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>24 (13%)</td>
<td>0 (0%/0%)</td>
<td>24 (100%/16%)</td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>40 (21%)</td>
<td>2 (5%/5%)</td>
<td>38 (95%/25%)</td>
<td></td>
</tr>
</tbody>
</table>

All lesions by groups (N=192)

| Nondysplasia           | 34 (18%)                        | 19 (56%/49%)                     | 15 (44%/10%)                     | <0.001  |
| Low grade              | 66 (34%)                        | 17 (26%/44%)                     | 49 (74%/32%)                     |         |
| High Grade             | 52 (27%)                        | 1 (2%/3%)                        | 51 (98%/33%)                     |         |
| SCC                    | 40 (21%)                        | 2 (5%/5%)                        | 38 (95%/25%)                     |         |

Low grade and nondysplasia (n=100)

| Nondysplasia           | 34 (34%)                        | 19 (56%/53%)                     | 15 (44%/23%)                     | 0.004   |
| Low grade              | 66 (66%)                        | 17 (26%/47%)                     | 49 (74%/77%)                     |         |

Nondysplasia (n=34)

| No history of dysplasia| 17 (50%)                        | 13 (76%/68%)                     | 4 (24%/27%)                      | 0.037   |
| History of dysplasia   | 17 (50%)                        | 6 (35%/32%)                      | 11 (65%/73%)                     |         |

<sup>1</sup> % of lesions in indicated histological group  
<sup>2</sup> % of indicated diagnosis that was FVR or FVL  
<sup>3</sup> % of FVR lesions or FVL lesions that had indicated histology
Molecular change and FV status

We next examined the data for associations between FV status and the presence of alterations at chromosome sites that had previously been related to progression risk in retrospective analysis [8, 25]. FVL lesions showed a higher frequency of loss on all 7 arms; however, of these associations only 3 were significant (Table 3-5). A greater proportion of FVL lesions displayed a loss at 3p (44% FVL group vs. 23% FVR group, P = 0.050), 9p (58% FVL group vs. 32% FVR group, P = 0.021) and 17p (45% FVL group vs. 24% FVR group, P = 0.050). Although there was an increased proportion of lesions with FVL that had loss at 3p and/or 9p plus other arm (loci at 4q, 8p, 11q, 13q or 17p), a high-risk pattern for progression, this association was not significant. However, FVL lesions were more likely to show multiple arm loss: loss at 2 or more arms (65% FVL group vs. 43% FVR group, P = 0.036) or 3 or more arms (48% FVL group vs. 20% FVR group, P = 0.006) suggesting a higher level of genetic instability in the tissue.
### Table 3-5. Molecular change and FV status of all lesions.

<table>
<thead>
<tr>
<th>LOH pattern&lt;sup&gt;1&lt;/sup&gt;</th>
<th>No. of lesions (%)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>FVR (%&lt;sup&gt;3&lt;/sup&gt;/%&lt;sup&gt;4&lt;/sup&gt;)</th>
<th>FVL (%&lt;sup&gt;3&lt;/sup&gt;/%&lt;sup&gt;4&lt;/sup&gt;)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3p (n=99)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>63 (64%)</td>
<td>27 (43%/77%)</td>
<td>36 (57%/56%)</td>
<td>0.050</td>
</tr>
<tr>
<td>Loss</td>
<td>36 (36%)</td>
<td>8 (22%/23%)</td>
<td>28 (78%/44%)</td>
<td></td>
</tr>
<tr>
<td>4q (n=87)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>69 (79%)</td>
<td>26 (38%/87%)</td>
<td>43 (62%/75%)</td>
<td>0.274</td>
</tr>
<tr>
<td>Loss</td>
<td>18 (21%)</td>
<td>4 (22%/13%)</td>
<td>14 (78%/25%)</td>
<td></td>
</tr>
<tr>
<td>8p (n=87)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>60 (69%)</td>
<td>22 (37%/73%)</td>
<td>38 (63%/67%)</td>
<td>0.629</td>
</tr>
<tr>
<td>Loss</td>
<td>27 (31%)</td>
<td>8 (30%/27%)</td>
<td>19 (70%/33%)</td>
<td></td>
</tr>
<tr>
<td>9p (n=100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>51 (51%)</td>
<td>23 (45%/68%)</td>
<td>28 (55%/42%)</td>
<td>0.021</td>
</tr>
<tr>
<td>Loss</td>
<td>49 (49%)</td>
<td>11 (22%/32%)</td>
<td>38 (78%/58%)</td>
<td></td>
</tr>
<tr>
<td>11q (n=89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>67 (75%)</td>
<td>26 (39%/81%)</td>
<td>41 (61%/72%)</td>
<td>0.444</td>
</tr>
<tr>
<td>Loss</td>
<td>22 (25%)</td>
<td>6 (27%/19%)</td>
<td>16 (73%/28%)</td>
<td></td>
</tr>
<tr>
<td>13q (n=91)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>75 (82%)</td>
<td>28 (37%/85%)</td>
<td>47 (63%/81%)</td>
<td>0.778</td>
</tr>
<tr>
<td>Loss</td>
<td>16 (18%)</td>
<td>5 (31%/15%)</td>
<td>11 (69%/19%)</td>
<td></td>
</tr>
<tr>
<td>17p (n=101)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>63 (62%)</td>
<td>26 (41%/77%)</td>
<td>37 (59%/55%)</td>
<td>0.050</td>
</tr>
<tr>
<td>Loss</td>
<td>38 (38%)</td>
<td>8 (21%/24%)</td>
<td>30 (79%/45%)</td>
<td></td>
</tr>
<tr>
<td>Any loss (n=101)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No loss</td>
<td>27 (27%)</td>
<td>12 (44%/34%)</td>
<td>15 (56%/23%)</td>
<td>0.242</td>
</tr>
<tr>
<td>Loss</td>
<td>74 (73%)</td>
<td>23 (31%/66%)</td>
<td>51 (69%/77%)</td>
<td></td>
</tr>
<tr>
<td>LOH pattern¹</td>
<td>No. of lesions (%)²</td>
<td>FVR (%³/⁴)</td>
<td>FVL (%³/⁴)</td>
<td>P value</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>≥2 arm loss (n=101)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or less</td>
<td>43 (43%)</td>
<td>20 (47%/57%)</td>
<td>23 (53%/35%)</td>
<td></td>
</tr>
<tr>
<td>2 or more</td>
<td>58 (57%)</td>
<td>15 (26%/43%)</td>
<td>43 (74%/65%)</td>
<td></td>
</tr>
<tr>
<td>≥3 arm loss (n=101)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 or less</td>
<td>62 (61%)</td>
<td>28 (45%/80%)</td>
<td>34 (5%/52%)</td>
<td></td>
</tr>
<tr>
<td>3 or more</td>
<td>39 (39%)</td>
<td>7 (18%/20%)</td>
<td>32 (82%/49%)</td>
<td></td>
</tr>
<tr>
<td>Molecular risk pattern⁵ (n=101)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No loss at 3p +/- or 9p</td>
<td>42 (42%)</td>
<td>19 (45%/54%)</td>
<td>23 (55%/35%)</td>
<td></td>
</tr>
<tr>
<td>Loss at 3p +/- or 9p + other arms</td>
<td>59 (58%)</td>
<td>16 (27%/46%)</td>
<td>43 (73%/65%)</td>
<td></td>
</tr>
</tbody>
</table>

¹ # in parentheses: # of informative cases for each chromosome loci  
² % of lesions that show retention or loss for indicated chromosome loci  
³ % of indicated LOH patterns that are FVR vs. FVL  
⁴ % of FVR or FVL lesions that show indicated LOH pattern  
⁵ Includes loss at evaluated loci on 4q, 8p, 11q, 13q, or 17p

**FV status in premalignant lesions, according to presence and degree of dysplasia**

The above analyses using all samples suggested associations between FV status and high-risk clinical features, histological progression and presence of molecular clones. This section describes the further analysis of the data, examining clinical and molecular data in premalignant lesions, according to the presence and degree of dysplasia.

We first examined clinical features with respect to FV status restricting the analysis to premalignant lesions, first looking at the group as a whole, and then at different histological categories.

We next examined clinical features and FV status within each histological subgroup. Since virtually all high-grade dysplasia showed FVL (51 out of 52), a comparison of the frequencies of clinical features in FVL and FVR categories was not appropriate. However, the majority of high-grade lesions were at a high-risk site (69%) and were nonhomogeneous (85%). Overall, 83% of lesions had 2 or more high-risk features.
In contrast, FV status did not significantly associate with any of the clinical features in either the nondysplastic or low-grade dysplasia groups. Of interest, for nondysplastic lesions, the presence of 2 or more clinical risk factors was significantly associated with FVL (93% FVL vs. 33% FVR, P = 0.002). However, sample numbers was small. Of note, 6 of the lesions with multiple risk factors had a history of dysplasia at the site, suggesting that this association might at least partially reflect lesion history.
### Table 3-6. Clinical risk factors and FV status of premalignant lesions.

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>No. of lesions (%)</th>
<th>Nondysplasia (n=34)</th>
<th>Low grade dysplasia (n=66)</th>
<th>High grade dysplasia (n=52)</th>
<th>All (no SCC) (n=152)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FVR (%)</td>
<td>FVL (%)</td>
<td>P value</td>
<td>FVR (%)</td>
</tr>
<tr>
<td>Risk site(^4) (n=152)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>67 (44%)</td>
<td>23 (34%/68%)</td>
<td>14 (61%/74%)</td>
<td>9 (39%/60%)</td>
<td>0.475</td>
</tr>
<tr>
<td>High</td>
<td>85 (56%)</td>
<td>11 (13%/32%)</td>
<td>5 (45%/26%)</td>
<td>6 (55%/40%)</td>
<td></td>
</tr>
<tr>
<td>Appearance (n=137)(^5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogeneous</td>
<td>50 (37%)</td>
<td>14 (28%/48%)</td>
<td>10 (71%/67%)</td>
<td>4 (29%/29%)</td>
<td>0.066</td>
</tr>
<tr>
<td>Nonhomogeneous</td>
<td>87 (64%)</td>
<td>15 (17%/52%)</td>
<td>5 (33%/33%)</td>
<td>10 (67%/71%)</td>
<td></td>
</tr>
<tr>
<td>Size (n=142)(^6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20mm</td>
<td>85 (60%)</td>
<td>24 (28%/80%)</td>
<td>12 (50%/80%)</td>
<td>12 (50%/80%)</td>
<td>1.000</td>
</tr>
<tr>
<td>≥20mm</td>
<td>57 (40%)</td>
<td>6 (11%/20%)</td>
<td>3 (50%/20%)</td>
<td>3 (50%/20%)</td>
<td></td>
</tr>
<tr>
<td>Number of clinical risk factors (n=142)(^7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 features</td>
<td>44 (31%)</td>
<td>11 (25%/38%)</td>
<td>10 (91%/67%)</td>
<td>1 (9%/77%)</td>
<td>0.002</td>
</tr>
<tr>
<td>≥2 features</td>
<td>98 (69%)</td>
<td>18 (18%/62%)</td>
<td>5 (28%/33%)</td>
<td>13 (72%/93%)</td>
<td></td>
</tr>
</tbody>
</table>
Finally, we examined LOH frequencies with respect to FV status in the premalignant lesions, again first looking at the group as a whole, and then at different histological categories.

When lesions were examined together, an increase in LOH frequency for FVL lesions in comparison with FVR lesions was observed for each LOH pattern (Table 3-7). Of these comparisons, LOH at 3p, 9p and 17p was on the verge of significance (3p LOH: FVL, 42%; FVR, 23%, P = 0.076; 9p LOH: FVL, 53%; FVR, 32%, P = 0.054; 17p LOH: FVL, 43%, FVR, 24%, P = 0.077); and loss on at 3 or more arms was significantly different (FVL, 47%; FVR, 20%, P = 0.009).

We next examined LOH patterns and FV status within each histological subgroup. Again, since only 1 of 52 high-grade lesions showed FVR, a comparison of LOH with FV status was not appropriate. Also, only 13 of the high-grade cases were assessed for LOH in this study, unfortunately, not including the FVR case. However, these cases were chosen randomly from the sample set and do provide an indication of LOH frequencies at different arms in these FVL lesions. There was a strong association with 5 LOH patterns: 3p (present in 46% of lesions), 9p (62%), 11q (58%), 17p (62%) and multiple arm loss (69% of lesions showed > 3 arms loss).

FV status did not significantly associate with any of the LOH patterns in either the nondysplastic or low-grade dysplasia groups although LOH at 3p and any loss approached significance in the low-grade group (3p LOH: FVL, 44%; FVR, 18%, P = 0.072; any loss: FVL, 87%; FVR, 65%, P = 0.076).
Table 3-7. LOH frequencies and FV status, by histological group.

<table>
<thead>
<tr>
<th>LOH pattern¹</th>
<th>No. of lesions² (%)</th>
<th>Nondysplasia (n=34)</th>
<th>Low grade dysplasia (n=66)</th>
<th>High grade dysplasia (n=52)</th>
<th>All (no SCC) (n=152)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All Nondys³ (%)</td>
<td>FVR (%)'s²/³</td>
<td>FVL (%)'s²/³</td>
<td>P value</td>
</tr>
<tr>
<td>3p (n=95)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>62/65%</td>
<td>21/34%/72%</td>
<td>13/62%/72%</td>
<td>8/38%/73%</td>
<td>1.000</td>
</tr>
<tr>
<td>Loss</td>
<td>33/35%</td>
<td>8/24%/28%</td>
<td>5/63%/28%</td>
<td>3/38%/27%</td>
<td></td>
</tr>
<tr>
<td>4q (n=84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>67/80%</td>
<td>24/36%/89%</td>
<td>15/63%/88%</td>
<td>9/38%/90%</td>
<td>1.000</td>
</tr>
<tr>
<td>Loss</td>
<td>17/20%</td>
<td>3/18%/11%</td>
<td>2/67%/12%</td>
<td>1/13%/10%</td>
<td></td>
</tr>
<tr>
<td>8p (n=85)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>60/71%</td>
<td>21/35%/81%</td>
<td>11/52%/69%</td>
<td>10/48%/100%</td>
<td>0.212</td>
</tr>
<tr>
<td>Loss</td>
<td>25/29%</td>
<td>5/20%/19%</td>
<td>5/100%/31%</td>
<td>0/0%/0%</td>
<td></td>
</tr>
<tr>
<td>9p (n=96)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>51/53%</td>
<td>24/47%/80%</td>
<td>15/63%/83%</td>
<td>9/38%/75%</td>
<td>0.660</td>
</tr>
<tr>
<td>Loss</td>
<td>45/47%</td>
<td>6/13%/20%</td>
<td>3/50%/17%</td>
<td>3/50%/25%</td>
<td></td>
</tr>
<tr>
<td>11q (n=86)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>64/74%</td>
<td>22/34%/85%</td>
<td>14/64%/82%</td>
<td>8/36%/89%</td>
<td>1.000</td>
</tr>
<tr>
<td>Loss</td>
<td>22/26%</td>
<td>4/18%/15%</td>
<td>3/75%/18%</td>
<td>1/25%/11%</td>
<td></td>
</tr>
<tr>
<td>11q (n=88)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>74/84%</td>
<td>24/32%/92%</td>
<td>15/63%/88%</td>
<td>9/38%/100%</td>
<td>0.529</td>
</tr>
<tr>
<td>Loss</td>
<td>14/16%</td>
<td>2/14%/8%</td>
<td>2/100%/12%</td>
<td>0/0%/0%</td>
<td></td>
</tr>
<tr>
<td>17p (n=97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ret</td>
<td>62/64%</td>
<td>25/40%/86%</td>
<td>14/56%/82%</td>
<td>11/44%/92%</td>
<td>0.622</td>
</tr>
<tr>
<td>Loss</td>
<td>35/36%</td>
<td>4/11%/14%</td>
<td>3/75%/18%</td>
<td>1/25%/8%</td>
<td></td>
</tr>
<tr>
<td>LOH pattern(^1)</td>
<td>No. of lesions(^2) (%)</td>
<td>Nondysplasia (n=34)</td>
<td>Low grade dysplasia (n=66)</td>
<td>High grade dysplasia (n=52)</td>
<td>All (no SCC) (n=152)</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------</td>
<td>----------------------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td>All Nondys(^3) (%)</td>
<td>FVR (%(^4)/%(^5))</td>
<td>FVL (%(^4)/%(^5))</td>
<td>All LGDys(^3) (%)</td>
<td>FVR (%(^4)/%(^5))</td>
</tr>
<tr>
<td>Any loss (n=97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No loss</td>
<td>27 (28%)</td>
<td>12 (44% /41%)</td>
<td>6 (50% /33%)</td>
<td>6 (50% /55%)</td>
<td>11 (41% /20%)</td>
</tr>
<tr>
<td>Loss</td>
<td>70 (72%)</td>
<td>17 (24% /59%)</td>
<td>12 (71% /67%)</td>
<td>5 (29% /46%)</td>
<td>44 (63% /80%)</td>
</tr>
<tr>
<td>22 arm loss (n=97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or less</td>
<td>42 (43%)</td>
<td>19 (45% /66%)</td>
<td>12 (63% /67%)</td>
<td>7 (37% /64%)</td>
<td>19 (45% /35%)</td>
</tr>
<tr>
<td>2 or more</td>
<td>55 (57%)</td>
<td>10 (18% /35%)</td>
<td>6 (60% /33%)</td>
<td>4 (40% /36%)</td>
<td>36 (65% /66%)</td>
</tr>
<tr>
<td>23 arm loss (n=97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 or less</td>
<td>61 (63%)</td>
<td>27 (44% /93%)</td>
<td>16 (59% /89%)</td>
<td>11 (41% /100%)</td>
<td>30 (49% /55%)</td>
</tr>
<tr>
<td>3 or more</td>
<td>36 (37%)</td>
<td>2 (6% /7%)</td>
<td>2 (100% /11%)</td>
<td>0 (0% /0%)</td>
<td>25 (69% /46%)</td>
</tr>
<tr>
<td>Molecular risk pattern(^6) (n=97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No loss at 3p +/or 9p</td>
<td>42 (43%)</td>
<td>18 (43% /62%)</td>
<td>11 (61% /61%)</td>
<td>7 (39% /64%)</td>
<td>20 (48% /36%)</td>
</tr>
<tr>
<td>Loss at 3p +/or 9p + other arms</td>
<td>55 (57%)</td>
<td>11 (20% /38%)</td>
<td>7 (64% /39%)</td>
<td>4 (36% /36%)</td>
<td>35 (64% /64%)</td>
</tr>
</tbody>
</table>

1 \# in parentheses: \# of informative cases for each chromosome loci

2 % of lesions that show retention or loss for indicated chromosome loci

3 Nondysplasia = Nondys, low grade dysplasia = LGDys, high grade dysplasia = HGDys

4 % of indicated LOH patterns that are FVR vs. FVL

5 % of FVR or FVL lesions that show indicated LOH pattern

6 Includes loss at evaluated loci on 4q, 8p, 11q, 13q, or 17p
TB retention and FV status

A total of 187 of the 192 lesions in this study also had TB data. For TB analysis, retention of the dye was scored as TB positive (TB+). Overall, 80% of the 187 lesions were positive for FV status (i.e., FVL) compared with only 50% for TB status (i.e., TB+). In this section, a comparison is made of the association of FVL and FVR with TB status, according to histological diagnosis.

Of 39 FVR lesions (negative for loss of autofluorescence), 37 (95%) were also negative for TB staining, supporting an association of TB negativity with FVR lesions (kappa = 0.371 (fair agreement, P<0.001; Table3-8 and Figure 3-1). In contrast, a significantly larger number of lesions were positive for loss of autofluorescence compared to those positive for the TB stain reaction. Only 91 of 148 (62%) FVL lesions were TB+. 
Table 3-8.  FV and TB interaction of all lesions

<table>
<thead>
<tr>
<th>Adjunctive device</th>
<th>No. of cases (%)</th>
<th>FVR (%(^1)/(^2))</th>
<th>FVL (%(^1)/(^3))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluidine blue (n=187)(^4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>94 (50%)</td>
<td>37 (39 %/95%)</td>
<td>57 (60%/38%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>93 (50%)</td>
<td>2 (2%/5%)</td>
<td>91 (98%/62%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) % of lesions with indicated feature that were FVR or FVL
\(^2\) % of FVR (or FVL) lesions that displayed indicated feature
\(^3\) TB data missing for 5 cases

Figure 3-1.  Venn diagram of the interaction of TB and FV status

Venn diagram of interaction of FV and toluidine blue status. FVL lesions are represented by the green circle; TB positive lesions are represented by the blue circle. FVL/TB+ lesions are represented by the green and blue overlapping area (n=91), FVL only within the green shaded area only (n=57); TB+ only within the blue shaded area only (n=2); FVR/TB- lesions are outside both circles (n=37).

Table 3-9 compares TB dye retention and FV status in different histological groups with Figure 3-2 presenting these data graphically. Similar to FV results, TB staining also showed a strong association with histological progression (\(P<0.001\)). However, the proportion of lesions that were positive for these two visualization procedures was strikingly different, and this was apparent across all histological groups (see Figure 3-2). FV identified more of the histologically high-risk lesions as positive (FVL), i.e., SCC and high-grade dysplasia. However, it also identified more of the low-grade and nondysplastic lesions.

Since history of a previous dysplasia was significantly associated with FVL among nondysplastic lesions (see section: FV status in premalignant lesions), we also compared TB data in nondysplastic lesions according to such history. Similar to the FV results, dysplasia history
was associated with an increase in TB positivity, although this association was not significant (% nondysplastic lesions showing TB stain: no history, 12%; history, 18%, \( P =1.000 \)). These data are different from the observations with FV (see section: FV status in premalignant lesions, Table 3-4), where FVL was present in 65% of nondysplastic lesions with a dysplasia history compared to only 24% of nondysplastic lesions without dysplasia history (\( P = 0.037 \)).

<table>
<thead>
<tr>
<th>Adjunctive device (^1)</th>
<th>No. of lesions (%) (^2)</th>
<th>Nondys (%) (^2, 3)</th>
<th>LGDys (%) (^2, 3)</th>
<th>HGDys (%) (^2, 3)</th>
<th>SCC (%) (^2)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluidine blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>94 (50%)</td>
<td>29 (85%)</td>
<td>43 (65%)</td>
<td>18 (35%)</td>
<td>4 (11%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>93 (50%)</td>
<td>5 (15%)</td>
<td>23 (35%)</td>
<td>33 (65%)</td>
<td>32 (89%)</td>
<td></td>
</tr>
<tr>
<td>Fluorescence visualization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVR</td>
<td>39 (21%)</td>
<td>19 (56%)</td>
<td>17 (26%)</td>
<td>1 (2%)</td>
<td>2 (6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FVL</td>
<td>148 (79%)</td>
<td>15 (44%)</td>
<td>49 (74%)</td>
<td>50 (98%)</td>
<td>34 (94%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) FV data was restricted to the 187 lesions with TB data available.  
\(^2\) % of lesions with indicated feature by histological diagnosis  
\(^3\) Nondysplasia = Nondys, low grade dysplasia = LGDys, high grade dysplasia = HGDys
Figure 3-2. Proportion of TB+ and FVL per histological diagnosis.

A graph showing proportion of adjunctive device results (TB+, FVL) and increasing histological diagnosis. The blue line points are the proportion of lesions staining TB positive per diagnosis; the green points are the proportion of lesions with a loss of FV per diagnosis.

As a final analysis, we determined the proportion of lesions with different histological diagnoses that were negative for both visualization approaches (FVR/TB-), positive for both approaches (FVL/TB+) or positive for only one of the approaches (Table 3-10). Figure 3-3 presents these data graphically.

Neither visualization approach was effective in identifying 2 SCC lesions and 1 high-grade dysplasia, suggesting that not all high-risk lesions are detected with these approaches. However, in comparison with TB staining, FVL did detect 2 SCCs and 17 (33%) high-grade dysplasia that were TB negative. These data suggest that FV is more sensitive with respect to detection of histologically high-risk lesions.

The largest differences in detection occurred among the nondysplasia and low-grade dysplasia. Sixteen (24%) low-grade lesions were negative for both approaches, compared with 18 (53%) of nondysplasia lesions. Twenty-two (33%) low-grade lesions were positive for both approaches, compared with 4 (12%) of nondysplastic lesions. Twenty-seven (41%) of low-grade dysplasia were positive only for FV (showed FVL), compared with 11 (32%) of nondysplasia.

A final interesting observation is that if a lesion is TB+, it will also be FVL. Only 2 of the 93 TB+ lesions were FVR, one nondysplastic lesion and one low-grade dysplasia.
Table 3-10. TB and FV combinations according to histology

<table>
<thead>
<tr>
<th>Adjunctive device</th>
<th>No. of lesions (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Nondys (%)&lt;sup&gt;1,2&lt;/sup&gt;</th>
<th>LGDys (%)&lt;sup&gt;1,2&lt;/sup&gt;</th>
<th>HGDys (%)&lt;sup&gt;1,2&lt;/sup&gt;</th>
<th>SCC (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both negative (FVR/TB-)</td>
<td>37 (20%)</td>
<td>18 (53%)</td>
<td>16 (24%)</td>
<td>1 (2%)</td>
<td>2 (6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TB+ only (FVR)</td>
<td>2 (1%)</td>
<td>1 (3%)</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>FVL only (TB-)</td>
<td>57 (31%)</td>
<td>11 (32%)</td>
<td>27 (41%)</td>
<td>17 (33%)</td>
<td>2 (6%)</td>
<td></td>
</tr>
<tr>
<td>Both positive (FVL/TB+)</td>
<td>91 (49%)</td>
<td>4 (12%)</td>
<td>22 (33%)</td>
<td>33 (65%)</td>
<td>32 (89%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> % of histological diagnosis with indicated feature(s)

<sup>2</sup> Nondysplasia = Nondys, low grade dysplasia = LGDys, high grade dysplasia = HGDys

Table 3-11. TB and FV combinations within nondysplastic lesions

<table>
<thead>
<tr>
<th>Adjunctive device</th>
<th>No. of lesions (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Nondys - no hx of dys (%)&lt;sup&gt;2,3,4&lt;/sup&gt;</th>
<th>Nondys - hx of dys (%)&lt;sup&gt;2,3,4&lt;/sup&gt;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both negative (FVR/TB-)</td>
<td>18 (53%)</td>
<td>12 (67%/71%)</td>
<td>6 (33%/35%)</td>
<td></td>
</tr>
<tr>
<td>TB+ only (FVR)</td>
<td>1 (3%)</td>
<td>1 (100%/6%)</td>
<td>0 (0%/0%)</td>
<td>0.099</td>
</tr>
<tr>
<td>FVL only (TB-)</td>
<td>11 (32%)</td>
<td>3 (27%/18%)</td>
<td>8 (73%/47%)</td>
<td></td>
</tr>
<tr>
<td>Both positive (FVL/TB+)</td>
<td>4 (12%)</td>
<td>1 (25%/6%)</td>
<td>3 (75%/18%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>2</sup> % of lesions with indicated feature

<sup>3</sup> Nondysplasia = Nondys; history of dysplasia = hx of dys

<sup>4</sup> % of lesions with indicated feature with history (or not) of dysplasia

<sup>4</sup> % of histological diagnosis that displayed indicated feature
Figure 3-3. Association of FV and TB status with histology.

1 Nondysplasia = Nondys, low grade dysplasia = LGDys, high grade dysplasia = HGDys

The proportion of each combination of adjunctive test per histological diagnosis. The green bars represent lesions which retained FV and stained TB-; the yellow bars represent lesions which showed a loss of FV but were TB-; red bars represent lesions which showed a loss of FV and stained TB +. FVR/TB+ is not shown in this graph it was rare, occurring in only 1% of the lesions studied.

Association of FV status with progression of lesions with little or no dysplasia

A decisive assessment of the significance of these associations of FV and TB with premalignant lesions lies in a determination of how well each predicts progression to cancer. Since high-grade dysplasias are treated with surgery in BC, association with outcome is not available. However, low-grade and nondysplastic lesions are followed over time in the longitudinal study, although average follow-up time is still short (32 ± 21 months).

To date, seven (7%) lesions have progressed to a high grade lesion or SCC, all from the low grade dysplasia group. None of the nondysplastic lesions has progressed. Each of these progressing lesions came from a separate individual (Table 3-12).

There was a significant association between the presence of FVL in a lesion and progression (P = 0.047). All progressing lesions showed FVL. Eleven percent of such lesions have progressed to date, within an average of 14 months.
Figure 3-4 is a plot of time-to-progression versus FV status. Since there was no progression for FVR lesions, it was not possible to determine an OR for this comparison.

Table 3-12. FV status and progression of low-grade and non-dysplastic lesions.

<table>
<thead>
<tr>
<th>Non and low grade dysplasia patients (N=100)</th>
<th>No. of lesions (%)</th>
<th>FVR (%) 1</th>
<th>FVL (%) 1</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression to HGDys 2 or SCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonprogressing</td>
<td>93 (93%)</td>
<td>36 (100%)</td>
<td>57 (89%)</td>
<td>0.047</td>
</tr>
<tr>
<td>Progressing</td>
<td>7 (7%)</td>
<td>0</td>
<td>7 (11%)</td>
<td></td>
</tr>
<tr>
<td>Follow-up time (months ± SD)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nonprogressing</td>
<td>32 ± 21</td>
<td>40 ± 22</td>
<td>31 ± 19</td>
<td></td>
</tr>
<tr>
<td>Progressing</td>
<td>14 ± 9</td>
<td>0</td>
<td>14 ± 9</td>
<td></td>
</tr>
</tbody>
</table>

1 % of FVR (or FVL) lesions that displayed indicated feature
2 High grade dysplasia = HGDys
Figure 3-4. FV and progression to a high-grade lesion

Probability of developing a high-grade dysplasia or SCC from a low-grade or nondysplastic lesion, according to fluorescence visualization status pattern for 100 lesions (36 FV retention; 64 FV loss).

All but one of the progressing lesions had TB staining. Six of 28 TB (21%) TB+ lesions have progressed to date, compared with 1 of 72 (1%) TB negative lesions (Table 3-13). Time-to-progression was significantly decreased for TB+ lesions when compared with TB- lesions (P <0.001, Figure 3-5). The hazard ratio based on the Cox regression for progression was 19.36 (95% CI 2.21 – 169.7).
Table 3-13. TB status and progression of low grade and non-dysplastic lesions.

<table>
<thead>
<tr>
<th>Non and low grade dysplasia patients (N=100)</th>
<th>No. of lesions (%)$^1$</th>
<th>TB- (%)$^1$</th>
<th>TB+ (%)$^1$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression to HGDys$^2$ or SCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonprogressing</td>
<td>93 (93%)</td>
<td>71 (99%)</td>
<td>22 (79%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Progressing</td>
<td>7 (7%)</td>
<td>1 (1%)</td>
<td>6 (21%)</td>
<td></td>
</tr>
<tr>
<td>Follow-up time (months ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonprogressing</td>
<td>34 ± 21</td>
<td>36 ± 21</td>
<td>29 ± 21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressing</td>
<td>14 ± 9</td>
<td>13</td>
<td>13 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ % of TB+ (TB-) lesions that displayed indicated feature

$^2$ High grade dysplasia = HGDys
Figure 3-5. TB and progression to a high-grade lesion

Probability of developing a high-grade dysplasia or SCC from a low-grade or nondysplastic lesion, according to toluidine blue staining pattern for 100 lesions (28 toluidine blue positive; 72 toluidine blue negative).

Finally, of interest, all 7 of the progressing lesions had 2 or more clinical features of risk \( (P = 0.043) \) and 5 of 7 (71%) had a loss at 9p in comparison with 32 of 76 (42%) non-progressing lesions \( (P = 0.233) \).
3.4 Discussion

Our results support the value of direct FV for clinical examination of oral lesions within a high-risk referral centre, providing the first evidence of an association of loss of FV with high-risk clinical features, histological progression and molecular change. The data show that FVL is significantly associated with high-risk clinical features: a nonhomogeneous appearance, location at a high-risk site and the presence of more than 1 clinical risk factor. FVL is also strongly associated with histological diagnosis, with percentage of lesions with FVL increasing in frequency with progression from nondysplasia to low-grade dysplasia, high-grade dysplasia and SCC. Finally, FVL lesions show a higher frequency of LOH at all 7 arms studied at loci at which loss had been previously associated with early disease and histological progression [23-28], although this elevation in LOH was significant only at 3p, 9p and 17p. FVL lesions are also more likely to have loss at 3 or more arms that FVR lesions (48% FVL group vs. 20% FVR group, P = 0.006) suggesting a higher level of genetic instability in the tissue.

<table>
<thead>
<tr>
<th>Table 3-14. Summary of clinical risk factors and histology.</th>
</tr>
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<tbody>
<tr>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Nondysplasia</td>
</tr>
<tr>
<td>No history of dysplasia</td>
</tr>
<tr>
<td>History of dysplasia</td>
</tr>
<tr>
<td>Low grade dysplasia</td>
</tr>
<tr>
<td>High grade dysplasia</td>
</tr>
<tr>
<td>SCC</td>
</tr>
</tbody>
</table>

The study also looked at the association of FV status with presence and degree of dysplasia. Eighty-nine of 92 (97%) high-grade dysplasias and SCCs were identified as having FVL: 27 of 28 severe dysplasia, 24 of 24 CIS, and 38 of 40 SCC. Thus FV detected a greater proportion of such lesions than any of the other clinical risk indicator examined including conventional
clinical features and TB staining (Table 3-14). The ability to detect high-grade dysplasia is critical to disease management, given the strong association of such lesions with risk of progression [28]. In British Columbia, we have found that 56% of lesions with severe dysplasia/CIS will progress to cancer in 3 years if left untreated and 70% in 5 years, leading to a recommendation in British Columbia that all such lesions be treated at diagnosis to prevent further development into invasive SCC (unpublished data). Thus the finding that FV will identify such change is encouraging.

We also looked at FV status among those lesions that had little or no dysplasia. Such lesions are more problematic and difficult to manage, since only a small proportion of such lesions will progress to cancer. A smaller proportion of low-grade dysplasia compared to high-grade dysplasia showed FVL: such change was present in only 74% of such lesions: 82% of moderate dysplasia and 67% of mild dysplasia. A seemingly high proportion of nondysplastic lesions (44%) also showed FVL; however, further examination of these samples showed that among this sub-group, half of the lesions had a prior history of dysplasia at the same site. This lesion history was associated with an increased likelihood for FVL: 65% of such lesions showed FVL compared to only 24% of nondysplastic lesions without dysplasia history (P = 0.037).

We looked at associations of other features with FV status among nondysplastic lesions and low-grade dysplasia. FV status did not significantly associate with any of the clinical features in these groups. Nor did FV status associate with LOH, although there was a trend towards an increase in LOH in the low-grade sub-group, with loss at 3p and any loss approaching significance (3p LOH: FVL, 44%; FVR, 18%, P = 0.072; any loss: FVL, 87%; FVR, 65%, P = 0.076). These data suggest the potential for an association of FV with genomic instability even among these histologically low-risk lesions and that a combination of molecular change and FVL could signal higher progression risk. However, further genetic analysis of FVL lesions is required to explore such a possibility and to determine how frequently FVL and genomic change are correlated in nondysplastic or low-grade dysplasia. Whether FV status will be an independent predictor of progression for such lesions is yet to be determined and this assessment will be dependent upon the further follow-up of the lesions in this study. However, early data are promising. To date, 7 of 57 (11%) lesions showing FVL have undergone progression compared with none of the 36 FVR lesions (P = 0.047).
This study is the first report in which FV and TB assessments were directly compared for ability to detect a wide range of histologies. Like FV, TB staining was also significantly associated with histological progression (P<0.001); however, FVL was a more frequent occurrence, present in 80% of lesions compared with 50% for TB staining. This association held across all histological groups, present in SCC, high-grade and low-grade dysplasia and among nondysplastic lesions (see Figure 3-2). FV detected 2 SCCs and 17 high-grade dysplasias that were negative for TB, suggesting that it had greater sensitivity for histologically high-risk lesions. However, FV also identified more of the low-grade and nondysplastic lesions as positive for FV loss. Twenty-seven (41%) of 66 low-grade dysplasia and 11 (32%) of 34 nondysplasia showed FVL. This would suggest an increased sensitivity for detection of low-grade dysplasia, but at the expense of decreased specificity, i.e., an increased detection of nondysplastic lesions. Of interest, 17 of 34 nondysplasia lesions had a history of dysplasia (had repeated biopsies of same site over time); among these, 8 (73%) were positive only for FV. This compares to 3 of 17 (27%) nondysplasia lesions that had no history of dysplasia, again only positive for FVL, not TB (P = 0.099). Although not statistically significant, these data suggest an intriguing possibility that in patients in high-risk clinics, FV may track some aspect of clinical change associated with a dysplastic history.

We also looked at concordance of the two approaches. Our data suggest that if a lesion is TB+, it will also be FVL. Only 2 of the 93 TB+ lesions were FVR, one nondysplastic lesion and one low-grade dysplasia. This is an interesting observation that could be clinically relevant, especially among lesions that have little or no dysplasia, by providing a method of cross checking FV positivity. Also 53% of the nondysplastic lesions were negative for both approaches. If such lesions showed no future progression, this would provide yet another way of confirming low-risk for these troublesome lesions. Finally, only 12% of such lesions were positive for both FV and TB status. Larger sample sizes and repeated analysis in other institutions are required to determine whether such a combination has an added potential for progression. Of interest, like FV, the association of TB stain with progression of these lesions was also positive (Figure 3-5, Table 3-13) in this small sample set, with all but 1 of the progressing cases positive for both TB and FV status.

The concept of integrating visualization approaches that target different underlying phenomena associated with cancer risk makes biological sense. FV relies on alterations to tissue morphology and biochemistry that affect the way in which specific wavelengths of light interact...
with tissue [14-16, 36, 37]. Naturally occurring fluorophores in the epithelium and stroma are excited when these wavelengths are absorbed, re-emitting light of a different wavelength. Among biochemical changes associated with alteration to fluorescence during cancer development is the reduction in autofluorescence from collagen cross-links, possibly due to the breakdown of the extracellular matrix. This change has been hypothesized to be due to collagen remodelling associated with alterations to matrix metalloproteinases (MMP) expression in host stromal cells as well as stromal remodelling associated with angiogenesis [38, 39]. Alterations to metabolic activity with dysplasia are accompanied by a change to flavin adenine dinucleotide (FAD), an important component of the electron transport chain that excite at these wavelengths in epithelial cells. In addition to these changes in fluorophores, alterations to epithelium thickness and nuclear morphology, and angiogenesis also play a role, by scattering of the excitation and emission light.

In contrast, TB is a metachromatic vital stain that targets nucleic acids. Hence, retention of the dye by the epithelium is most likely associated with an increase in concentration of nucleic acids in neoplastic and premalignant tissue. There is also some speculation that changes to tissue morphology during carcinogenesis may play a role, with defects in cellular intercellular barriers increasing penetration between cells and permitting stain in deeper layers of the epithelium [40, 41].

Thus, the two approaches appear to mark different, yet related tissue change. Combining analyses may be especially important to improving the definition clinically of low-grade and nondysplastic lesions of greater concern. Whether this combination will reduce false positives in such lesions is yet to be determined.

In summary, FV was found to be a very useful adjunctive tool when used by experienced clinicians in high-risk clinics for the determination of histologically high-grade oral premalignant lesions and cancers. An understanding of the value of using FV assessment in low-grade and nondysplastic lesions will require larger samples sets, more follow-up time and input from investigators at other sites. The presence of FVL in some nondysplastic inflammatory or benign lesions emphasizes the important fact that FV does not replace clinical knowledge but aids it as an adjunctive tool. The high-risk population used in the current study, however, is not an appropriate population for the study of FVL prevalence in inflammatory or nondysplastic lesions since such lesions could still represent premalignancies in this patient population. Presently, we
are conducting a study in community general practices where confounding due to reactive and inflammatory lesions is higher and where such lesions are more likely to be low-risk. Taken as a whole, the data are promising and support further study into how to best use visualization devices in high-risk and community settings.
3.5 References


Chapter 3 Appendix 1

**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?
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:

<table>
<thead>
<tr>
<th></th>
<th>At home?</th>
<th>At work?</th>
<th>In public places?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>No</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

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

How often are you regularly exposed to smoke of others:

<table>
<thead>
<tr>
<th></th>
<th>Less than once a month</th>
<th>More than once a month but less than once a week</th>
<th>At least once a week</th>
<th>Daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>At home?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>At work?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>In public places?</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Before Age 20 years</th>
<th>In your 20's</th>
<th>In your 30's</th>
<th>In your 40's</th>
<th>In your 50's</th>
<th>60’s &amp; older</th>
</tr>
</thead>
</table>
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 Specify site: ______
- □ Brothers/sisters Specify site: ______
- □ Daughters/sons Specify site: ______
- □ Grandparents Specify site: ______
- □ Aunts/uncles related by birth not marriage Specify site: ______

20070820
LOCATION OF LESION AND SAMPLES:

Patient Name: ____________________________ (Surname) ____________________________ (First Name) ____________________________ Date: ____________________________

EXAMINER’S SIGNATURE ____________________________ Date: ____________________________

PRINCIPAL INVESTIGATOR’S SIGNATURE ____________________________ Date: ____________________________

EACH GRID BLOCK REPRESENTS 10mm x 10mm

INDICATE LESION LOCATION
CHAPTER 4 PROJECT III: EXPERIENCES FROM THE DENTAL OFFICE: INITIATING ORAL CANCER SCREENING

Laronde DM, Botorff JL, Hislop TG, Poh CY, Currie B, Williams PM, Rosin MP


“You tend to forget it because you’re focusing on the crowns and bridges and fillings and implants and stuff like that, and you kind of leave all that sort of education behind.”

A vital component of the BC Oral Cancer Prevention Program is to promote dialogue among community dental professionals about their experiences with oral cancer screening. To begin this effort in British Columbia, we conducted two focus groups in February 2007 involving 12 dental professionals from well-established dental offices in greater Vancouver. The staff in these offices had participated in a 1-day workshop in September 2006. This workshop on oral cancer screening included a review of risk factors, procedures for conducting screening examinations and a hands-on clinical session with volunteer patients with high-grade dysplasia or squamous cell carcinoma. The purpose of the focus groups was to learn about their experiences with screening following this workshop. In this paper we will share the views of these dental professionals about integrating screening into their practices, summarize their concerns and solutions, and establish what is needed to support this effort.

There is too little time?

Some dental professionals suggested that time may be a reason for their peers not performing oral cancer screening examinations. One dentist commented that most dental offices are very busy and the thought of adding oral cancer screening to the existing workload would deter some from integrating screening into their practice. In addition to the time
needed to complete the examination, some of the dental professionals in our focus groups found that extra time was needed to explain the process to patients. One dental hygienist who recently became involved in screening explained:

“*You’re asking questions they’ve [the patient] not heard before, which raises questions from them, and so you’re trying to stay focused but you have to answer their concerns.*”

The dental professionals in our focus groups found ways to integrate screening into their practices that were not time-consuming. They developed printed information sheets for patients, involved dental hygienists, and developed short simple responses to common questions. When asked what advice they would give to other dental offices considering the introduction of screening, one dentist remarked:

“I would say it [oral cancer screening] doesn’t really take very long. If you do it a whole bunch you get really fast at it and you learn a bit more about what’s normal, and you might not always know what something is….but you’ll know if it’s something that you don’t see all the time.”

**What should I say to my patients?**

Another reason commonly given for not screening is that dental professionals are unsure how to talk to their patients about oral cancer and why they are screening for it. As one person explained, “*It was very helpful to detect at a very early state of the cancer but it’s hard when we need to explain to the patient what we need to do, and why*”. However, the dental professionals in the focus groups had some tips for their peers with this concern. Some suggested developing “*a script*” to follow or having literature available chairside - “*something simple*” for the patient to read while waiting. It was suggested by a dentist that an information sheet should be available in multiple languages and contain factual information: A dental hygienist suggested that “*It should include some statistics …comparing oral cancer to other cancers, because people are learning more about it and so this helps put it into perspective.*”

There was consensus about the need to keep explanations to patients simple. One dentist stated: “*I spent way too much time going into what I was doing and I probably shouldn’t have, so it maybe would have been better to maybe have a little script.*” Another dentist developed a script after the workshop that had the positive result of patients asking questions and opening up opportunities to discuss oral cancer that weren’t present previously:
“The message I got is that half of this battle is patient education and awareness, and that’s part of what your campaign is about. I went home and I put a one-page script together about what we were doing and why we were doing it, how we were involved and what the statistics were. Patients read that, they did the form out before they came in and then we did the normal screening. But half the time we were doing these screenings and they don’t know why we are doing it, they just think we’re playing football with their mouth. So this then raised their awareness and started them asking questions about oral cancer that they didn’t before. So to me, that gave me an opportunity to have dialogue, which is quite different than me telling you what’s the problem versus you asking me what the problem is and giving me permission to tell you.”

How will my patients respond?

Another concern often raised as a barrier to screening is how patients will respond to the new behaviour. Experiences shared by the focus group participants suggest that patients were curious and sometimes surprised. One dental assistant commented, “Like most people wondered, ‘Why have this screening, right? I have nothing.’” A dentist who practiced close to the Vancouver Downtown Eastside, an area renown for high-risk behaviour, provided the following explanation:

“It’s just astonishing how many new patients will say, oh, nobody ever did that to me before, and sometimes I look into who was the last dentist and it was one of my classmates, and I think what the hell, we all learned the same thing about what you’re supposed to do, and what’s thorough and what isn’t.”

The consensus from the dental professionals was that patient responses to screening were overall very positive. Patients often said, “Oh this is great, you know, I’m really glad that you’ve done this.” Other unexpected positive outcomes were that the dental office and staff were viewed by patients as progressive, and that the introduction of screening tended to “open up” discussions about friends or previous histories regarding oral cancer that would not have otherwise taken place.

Is oral cancer screening my responsibility?

The role of dental practitioners as oral cancer screeners was also discussed. One dentist considered the lack of oral cancer screening awareness among dental professionals may be related to the fact they are “not medical doctors” and usually do not deal with issues like cancer. The lack of clear financial incentives was also mentioned as a potential barrier. Two different approaches were suggested to encourage dental professionals to get involved in oral cancer
screening. The first related to increasing public awareness to create a demand for oral screening:

“Because they [the public] don’t know about it, and we haven’t told them about it, so there’s no expectation, but if the public comes in and say are you going to do an oral cancer check today, the guy is going to do it.”

The second approach related to appealing to dental professionals on an ethical level:

“I think we have to appeal to our practitioners on an ethical level more than anything else, because monetarily you’re not going to benefit a lot from it, but from an ethical standpoint, I mean what’s our first mandate, it’s public health.”

The need for continuing education?

Many of the dental professionals stated that involvement by the regulatory bodies, making oral cancer screening courses mandatory as part of continuing education, may be necessary to guarantee the dental professionals’ role in oral cancer screening. One participant advocated: “So make it a requirement through the college. I mean if you really want to educate the practitioners you’ve got them there, and there’s no ands, ifs or buts.” The focus group participants also discussed the importance of regular educational opportunities to support this change in practice. One dentist likened it to the need for CPR training:

“Well, I mean, why, if you educate the public and the public comes to demand and accept that in a dental office, that’s one way, but how do you get to the dentist. I mean, why don’t you consider going to the college level? We have continuing education that is due every three years, make it in one of those days every three years that you have to do an oral cancer, one of our education days, seven hours, has to be oral cancer. Once every three years. I mean, we have to do CPR and how often do we have to do CPR?”

Many of the dental professionals in our focus groups offered suggestions for future continuing oral cancer screening education. Participants were interested in learning more information on the biopsy procedure, referral pathways and guidelines for screening. They suggested that a clinical session be included involving patients with other mucosal conditions and variations of normal tissue. Suggestions on how to integrate oral cancer screening into the busy dental practices were also thought to be beneficial in supporting dental professionals who were interested in offering oral cancer screening. Educating all office staff, not just the dentists
and hygienists, was thought to be important. They suggested this could be facilitated through courses offered at conventions, study clubs or in-office sessions.

In summary

The dental professionals in our focus groups were successful in integrating oral screening into their practices in ways that addressed commonly sited barriers. Their experiences provide helpful advice for others who want to introduce oral screening. The importance and enthusiasm for this practice is perhaps most clearly captured in this dentist’s remarks:

“I mean, we’re talking ethical things here, the general health of patients, our business, people…. If I do my little quick check I’ll probably find it and it might make a big difference, so – yeah, for that one person.”

Acknowledgements:

We would like to acknowledge other members of the BC OCPP team (Heather Biggar, Samson Ng, Lewei Zhang, Eunice Rousseau, Anita Fang) the dental practitioners and the patient volunteers who participated in this project. Funding was provided by the BC Cancer Foundation. Ms. Laronde is supported by a Michael Smith Foundation for Health Research/BC Cancer Foundation Senior Trainee Award.
CHAPTER 5 PROJECT IV: DECISION-MAKING ON DETECTION AND TRIAGE OF ORAL MUCOSA LESIONS IN COMMUNITY DENTAL PRACTICES

5.1 Introduction

Globally, 274,000 new cases of oral cancer are detected each year with 127,000 deaths (IARC, GLOBALCAN, 2002) [1]. There has been only a modest improvement in survival over the last few decades, despite advances in therapy. A primary reason for this poor prognosis is late diagnosis: data from SEER analysis has shown that when the disease is detected early (stage I), the 5-year survival is 83%, in contrast survival for late-stage disease (Stage 4) is only 28% [2]. Unfortunately, the majority of cases continue to be identified late worldwide. Proportions vary by geographic region; in the United States, 67% of oral cancers are diagnosed after the disease has metastasized regionally or distantly [2] while in India more than 75% of oral cancers are diagnosed at a late stage [3].

Excitement about the potential impact of screening on the disease outcome has been fueled by results of the first oral cancer screening randomized controlled trial (RCT). This large-scaled randomized study of 168,000 individuals in India, reported an association of screening by community health workers with increased early stage diagnosis and decreased morbidity and mortality [4]. Prevalence of early stage disease in the screening arm was 72.3% compared with 12.5% in the control group. Most importantly, there was a 32% reduction in mortality among those receiving screening with data significant for high-risk patients (users of tobacco or alcohol).

These data have led to a renewed debate in the literature about the effectiveness of oral cancer screening in community settings (see recent reviews [5-9]) as well as the most appropriate venue in which to provide such service. Downer et al. [5] have recently published results of a meta-analysis of eight prospective studies in which the performance of the conventional oral exam was compared between screeners who were general dentists (4 studies) or trained health workers (remaining 4) and, as a “soft” gold standard, the oral specialist. A
range of values were observed for sensitivity (59 to 97%) and specificity (75 to 99%) with overall
sensitivity and specificity from the meta-analysis being 85% and 97% respectively. These data
compare favourably to other screening programs during their early development [10,
11]. However, given the low prevalence of this disease in developed countries, opportunistic
screening in general practitioner clinics is recommended (the Cochrane Review [12], the
American Cancer Society [13], the Surgeon General of the US [14], the Canadian Task Force on
the Periodic Health Examination [15] and the UK working group on screening for oral cancer and
precancer [16]) as part of regular clinic activity and may provide the most cost-effective
approach [17]. Dental practices are promising venues. Within the United States, 69% of
Americans [18] over the age of 18 visit a dental office annually. Similar data are reported for
Canada and more specifically, for British Columbia, where 65% over the age of 12 attend a
dental office (dentist, dental hygienist or orthodontist) at least yearly [19]. Dental practitioners
already self report as conducting oral exams: in a study of US dentists, 81% reported screening
all their patients over the age of 40 at the first appointment and 78% screened this group at
recall appointments [20], whereas in Canada, these percentages are 71% and 51% respectively
[21]. To date, however, efforts to standardize such behaviour and its integration into day-to-day
practice have been limited.

As a first step in this direction in British Columbia, the College of Dental Surgeons of
British Columbia and the British Columbia Oral Cancer Prevention Program released a set of
guidelines that were released along with protocols in March 2008 [22]. These guidelines
recommended a systematic approach to the evaluation of the head and neck and oral region
that included a methodical gathering of background information and a step-by-step clinical
examination. This methodical process was felt to be important given the many mucosal
conditions that have similar appearance with “quick checks” providing insufficient information
that could result in misdiagnosis.

The present study had two objectives. The first and primary objective was to evaluate
this step-by-step process in 15 community dental practices. The study involved a training
workshop for participants on screening protocols with hands-on demonstration, followed by
collection of data on screening activity in their practices for the next 11 months. We sought to
validate a decision tree based on the screening protocol for ability to facilitate detection of
mucosal change, to determine how well it assisted practitioners in differentiation of high- from low-risk lesions as well as in decisions for appropriate and timely follow-up.

A secondary objective was to begin the process of establishing a framework for evaluating adjunct tools in community settings. The growing advent of adjunct tools that are targeted at facilitating the conventional oral examination has been a further driving force for change in screening activity in community practices. Such devices are diverse in approach, including the use of toluidine blue, brush cytology, reflectance visualization and more recently autofluorescence imaging with others in development. To date, there has been little validation of these tools in community settings, with most data on usage of the devices coming from high-risk referral clinic settings with experienced personnel.

In this study, we introduced one such tool: a hand held device, used to assess alterations to tissue fluorescence. Loss of tissue autofluorescence has been associated with the identification of cancer and premalignant lesions at several sites, including the lung, cervix and oral cavity. While FV has been found to be associated with histological progression in the oral cavity [23], occult lesions [24] and enhanced surgical margins [25], these studies have all occurred within the high-risk referral clinic settings with experienced personnel. We introduced fluorescence visualization (FV) to these practitioners and asked them to use this technology as a final adjunct step to clinical evaluation of patients, after the completion of the conventional examination. Our goal was to determine how these practitioners used FV as they worked through the step-by-step procedure. The intent was to use information collected in this study to lay the initial framework for transfer of this and other new technology into community dental practices for evaluation of efficacy, ensuring that such a transfer would be integrated into the conventional oral examination.
5.2 Materials and Methods

Study participants

This study was reviewed and approved by the British Columbia Cancer Agency Research Ethics Board and the Simon Fraser Research Ethics Board. Dental practices participating in this study were chosen from those responding to a notice in a local dental association publication that described the study and requested volunteers. Each responding dentist was contacted by telephone (DL) and was given a more in-depth description of the project and its timelines. Among responders, eligibility was restricted to practitioners from established practices in the Greater Vancouver area that agreed to follow the study protocol and that were available on the day of the workshop. A total of 18 dentists participated from 15 offices (2 offices had 2 dentists participate) with each dentist signing informed consent.

The project included 3 components: 1) a one-day workshop to orient participants to the study and train them to study protocols; 2) subsequent follow-up of screening activities in each dental office, with facilitation and referral forward to dysplasia clinics for patients requiring further assessment; and 3) a final evening meeting that brought participants together to both present study results and gain input on their experiences with screening during the follow-up period. The latter information was collected through focus group discussions. This chapter will summarize key points that came up within these focus groups on study protocol and barriers and facilitators to screening; however, a full description of this focus group will be presented in a later publication.

Description of Workshop

The purpose of the workshop was to assess and improve participants’ knowledge of oral cancer, to calibrate study participants on oral cancer screening behaviour and to review study protocol. The workshop agenda is attached in Chapter 5 Appendix 1. Participants were encouraged to bring a staff member or associate to facilitate the later integration of the study protocol into their dental practices. Thirty people attended the workshop: 18 dentists, 8 registered dental hygienists (RDH) and 4 certified dental assistants (CDA).

We first used a short quiz, to assess the participating clinician’s knowledge of oral cancer risk factors (Chapter 5 Appendix 2), and a self-administered questionnaire, to collect
demographics of the clinician and characterize current screening activities (Chapter 5 Appendix 3). Seventeen of the 18 dentists completed the quiz and questionnaire. This quiz and questionnaire were largely based on earlier surveys of oral health professionals by Yellowitz et al, 2000 [26], and Horowitz et al, 2000 [20], in order to allow for a later comparison of our data to this larger sample set. Information collected from each study participant included: age, gender, years of practice, the number of hours worked per week and knowledge of oral cancer risk factors. Participants were also asked if they felt adequately trained in how to provide an oral cancer screening (both intraoral and extraoral), and on how to counsel on tobacco or alcohol cessation. An additional query was on interest in and frequency of attendance at continuing education courses (not restricted to oral cancer screening) and whether they currently participated in a dental study club. Final questions addressed characteristics of each clinician’s dental practice: type (general or specialty practice), support staff (e.g., whether they employed RDHs), the estimated number of patients seen per week, and estimated number of oral cancer exams among new and recall patients per week.

The second part of the workshop included presentation of a short review of oral cancer statistics, etiological factors, clinical risk factors and oral histopathology. An introduction to fluorescence visualization (FV) was given as an example of a new adjunct procedure to oral cancer screening. This was followed by presentation of the step-by-step protocol to be used in this study for clinical assessment of patients, including extraoral and intraoral examination (see following section for details). The latter has been published in the Journal of the Canadian Dental Association (see Williams et al, 2008 [27]; also http://www.orcanet.ca/) and follows steps outlined in the published BC Oral Cancer Screening Guidelines (2007). Finally, the referral pathway for suspicious lesions and follow-up procedures to be used in this study was detailed.

The workshop concluded with a hands-on clinical session where each participant watched and then performed an oral cancer screening exam under both white light and FV conditions. Patients with active disease were available for the participants to screen. During this part of the workshop, ideas were presented on different approaches that could be used to talk to patients about screening and a demonstration was given on how to fill out study forms on patients to be screened within their daily practices (see Chapter 5 Appendix 4 for forms).
Assessment of oral cancer screening activities during follow-up of dental practices

After completion of the workshop participants were asked to screen all adult patients 21 years of age and older for a period of 11 months. A study facilitator (DL) made regular visits to each dental practice to do quality control on data acquisition and to address any questions on study protocol that came up during the follow-up period. During the duration of the study the participating dentists were also contacted regularly via email. Patient screening forms were collected on an approximately 2 month interval. Each patient screened was given a unique identifier in the form of a study identification number to ensure patient confidentiality. Linkage of name to code only became necessary when the patient was referred to the follow-up clinic at which point they were accrued to a research protocol prior to biopsy.

Figure 5-1 gives an overview of the step-by-step protocol recommended to participating dental clinics for clinical examination of all patients. Dentists were asked to complete the screening at time of new patient or recall (i.e., 6 month check-up – hygiene appointment) as this would facilitate integration into regular care. The protocol included:

Step 1. Patient History. This step involved collection of patient demographics (gender, age), personal and family history of oral cancer, and oral habits (tobacco use and alcohol consumption). This information was recorded for each patient on the study form (Chapter 5 Appendix 4).

Step 2. Visual Screening Examination. This step included both extraoral and intraoral examinations. The extraoral examination included inspection and palpation of the head and neck region looking for asymmetry and swelling or tenderness. Study participants were asked to refer to a medical doctor any patients with fixed, firm or unexplained lymph nodes or asymmetries. An intraoral exam under incandescent (white light) conditions was completed next. If an anomaly was present, participants were asked to document the site, colour, texture and appearance of the lesion by checking off the appropriate boxes on the screening form and drawing the anomaly’s location on an oral cavity diagram. Colour options were white, red, brown and other. Texture options were smooth, rough, nodular, ulcerated or other. Lesion appearance was defined as “homogeneous” when it was uniform in color and appearance, white with a smooth, thin or slightly fissured texture. “Nonhomogeneous” lesions included those with a rough or speckled surface and/or red or white and red in colour (Williams et al,
2008). We requested that participants not include on the form amalgam tattoos, Fordyce’s granules, vascularities and pigmentation due to skin colour.

Step 3. Direct FV. All patients were then screened using an FV imaging device and FV status was recorded on the screening form. The FV examination followed the same methodical examination of all oral mucosa tissue as the conventional exam, this time done under reduced room lighting and with a handheld autofluorescence imaging device, marketed as the Velscope™, (LED Dental, Inc., White Rock, British Columbia, Canada). This device uses a blue/violet light (400 – 460 nm wavelengths) to illuminate oral tissue, with long-pass and notch filters to allow clinicians to directly view fluorescence. Lesions that retained the normal green autofluorescence under FV were classified as having FV retained (FVR). Tissue that showed a reduction in the normal pale green, appearing as dark patches were categorized as having FV loss (FVL). In cases where the clinician was unsure of FV loss, lesions were categorized as FV equivocal (FVE).

Step 4. Lesion Assessment. Participants then assessed the risk of an anomaly. Low risk lesions included obvious trauma, aphthous lesions, melanotic macules, candidiasis (including benign migratory glossitis) and geographic tongue. Anomalies without apparent cause, non-healing ulcers, red or white patches and lichenoid lesions were considered high-risk lesions. It should be noted that these lichenoid lesions were later re-classified as intermediate risk lesions for the purpose of data analysis. This reclassification was done because lichenoid lesions have a variation in clinical presentation from faint white striae to red and erosive and some of these lesions have increased cancer risk. Lesions in this latter group would require further follow-up for clinical management. Participants were further asked to document sites which appeared clinically normal but had a loss of FV (FVL).

Lesion follow-up

Patients with low-risk lesions that were not obviously due to trauma or another benign condition were asked to return for reassessment in 3 weeks, allowing time for benign reactive or inflammatory lesions to resolve, thus minimizing unnecessary referrals and biopsy. If the lesion was still present the dental practice was requested to notify the study’s community facilitator (DL, a dental hygienist) who reassessed the patient’s lesion within the dental office. The community facilitator referred any suspicious lesions to the Oral Mucosal Disease (OMD) clinic
at Vancouver General Hospital (VGH). In some cases the dental offices directly referred patients to this clinic. High-risk lesions still present at 3 weeks were either reassessed by the community facilitator (if the dentist was unsure) or referred directly by the dentist to the OMD clinic. Oral medicine specialists at OMD determined if a biopsy or further follow-up was warranted.
**Statistical analysis**

Descriptive statistic analysis (mean, median and range) was used to describe data on knowledge and baseline screening behaviour of dentists collected at the initial workshop. Patient screening forms were imaged and then uploaded directly into a Microsoft Excel study database using Teleform (version 10.1, 2006, Vista, California). Data analysis was performed with SPSS software, version 16.0 for Windows, 2007 (SPSS Inc., Chicago, Illinois).
5.3 Results

Description of dental practitioners and practices at study entry

Tables 5-1 – 5-4 summarize data obtained from 17 of the 18 clinicians participating in this study during the initial workshop via self-reported questionnaires (1 questionnaire and quiz not completed). Participating dentists were on average 47 years of age (34 – 62) and 59% were male. They had been practicing dentistry for a mean of 21 years (4 – 39). All participants worked in a general type practice not a specialty. Three quarters of the dentists employed at least one registered dental hygienist. This cohort of dentists worked an average of 35 hours per week (23 – 50) and reported seeing an average of 69 patients per week (20 – 125). They estimated that the average number of new patient and recall dental exams (check-ups) completed each week was 5 (2 - 10) and 30 (4 – 80), respectively. Eleven (65%) of the participants were members of a study club. All but 2 of the respondents claimed to attend CE sessions at least bimonthly.
Table 5-1. Demographics of dental practitioners and practice information

<table>
<thead>
<tr>
<th></th>
<th>Dentists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>Median (range)</td>
<td>50 (34 - 62)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 (41%)</td>
</tr>
<tr>
<td>Male</td>
<td>10 (59%)</td>
</tr>
<tr>
<td>Years of practice</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>21 ± 9</td>
</tr>
<tr>
<td>Median (range)</td>
<td>18 (4 - 39)</td>
</tr>
<tr>
<td>Employs RDH(^1) (N=16)</td>
<td>12 (75%)</td>
</tr>
<tr>
<td>Hours per week worked</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>Median (range)</td>
<td>32 (23 - 50)</td>
</tr>
<tr>
<td>Patients per week</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>69 ± 32</td>
</tr>
<tr>
<td>Median (range)</td>
<td>78 (20 -125)</td>
</tr>
<tr>
<td>Exams</td>
<td></td>
</tr>
<tr>
<td>New patient</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Median (range)</td>
<td>4 (2 - 10)</td>
</tr>
<tr>
<td>Recall</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>30 ± 19</td>
</tr>
<tr>
<td>Median (range)</td>
<td>33 (5 - 80)</td>
</tr>
<tr>
<td>Study club</td>
<td></td>
</tr>
<tr>
<td>Member</td>
<td>11 (65%)</td>
</tr>
<tr>
<td>Not a member</td>
<td>6 (35%)</td>
</tr>
</tbody>
</table>

\(^1\) RDH – Registered Dental Hygienist

Participants also self-reported their current screening behaviour via questionnaire (adapted from Horowitz et al, 2000 [20]). Table 5-2 provides an overview of the extraoral results. On new patients, 13 of 17 respondents stated they performed an extraoral exam on 50% or more of their patients 40 years and younger (mean = 70%) and 14 of 17 performed extraoral exam on 50% or more of their patients 40 years and older (mean=73%). Recall patients received fewer extraoral exams. Nine dentists claimed that 50% or more patients
under the age of 40 and 10 of 17 performed extraoral exams on 50% or more patients 40 and older received at recall (mean = 42% and 50%, respectively). In addition, only 6 of 17 dentists (65%) reported documenting extraoral exams in the dental chart on a regular basis.

As seen in table 5-3, intraoral exams on new patients were more frequently completed than extraoral exams. For new patient intraoral exams all but 1 dentist stated that their office performed an intraoral exam on 50% or more of new patients not yet 40 and all claimed to perform this exam on new patients 40 and older more than 50% of the time. The majority reported performing intraoral exams on 100% of their new patients (12 of 17 under 40, 13 of 17 when 40 and older). Similar results were reported for intraoral exams performed at recall appointments. Only 1 dentist did not examine 50% or more of patients under 40 and all examined 50% or more of patients over 40. Nine claimed 100% of recall patients receive intraoral exams regardless of age. Eleven of the 17 (65%) dentists claimed to regularly document intraoral exams in the dental chart. One participant commented that they only entered the

<table>
<thead>
<tr>
<th>Extraoral exam (EO)</th>
<th>Reported screening practices Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New patient</td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>70 ± 38 (0 - 100)</td>
</tr>
<tr>
<td>≥40 years</td>
<td>73 ± 34 (0 - 100)</td>
</tr>
<tr>
<td>Recall</td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>42 ± 41 (0 - 100)</td>
</tr>
<tr>
<td>≥40 years</td>
<td>51 ± 37 (0 - 100)</td>
</tr>
<tr>
<td>Document extraoral exam in the chart</td>
<td>N (%)</td>
</tr>
<tr>
<td>Do not document EO regularly</td>
<td>6 (35%)</td>
</tr>
<tr>
<td>Document EO regularly</td>
<td>11 (65%)</td>
</tr>
</tbody>
</table>
exam results if there was an abnormal result. Participants stated that 94% (60 – 100%) of their edentulous patients were being screened for oral cancer.

<table>
<thead>
<tr>
<th>Table 5-3.</th>
<th>Self-reported intraoral screening practices at study entry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraoral exam (IO)</strong></td>
<td><strong>Reported screening practices</strong></td>
</tr>
<tr>
<td></td>
<td><strong>% Mean ± SD (% range)</strong></td>
</tr>
<tr>
<td>New patient</td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>89 ± 26 (25 - 100)</td>
</tr>
<tr>
<td>≥40 years</td>
<td>93 ± 16 (50 - 100)</td>
</tr>
<tr>
<td>Recall</td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td>85 ± 26 (0 - 100)</td>
</tr>
<tr>
<td>≥40 years</td>
<td>95 ± 13 (50 - 100)</td>
</tr>
<tr>
<td>Edentulous patients</td>
<td>94 ± 11 (60 - 100)</td>
</tr>
<tr>
<td><strong>Document intraoral exam in the chart</strong></td>
<td><strong>N (%)</strong></td>
</tr>
<tr>
<td>Do not document IO regularly</td>
<td>6 (35%)</td>
</tr>
<tr>
<td>Document IO regularly</td>
<td>11 (65%)</td>
</tr>
</tbody>
</table>

An important aspect of screening for oral cancer and OPLs is assessing risk habit information. We next asked the dentists what risk habit (tobacco and alcohol) information they collect in their practices on a regular basis (Table 5-4). All the participants (100%) reported collecting information regarding the current use of tobacco (current smoker) amongst their patients, 88% collected information about past tobacco use (former smoker). Only 53% reported collecting information on the type of tobacco used (cigarette, cigar, and pipe) and how long the patient had smoked.

Very few of the practices collected alcohol information. Only 3 (18%) participants reported asking patients about current alcohol use (current drinker) and 2 (12%) asked about alcohol use in the past (former drinker). None of the offices collected information about the type of alcohol consumed or how long the patient had been consuming alcohol.
Table 5-4. Self-reported collection of habit and cancer history at study entry

<table>
<thead>
<tr>
<th>Risk habit information</th>
<th>No. collected information (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco</td>
<td></td>
</tr>
<tr>
<td>Present use</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Past use (N=16)</td>
<td>14 (88%)</td>
</tr>
<tr>
<td>Amount and duration</td>
<td>9 (53%)</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
</tr>
<tr>
<td>Present use</td>
<td>3 (18%)</td>
</tr>
<tr>
<td>Past use</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Amount and duration</td>
<td>0</td>
</tr>
<tr>
<td>History of oral cancer</td>
<td></td>
</tr>
<tr>
<td>Personal</td>
<td>16 (94%)</td>
</tr>
<tr>
<td>Within family</td>
<td>13 (76%)</td>
</tr>
</tbody>
</table>

The assessment of the knowledge base of participants on oral cancer risk factors used a short quiz, modified from Yellowitz et al, 2000 [26] (Chapter 5 Appendix 2). At study entry, all of the study dentists identified that leukoplakia is a white patch, that early detection of oral cancer is the most significant factor in the long-term survival of oral cancer, and that the proper order of histological progression is dysplasia, CIS, SCC. In regards to awareness of the most common type of oral cancer, 88% correctly identified SCC. Eighty-two percent correctly identified the dorsal surface of the tongue as the least common site for oral cancer of the options provided. In assessing the clinical risk of an oral lesion, 76% of participants correctly identified red lesions as having a higher risk of cancer than white lesions, nonhomogeneous lesions having a greater risk than homogeneous lesions and that the lateral tongue was a higher risk site than the buccal gingival. Oral cancer is diagnosed in approximately 420 British Columbians annually (CCS, 2009) [28] and 35% were able to identify the range containing that number (301 – 500). Only 18% were able to identify that palpation of lymph nodes, looking for asymmetries and examining the skin of the lips and face were parts of the extraoral exam (of the options provided) and 12% correctly answered that the ventral tongue is the most common site for an SCC in a patient with no known risk factors.
Figure 5-2. Percentage of dentists who responded correctly to questions about oral cancer screening. (Based on Yellowitz et al, 2000) [26]

Figure 5-3. Percentage of dentists who responded affirmatively to indicated factors as being associated with risk for oral cancer. Older age, alcohol, tobacco and a prior history of oral cancer are known risk factors for older cancer (green bars). Obesity, spicy foods, hot foods and beverages, poor oral hygiene and poorly fitting dentures have not been found to be risk factors for oral cancer (red bars). (Based on Yellowitz et al, 2000 [26])
Dentists were also questioned on their personal view on the adequacy of their training on different aspects of the oral cancer screening exam (adapted from Horowitz et al, 2000 [20]). When asked about intraoral exams, 71% of the dentists agreed or strongly agreed that they had received adequate training. Palpating lymph nodes is a major aspect of an extraoral exam and 77% of the dentists agreed that they had received adequate training in this skill. However, few of the dentists felt adequately prepared to offer tobacco cessation counselling (53%) and none felt they had received adequate alcohol cessation training. Still 53% felt that their oral cancer education while in dental school was good or very good. Only 41% felt that oral cancer screening exams were similarly weighted to other clinical activities during their training.
Table 5-5. View of clinicians on adequacy of training for different activities

<table>
<thead>
<tr>
<th>Adequacy of training</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cancer screening</td>
<td></td>
</tr>
<tr>
<td>Strongly agree or agree</td>
<td>12 (71%)</td>
</tr>
<tr>
<td>Strongly disagree or disagree</td>
<td>5 (29%)</td>
</tr>
<tr>
<td>Palpating lymph nodes</td>
<td></td>
</tr>
<tr>
<td>Strongly agree or agree</td>
<td>13 (77%)</td>
</tr>
<tr>
<td>Strongly disagree or disagree</td>
<td>4 (24%)</td>
</tr>
<tr>
<td>Tobacco cessation</td>
<td></td>
</tr>
<tr>
<td>Strongly agree or agree</td>
<td>3 (18%)</td>
</tr>
<tr>
<td>Strongly disagree or disagree</td>
<td>12 (71%)</td>
</tr>
<tr>
<td>Don't know</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Alcohol cessation (N=16)</td>
<td></td>
</tr>
<tr>
<td>Strongly agree or agree</td>
<td>0</td>
</tr>
<tr>
<td>Strongly disagree or disagree</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>Rate oral cancer screening training</td>
<td></td>
</tr>
<tr>
<td>Very good or good</td>
<td>9 (53%)</td>
</tr>
<tr>
<td>Very poor or poor</td>
<td>6 (35%)</td>
</tr>
<tr>
<td>Not sure/don't recall</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Did oral cancer screening examinations similarly weighted to other clinical exam procedures?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (41%)</td>
</tr>
<tr>
<td>No</td>
<td>7 (41%)</td>
</tr>
<tr>
<td>Not sure/don't recall</td>
<td>3 (18%)</td>
</tr>
</tbody>
</table>

Screening activity of dental practices during the study

The total number of screening exams completed in each of the 15 dental offices during the course of this study is shown in Figure 5-4. A total of 2631 exams were completed over the
study duration; however, of these 48 had incomplete or no documentation of clinical data, 33 patients were under 21 years of age (not eligible for the study) and 8 patients had a history of oral cancer (0.3%). These exams were removed from the sample set resulting in 2542 screening exams for further analysis.

Overall, the average number of exams per office was 169 ± 98, with a wide variation in screening activity between clinics (range: 17 – 367). In the following sections, data from each of the steps of the screening protocol will be presented.

![Figure 5-4](image.png)

Figure 5-4. The total number of patients screened per office against the average patients screened per office (blue horizontal line).

**Step 1. Patient History**

The first step of the oral cancer screening exam involved collection of patient history including a medical history, patient demographics and a review of known risk habits. This data was entered on the study screening form (Chapter 5 Appendix 4). Of the 2542 patients assessed, data on these features was 95-99% complete. These data showed that the study population had slightly more females than males (58% female) (Table 5-6) with an average age of 52 ± 15 years (21 – 95 years). Of these, 79% of patients were 40 years of age and older, considered to be a key demographic to screen according to the BC Guideline for early detection of oral cancer (2008). Three percent had a family history of oral cancer. Less than half (40%)
had a history of smoking; 27% of these smokers (11% of all patients) were still smoking at the
time of the exam. Among smokers, most individuals smoke or smoked cigarettes with only 6%
and 4% with a history of smoking cigars or pipes, respectively. In regards to the duration of the
tobacco habit, 37% have smoked less than 10 years, 33% smoked 11-20 years and 30% have
smoked more 21 or more years. Seventy percent of former smokers had quit 10 or more years
prior to their screening exam. Only a small amount of patients had a history of chewing tobacco
(2%).

Almost 60% of patients had a history of drinking more than 2 drinks per week for one
year or more. Wine was the alcohol drunk by most patients who consumed alcohol (84%)
followed by beer (61%) and then spirits (51%). Only a small percent (1-2%) in each category
consumed more than 21 drinks per week. Half of the patients who drank alcohol had done so
for 21 or more years.
### Table 5-6. Demographics and risk habit information for all patients

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Total respondents</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>2542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>2528 (99%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>1069 (42%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>1459 (58%)</td>
<td></td>
</tr>
<tr>
<td>Age at screening (years)</td>
<td></td>
<td>2432 (96%)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>52 ± 15</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>21-95</td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td></td>
<td>2432 (96%)</td>
<td>531 (21%)</td>
</tr>
<tr>
<td>≥40</td>
<td></td>
<td>1901 (78%)</td>
<td></td>
</tr>
<tr>
<td>Family history of oral cancer</td>
<td></td>
<td>2477 (97%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>2411 (97%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>66 (3%)</td>
<td></td>
</tr>
<tr>
<td>Tobacco</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever Smoker</td>
<td>2477 (97%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>1475 (60%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>1002 (40%)</td>
<td></td>
</tr>
<tr>
<td>Smoking category</td>
<td>2477 (97%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td></td>
<td>1475 (60%)</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td></td>
<td>269 (11%)</td>
<td></td>
</tr>
<tr>
<td>FS</td>
<td></td>
<td>733 (30%)</td>
<td></td>
</tr>
<tr>
<td>Amount (packs) (N=1002)</td>
<td>886 (88%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1/2</td>
<td></td>
<td>389 (44%)</td>
<td></td>
</tr>
<tr>
<td>1/2-1</td>
<td></td>
<td>313 (35%)</td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td></td>
<td>184 (21%)</td>
<td></td>
</tr>
<tr>
<td>Duration (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>933 (93%)</td>
<td>343 (37%)</td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td></td>
<td>309 (33%)</td>
<td></td>
</tr>
<tr>
<td>≥21</td>
<td></td>
<td>281 (30%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever drinker</td>
<td>2488 (98%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinker</td>
<td></td>
<td>1019 (41%)</td>
<td></td>
</tr>
<tr>
<td>Ever drinker</td>
<td></td>
<td>1469 (59%)</td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>Total respondents</td>
<td>No. (%)</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>Amount (drinks/week) (N=1469)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>889 (61%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14/wk</td>
<td>844 (95%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-20/wk</td>
<td>26 (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥21/wk</td>
<td>19 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>1231 (84%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14/wk</td>
<td>1185 (96%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-20/wk</td>
<td>31 (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥21/wk</td>
<td>15 (1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirits</td>
<td>750 (51%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14/wk</td>
<td>721 (96%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-20/wk</td>
<td>21 (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥21/wk</td>
<td>8 (1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (N=1469) (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1350 (92%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>299 (22%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td>383 (28%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥21</td>
<td>668 (50%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Step 2. Visual screening: Extraoral and intraoral examination**

Of the 2542 screenings completed, 2354 (93%) had an extraoral exam. Of those, 134 (6%) patients had palpable lymph nodes, 2 of which were referred to their medical doctor. Also noted was a patient with an enlarged thyroid awaiting thyroid surgery and a patient with a history of chronic lymphocytic leukemia. Comments for positive lymph nodes included recent illness, infection, mobile and soft nodes, and long-term history with medical follow-up, the remaining 77 had no explanation for positive nodes.

Intraoral examination of the 2542 patients resulted in the identification of 389 lesions, each in a separate patient, representing the presence of an anomaly in 15% of cases. Figure 5-5 is a graphic display of the flow of patients through the critical steps of this risk assessment strategy.
Of the 389 lesions, 350 (90%) were classified by the clinicians as low risk and only 39 (10%) were high risk. Nineteen of the high risk lesions were lichenoid and reclassified as intermediate risk (5%) at analysis, leaving 20 (5%) high risk lesions. Low risk lesions were further broken down into 246 (70%) lesions with trauma and nonspecific ulcer, 34 (14%) with geographic tongue and 70 (28%) with ‘candidiasis and other’ (melanotic macule, amalgam tattoo, scar, fistula, nevi, papilloma, pigmentation and mucocele).

Table 5-7.  Screening exams performed and lesions identified for each practice.

<table>
<thead>
<tr>
<th>Office</th>
<th>Total screened</th>
<th>Total No. of lesions</th>
<th>Low risk</th>
<th>Intermediate risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>All Low risk</td>
<td>Trauma and nonspecific ulcer</td>
<td>Geographic tongue</td>
</tr>
<tr>
<td>1</td>
<td>155 (6%)</td>
<td>19 (12%)</td>
<td>13 (68%)</td>
<td>7 (50%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>2</td>
<td>186 (7%)</td>
<td>14 (4%)</td>
<td>12 (86%)</td>
<td>10 (83%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>3</td>
<td>97 (4%)</td>
<td>22 (6%)</td>
<td>18 (82%)</td>
<td>10 (56%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>4</td>
<td>240 (9%)</td>
<td>60 (15%)</td>
<td>52 (87%)</td>
<td>46 (88%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>5</td>
<td>17 (1%)</td>
<td>4 (1%)</td>
<td>4 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>6</td>
<td>212 (8%)</td>
<td>57 (15%)</td>
<td>56 (98%)</td>
<td>39 (70%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>7</td>
<td>47 (2%)</td>
<td>14 (4%)</td>
<td>11 (79%)</td>
<td>6 (55%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>8</td>
<td>105 (4%)</td>
<td>3 (1%)</td>
<td>3 (100%)</td>
<td>2 (67%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>9</td>
<td>137 (5%)</td>
<td>26 (7%)</td>
<td>26 (100%)</td>
<td>21 (81%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>10</td>
<td>54 (2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>11</td>
<td>243 (10%)</td>
<td>23 (6%)</td>
<td>19(83%)</td>
<td>16 (84%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>12</td>
<td>168 (7%)</td>
<td>38 (10%)</td>
<td>35 (92%)</td>
<td>27 (77%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>13</td>
<td>367 (14%)</td>
<td>64 (16%)</td>
<td>62 (97%)</td>
<td>46 (74%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>14</td>
<td>314 (12%)</td>
<td>40 (10%)</td>
<td>35 (88%)</td>
<td>9 (26%)</td>
<td>14 (40%)</td>
</tr>
<tr>
<td>15</td>
<td>199 (8%)</td>
<td>5 (1%)</td>
<td>4 (80%)</td>
<td>3 (75%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>All</td>
<td>2542 (100%)</td>
<td>389 (15%)</td>
<td>350 (90%)</td>
<td>246 (70%)</td>
<td>34 (14%)</td>
</tr>
</tbody>
</table>

The clinicians further categorized these lesions as to need for follow-up. Of the 350 low risk lesions, the dental offices did not feel the need to reassess 214 (61%) lesions; this decision was based on knowledge of cause for the lesion, for example, trauma such as burns, linea alba and other cheek biting, aphthous ulcers, herpetic lesions, denture sores, pigmentation,
mucoceles, flossing trauma, varicosities, fistulas, candidiasis and geographic tongue. One hundred and thirty six (39%) individuals were asked to return in 3 weeks time to reassess the lesion site. All but 12 complied with this request (91%). The 3 week reassessment further eliminated another 88 lesions which had resolved within that time period leaving 36 lesions to be evaluated by the community facilitator or referred directly to the Oral Mucosal Disease Program (OMDP) at VGH for assessment by oral medicine specialists. Four of these 36 lesions were felt to require further assessment: 3 were biopsied and one is in follow-up. Of these biopsies, 1 low-grade dysplasia, 1 melanotic macule and 1 focal mucositis with melanin incontentia were identified. It should be noted that the latter referral and biopsy of the low risk patient with melanin incontentia was done at the request of the patient out of concern over a family history of oral cancer and not due to a concern by the clinician of the presence of cancer risk.

Of 19 lesions with lichenoid characteristics, re-classified as intermediate risk at analysis, 12 were asked to return for reassessment in 3 weeks. All 12 returned and 9 (75%) were rescheduled for review by the facilitator or at OMDP. Three of these lesions required further assessment resulting in 1 biopsy (lichenoid mucositis). The 2 patients who did not undergo biopsy are in follow-up and being monitored at OMDP.

Eighteen (90%) of the 20 lesions dentists felt were at high risk for cancer were asked to return for a 3 week reassessment and 17 (94%) complied. Nine of the lesions were still present at the 3 week visit and these patients were seen by the facilitator or referred directly to OMDP. Four (44%) patients were felt to require further follow-up and 2 have since been biopsied resulting in 2 low-grade dysplasias.
Figure 5-5. The triage of all patients with lesions from detection through to biopsy. Green columns are lesions that were categorized as low risk, yellow intermediate risk and red are the high risk lesions.

FV status of low-risk lesions

We also collected information on FV status for lesions identified within the study. Within the low risk group, 125 (51%) of the trauma and nonspecific ulcers (aphthous, herpetic) had a loss of FV (FVL), 17 (7%) were FV equivalent (FVE) and 81 (33%) retained FV (FVR).

Geographic tongue was found to be FVL in 14 (41%) of cases, FVR in 20 (59%) and none were determined to be FVE. In the candidiasis and other category, 36 (51%) were FVL, 25 (36%) were FVR and 7 (10%) were FVE. FV status was missing for the remaining 25 (7%) low-risk lesions,
including 23 trauma and 2 cases of candidiasis. Three of the lesions categorized as trauma with no FV data were reviewed by the facilitator and one showed a loss of FV at review and referred forward. This lesion was biopsied and found to be mild dysplasia.

Figure 5-6. Association of FV status with clinical diagnosis in lesions categorized as low-risk. Trauma and nonspecific ulcers are in orange, geographic tongue in purple and candidiasis and other are in pale yellow.

Figure 5-7 displays the triage information for these low risk data, shown by clinical diagnosis and according to FV status. It is important to note that these low risk lesions (trauma, geographic tongue, candidiasis, aphthous ulcer herpetic ulcer, etc) are known confounders of FV assessment and clinicians had been trained to exclude such seemingly positive findings on the basis of their white light assessment. However, if there was any level of uncertainty the clinician was instructed to reassess the patient in 3 weeks. Within the FVL subset, 93 of the 175 total lesions were asked to return and 86 (92%) complied. At that time, 18 (21%) of 86 ‘low risk’ lesions that originally showed FVL remained FVL, 2 (2%) were FVE and the rest were FVR. With respect to low risk lesions that were originally FVR (24), 12 were asked to return with 10 (83%) of the patients complying. None of these lesions were recorded as FVE or FVL at the 3 week reassessment. With respect to low risk lesions originally showing FVR, only 19 (15%) of the 126 low risk FVR lesions were asked to return in 3 weeks and they all complied. Two lesions had a change in FV status and were FVL or FVE. Overall, the call back of patients at 3 weeks was critical, resulting in a significant reduction of FVL lesions by 92% in trauma and nonspecific ulcers, by 86% in geographic tongues and by 72% among candidiasis and others.
Figure 5-7. The triage flow of low risk lesions through assessment, reassessment at 3 weeks, referral and biopsy, if any. a) Trauma and nonspecific ulcers; b) Geographic tongue; c) Candidiasis. There were 25 lesions with no FV data available (23 trauma and 2 candidiasis), 12 of 13 complied with request to reassess. Three were seen by the facilitator, of which one was referred and biopsied (low-grade dysplasia). One was referred directly and not judged high risk.
Nine of 20 (45%) high risk lesions and 8 of 19 (42%) intermediate risk lesions were FVL at initial assessment with 7 (37%) of intermediate risk and 6 (30%) of high risk lesions showing FVR (Figure 5-8). The remaining lesions in each of the intermediate and high risk groups were scored FVE.

Within the intermediate category, 6 of 8 (75%) of the FVL lesion patients were asked to return, all complied, and 67% maintained FVL status. Four of these lesions were seen by the facilitator who referred to OMD. One patient was referred to OMDP directly. None of these lesions were judged significant by the clinic specialists (no biopsies). The intermediate risk lesion that was FVE at initial viewing was reassessed and judged FVR, but still referred to OMDP but not biopsied. Of the 3 intermediate risk patients initially judged as FVR, all 3 were asked to return for reassessment and all complied. One lesion had changed from FVR to FVL. Of these 3 lesions, 2 lesions were seen by the facilitator and referred to OMDP, one was biopsied resulting in a diagnosis of lichenoid mucositis. One patient was referred directly to OMDP with no resulting biopsy. There were 3 lesions categorized as intermediate risk whose initial FV status was not available. Two patients were asked to return and both complied and were FVR at the 3 week reassessment. Neither of these patients were seen by the facilitator or referred to OMDP directly.

Of the high risk lesions all 9 patients with FVL complied with the request for 3-week reassessment. At 3 weeks, 6 lesions remained FVL and 1 was FVE. One lesion was seen by the facilitator and referred forward but not biopsied (still in follow-up). Three lesions were referred directly to OMDP, 2 were biopsied and both were low-grade dysplasias. The single FVE lesion was seen by the facilitator but not referred forward. Four of 6 (67%) FVR lesions were asked to return and all complied. The facilitator was called to see 1 lesion which was referred to OMDP but no biopsy was performed.
Figure 5-8. The triage flow from intermediate and high risk lesions identified through reassessment, referral and biopsy, if any. a) Intermediate risk; b) High risk. Three intermediate lesions and 4 high-risk lesions had no FV data. Two of 2 lesions complied with a 3 week reassessment and none were seen by the facilitator or referred to OMD. Three of 4 cases high-risk cases complied with a 3 week reassessment, 3 were referred directly to OMD and none were biopsied.
There were 25 cases which were FVL with no visible clinical lesion, 9 were asked to return and 8 complied, 1 was referred directly to OMDP and 3 were seen by the facilitator. None of the cases were considered high-risk. These sites were associated with subclinical linea alba, denture sore spots, scar tissue and vascularities. Twelve cases with no clinical lesions were judged FVE, 5 were asked to return in 3-weeks and all complied. All were FVR at reassessment and 1 was reviewed with the facilitator.

In summary, overall, 389 lesions were noted, 350 low risk, 19 intermediate risk and 20 high risk. Six biopsies were performed resulting in 3 low-grade dysplasias (1in ~850), 1 lichenoid mucositis, 1 melanotic macule and 1 focal mucositis with melanin incontentia.
5.4 Discussion

This paper represents one of the first studies to explore the process by which dental practitioners make decisions on clinical anomalies. Participants were presented with a fixed protocol and a decision tree and followed over time to evaluate its utility. Frequent contact was maintained with the study group through visits of a community facilitator. Referral forward to oral specialists was monitored and the results of any ensuing biopsies were collected. Biopsy decisions were made by specialists at the referral centre. The study resulted in the identification and evaluation of key decision points as well as an indication of where the process breaks down and possible ways in which it can be improved. The end is the collection of data that will provide a framework for conventional screening activity in the dental community.

The participants were all general dentists, working in urban or suburban practices, with a wide range in both practice experience (4 – 39 years) and patient load. Overall, an average of 169 exams were performed per office, with from 17 to 367 exams per clinic, suggesting a significant variation in the amount of screening activity in the 15 offices.

One of the key questions addressed in the study analysis was whether the step-by-step protocol facilitated the screening process. We examined the flow of patients through the protocol for the 2542 patients evaluated in this study, to determine how well decisions were made at each step with respect to collection of critical information and detection, risk classification and triage of lesions.

Medical and risk factor history for the 2542 screened in this study was 95 - 99% complete. The study population had slightly more females than males (58% female) with primarily middle-aged individuals (79% were 40 years of age and older). Risk due to lifestyle was fairly low, with 40% reporting a history of smoking; of these smokers, only 27% were still smoking at the time of exam (11% of study group).

This smoking prevalence was similar to that reported for the general British Columbian population (15% ever smokers, 14% aged 45 years and older) (2008 Canadian Tobacco Use Monitoring Survey (CTUMS) [29].) Consumption for the average smokers is less than a pack a day (14 cigarettes/day) [29], again similar to average tobacco use in this study group, where 44% of smokers reported less than ½ pack per day and an additional 30%, ½ to 1 pack per day.
Alcohol intake was also fairly low, with only 40% of individuals consuming alcohol; of these, only 2% drinking would be categorized as high risk (21 or more drinks of alcohol per week), using intake categories that have been associated with oral cancer risk in Lim et al, 2003 [30].

Ninety-three percent of individuals had an extraoral exam. Of these, 134 (6%) had palpable lymph nodes; two were referred to their medical doctor, 59 had an associated illness or medical history. There was no explanation for the remaining 77 individuals. Extraoral examination remains a difficult procedure for the clinicians and further work is required to better guide this step.

Intraoral examination of these 2542 patients resulted in the identification of 389 lesions, which represents 15% of cases. This value was similar to a previous report in the UK of 18 dental practices where 2265 patients were examined with 14.1% showing an anomaly. Classification into different levels of risk represented a key decision point. Of interest, the vast majority of these cases (350, 90%) were classified by the dentists into the low-risk category. Of the 39 high-risk lesions, 19 lesions (0.75% of 2542) were lichenoid in appearance and these were classified at analysis into an intermediate risk group, leaving 20 high-risk lesions (0.79% of 2542).

A second key decision point involved identifying low-risk lesions with known cause. Generally, the dentists were able to rule out a large percentage of these lesions due to known causes of trauma such as burns, cheek biting, linea alba, aphthous ulcers and herpetic ulcers, denture sores, or common benign lesions, such as amalgam tattoos, of which they are familiar. Only 136 patients with low risk lesions (39%) were asked back for a 3-week reassessment by the dentist. In contrast, the majority of the patients with intermediate (12 of 19 cases, 63%) or high-risk lesions (18 of 20, 90%) were asked to return for reassessment.

The request for a 3-week reassessment was another critical point. All together the 3-week reassessment eliminated the apparent unnecessary referral of 99 of 166 (60%) lesions for assessment of lesions, as these lesions resolved (88 low-risk, 3 intermediate-risk, 8 high-risk). Of note, compliance to reassessment was fairly high with only 13 (8%) of 166 patients failing to return for the 3 week reassessment appointment.

A final key decision involved the request for assessment of lesions by the study facilitator. This confirmation saved the patient the time and anxiety of further unnecessary assessments and at the same time reduced by 73% (30 reduced to 8 lesions) the number of
patients referred to the OMDP clinic. Of the 8 facilitator-referred patients 3 were biopsied with 1 dysplasia, 2 lichenoid mucositis, 2 patients in follow-up and 1 patient with hyperplastic lymphoid tissue on the tonsil (patient requested referral). An added value of this community facilitation was that clinicians were able to witness the facilitator discussing referral issues with the patient. The discussion of referrals and biopsies with patients was raised as a difficulty (and a barrier to screening) by clinicians in a previous study [31].

Of interest, 8 of the practices chose to refer some patients (24) directly to the OMDP clinic or to a specialist’s office, without a pre-screen by the study facilitator. Four of these patients were not initially screened or their forms were not turned over to the researchers. Two refused an appointment at the referral clinic, 2 had irregular presentations of benign lesions (Fordyce’s granules and geographic tongue) and 3 had lichenoid lesions. Three patients required further diagnostic information by biopsy. This leaves unnecessary referral of 10 patients into the OMDP clinic or other specialist. These lesions were trauma (4), melanotic macule (2), vascularity, median rhomboid glossitis and 2 lesions which had resolved.

It is noted that with the decision tree used in the study, the decision to biopsy was made by a specialist in the referral clinic. Overall, the study resulted in the referral forward of 32 (8 from facilitator, 24 direct referral) patients to the clinic. Six of 30 cases who complied with referral were biopsied, with 3 dysplasia identified.

As a secondary objective, we began the process of laying a framework for transfer of new technology into community dental practices, ensuring that such a transfer would be integrated into the conventional oral examination. Practitioners introduced to FV during the workshop and supplied with a device for use in their practice. They were instructed to conduct an FV examination at the completion of each conventional white-light exam and to record observations made. As this was the first study to introduce FV technology into a community screening framework, our questions mainly focused on how these practitioners used FV as they worked through the step-by-step procedure.

The data show that within low risk lesions, a large proportion of FVL lesions (93%) were transient: the lesion no longer showed a loss of FV at the 3-week reassessment. This may be due to the removal of a chronic trauma source or healing of an acute episode of trauma or inflammation. Low-risk lesions with persisting FVL were more likely to be referred or reviewed
by the facilitator and be a potentially troublesome lesion that was referred forward for review to the OMDP clinic (10 of 18). Of interest a small number of low-risk lesions which retained FV were still presented to the facilitator implying that the clinicians’ did try to integrate their clinical judgement with FV status when making decisions on 3-week reassessment and referral forward for further assessment. Another interesting point was that all of the lesions that were initially FVE (i.e., 26 of 26, 100%) had a resolution of the FV loss at 3-week reassessment visit. FVE appears to be a label of uncertainty and reassessing the site may have made the FV status more definitive to the clinician. The use of such a category is not unusual given that this is new technology and that the learning curve for its use would be a gradual process. In contrast, very few FVR lesions were called back for a 3 week reassessment. The clinician may have regarded the FV status as confirmation of low risk. Interestingly, the FV status of intermediate and particularly high risk lesions did not change much at the 3 week reassessment appointment. This confirms the clinicians’ decision to categorize these lesions as intermediate and high risk. In regard to sites which were FVL but with no apparent clinical lesion (occult lesions), since this study was a learning opportunity on how to use FV there was not enough certitude that it was being used correctly to properly evaluate such lesions. Of the cases in which such lesions were seen by the facilitator or at OMDP, all were considered low risk. A conventional visual examination only is based on subjective analysis, interestingly, based on the higher percentage of FVL lesions moving forward, the addition of FV appeared to factor into the OHPs decision to re-evaluate and refer lesions yet not overshadow their clinical judgement.

Among lessons learned in this study was the importance of studying how community dentists make decisions on oral lesions that they encounter in their daily practice in order to better facilitate education processes that are needed to guide screening activity into high quality, standardized exams. It is important not to rely on didactic training processes to disseminate such information, e.g., lectures or written media. A hands-on experience in the presence of a calibrated trainer is critical. For example, this is best reflected in the low compliance with extraorals exams self reported at study entry among the study dentists and is consistent with the literature on this issue [20, 21]. Furthermore, this hands-on support needs to be reinforced as dentists begin regular screening. Study participants noted that the provision of a facilitator in this study helped reduce uncertainty as they integrated protocol into their clinics, especially for those lesions that are low-risk. Over time, they were more confident in
their decision-making and more aware of confounders and common benign and reactive lesions. In addition, a check by a facilitator reinforced protocol.

We identified several components of the education process that appeared to facilitate change and to build confidence. Reinforcing the use of a methodical, consistent approach for each patient is critical, with an emphasis that short-cuts can create misdiagnosis. The rationale and importance of each step needs to be emphasized. This is true not just for the physical exam but also for the medical history; for example, although 17 dentists in this study reported collecting current tobacco usage from their patients, only 53% enquired as to amount and duration of tobacco smoked. Alcohol usage is infrequently collected (only 3 of 17 reported even enquiring as to current alcohol usage), despite the growing concern in the literature with respect to oral cancer risk [32-34]. Finally, documentation of habit is poor. This difficulty has been noted previously in earlier studies [20, 21] but little has changed in actual practice. The lack of documentation of this data and of oral cancer screening itself is also a concern [35-37]. Only 65% of the dentists’ state claim to document the completion of these exams regularly and some noted documenting only when abnormal findings are discovered.

In summary, the data presented in this paper supports the need for a well-defined decision trees to facilitate oral cancer screening programs as well as their evaluation prior to dissemination; guidelines can only be viewed as a starting point towards standardizing such activity. It also indicates the need for hands-on education processes by established and calibrated screeners to reinforce such activity in dental practices. Moving such activity into study group settings located within a community practice might be an attractive method for providing this service; such a venue and others should be explored. The provision of the results of such activity should be communicated back to the community to further guide the evolution of high quality screens. Key decision points that need strengthening are: the evaluation of the extraoral exam; classification of lesions by degree of concern (in this study, into low-, intermediate- and high-risk), and the importance of a re-assessment at 2-3 weeks. However, overall the end result of this study was positive: 3 dysplasias were identified in the 2542 patients screened (1 in ~ 850).

In conclusion, it is important to emphasize that the fine-tuning and tailoring of education strategies to the community is only a first step. Such activity most often results in a change to the management system for the disease, including the development of well defined
referral pathways, with relay of this information to the community, and the assurance of appropriate infrastructure and guidelines for the further assessment and treatment of cases identified in the screening process. Hence, the streamlined linkage of screening, referral and treatment is critical.
5.5 References


Chapter 5 Appendix 1

Oral Cancer Screening Initiative for Dentists: Workshop Agenda

Saturday session: 8:30 – 4:30
Didactic morning and clinical afternoon

8:30 – 9:00 Sign in and pre-test
Introduction (MR)
- Of educators and trainees (trainee experiences)
- The OrCa screening program
Goals of the program

9:00 – 9:45 Etiology and epidemiology of oral cancer (DL)

9:45 – 10:15 Clinical lesions and clinical risk factors (size, texture, appearance, etc) (MW)

10:15 – 10:30 Break

10:30 – 11:00 Visualization Techniques (SN)
History and development
Mechanism of action
Early results from BCCA

11:00 – 12:00 Histopathological risk factors of oral cancer (CP)
- What is oral cancer and oral precancer?
  - Squamous cell carcinoma
  - Squamous dysplasia
- Histopathological review (Gold standard)
- Progression risk associated with dysplasia

12:00 – 1:00 Lunch

1:00 – 1:30 Image review (SN)

1:30 – 2:15 Transfer to BCCA
Clinical demonstration and practice session Group A
Study Protocol (DL) Group B
Referral pathway
Dialogue with patients
Break

2:15 – 2:30 Transfer time Holiday Inn ↔ BCCA clinic

2:30 – 3:15 Clinical demonstration and practice session Group B
Study Protocol Group A
Break
Referral pathway
Dialogue with patients

3:15 – 3:30 Transfer to Holiday Inn clinic

3:30 – 4:00 Question period

4:00 – Hand out VELScopes and supplies
Chapter 5 Appendix 2

Oral Cancer Screening Initiative for Dentists: Knowledge Quiz

Form 2

1) Of the following which is the LEAST common site for the development of an oral squamous cell carcinoma?
   a) Soft palate
   b) Dorsal tongue
   c) Lateral tongue
   d) Ventral tongue
   e) Floor of the mouth

2) Which of the following indicates the probable development of oral squamous cell carcinoma?
   a) Carcinoma-in-situ → mucosal dysplasia → invasive squamous cell carcinoma.
   b) Mucosal dysplasia → carcinoma-in-situ → invasive squamous cell carcinoma.
   c) Invasive squamous cell carcinoma → carcinoma-in-situ → mucosal dysplasia.
   e) Carcinoma-in-situ → invasive squamous cell carcinoma → mucosal dysplasia.

3) "Leukoplakia" is synonymous with which of the following?
   a) Red patch
   b) White patch
   c) Hyperkeratosis
   d) Carcinoma-in-situ
   e) Mucosal dysplasia

4) For patients without known risk factors, which is the most common site for the occurrence of oral squamous cell carcinoma?
   a) Gingiva
   b) Ventral tongue
   c) Buccal mucosa
   d) Floor of mouth
   e) Soft palate and tonsillar pillars

5) What is most common type of oral cancer?
   a) Squamous cell carcinoma
   b) Adenoid cystic carcinoma
   c) Verrucous carcinoma
   d) Osteosarcoma
   e) Mucoepidermoid carcinoma
6) Which of the following statement is incorrect, in terms of the clinical risk assessment of leukoplakia or erythroplakia?

a) Erythroplakia has higher risk than leukoplakia because it often has higher degree of histological changes
b) Diffuse nonhomogenous lesion has higher risk than discrete homogenous lesion
c) Lesion at lateral tongue has higher risk than lesion at buccal gingival
d) All of the above are correct

7) Which following statement is correct?

a) All of the precancers can be seen by naked eyes if we examine the oral cavity carefully
b) If we can identify the oral lesion at risk as early as possible, it usually has a better prognosis and less morbidity

c) Toluidine blue is a visual aid that can be used to replace histology for the risk assessment

8) A comprehensive head and neck (extraoral) examination includes (circle all that apply):

a) Look for asymmetries
b) Palpate lymph nodes of the head and neck
c) Palpation of the masticatory musculature
d) Exam the skin of the lips and face

9) What is the most significant factor in the long-term survival of oral cancer?

a) Early detection
b) Location of lesion
c) Number of lesions
d) Tobacco cessation
e) Age at time of initial diagnosis

10) Approximately, how many British Columbians are diagnosed with oral cancer each year?

a) <100
b) 100 – 300
c) 301 – 500
d) 501 – 700
e) >700

11) Which of the following are considered etiological risk factors for oral cancer? (Please circle all that apply)

a. Tobacco   f. Hot foods and beverages
b. Alcohol   g. Poor oral hygiene
c. UV damage (lip) h. Older age
d. Obesity   i. Poor fitting dentures
e. Spicy foods j. Prior history of oral cancer
Chapter 5 Appendix 3

Oral Cancer Screening Initiative: Current Screening Practice

**Personal information:**

Age: _____
Gender: ____
How many years have you been in practice? ________
Type of practice: general dentistry or specialist (please specify speciality) ________
DMD □ | RDH □ | CDA □
DMDs: Do you employ a registered dental hygienist? ______
How many hours per week do you work? ______
Approximately how many patients do you see per week? _____
New patient exams_____
Recall exams ___
Standard exams _____
How often do you attend CE courses? ______
Do you belong to a study club? _____
What type of CE courses interest you?
____________________________________________________________________________________
____________________________________________________________________

**Oral Cancer Risk Factors**

_Do you collect the following information when completing a patient health history?_

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<td>Family history of cancer?</td>
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**Oral Cancer Screening**

Please provide your best estimate of the percentage of adult patients (≥19 years) for whom your office completes an oral cancer screening examination at the INITIAL (emergency or scheduled) and RECALL appointment.

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Do you record the exam in the treatment record?  Y □  N □  NA □.

(NA - do not perform oral cancer screening examinations.)

Please provide your best estimate of the percentage of adult patients (≥19 years) for whom your office provides a head and neck examination (extraoral) at their INITIAL (emergency or scheduled) and RECALL appointments.

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Do you record the exam in the treatment record?  Y □  N □  NA □.

(NA - do not perform oral cancer screening examinations.)

Please provide your best estimate of the percentage of your edentulous patients for whom you provide an oral cancer screening examination. ____%

**Opinions about Oral Cancer Education and Training**

Please indicate the extent to which you personally agree or disagree with each of the following statements (CHECK ONE RESPONSE PER LINE)

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In your opinion, did your dental/hygiene school treat oral cancer screening examinations similar to other procedures in terms of training and clinical requirements? (CHECK ONLY ONE)

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How would you rate your dental/hygiene school training regarding oral cancer screening examinations? (CHECK ONLY ONE)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>□</td>
<td>1. Very good</td>
</tr>
<tr>
<td>□</td>
<td>2. Good</td>
</tr>
<tr>
<td>□</td>
<td>3. Poor</td>
</tr>
<tr>
<td>□</td>
<td>4. Very poor</td>
</tr>
<tr>
<td>□</td>
<td>5. Not sure</td>
</tr>
</tbody>
</table>
Chapter 5 Appendix 4

Risk Assessment Form

Date of birth (YYYYMMDD):

Gender: ☐ Male  ☐ Female

Have you ever had cancer of the mouth: ☐ Yes  ☐ No
Do you have a family history of cancer of the mouth: ☐ Yes  ☐ No

Tobacco history (cigarettes, cigars, pipes):

Are you a smoker: ☐ Yes  ☐ No
Are you an ex-smoker: ☐ Yes  ☐ No

If yes to any of the above, complete the appropriate column(s):

<table>
<thead>
<tr>
<th>Cigarettes</th>
<th>Cigars</th>
<th>Pipes</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1/2 pack</td>
<td>&lt; 5</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>1/2 - 1 pack</td>
<td>5 - 10</td>
<td>3 - 6</td>
</tr>
<tr>
<td>&gt; 1 pack</td>
<td>&gt; 10</td>
<td>&gt; 6</td>
</tr>
</tbody>
</table>

For how many years did you smoke?

| <= 10 | <= 10 | <= 10 |
| 11 - 20 | 11 - 20 | 11 - 20 |
| => 21 | => 21 | => 21 |

Former smoker, how many years ago did you quit?

Do you or have you ever used chewing tobacco?

Yes  No

Alcohol history: Have you now or in the past consumed more than 2 drinks per week: ☐ Yes  ☐ No

If yes to any of the above, complete the appropriate column(s):

<table>
<thead>
<tr>
<th>Beer (6oz)</th>
<th>Wine (4oz)</th>
<th>Spirits (1oz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>1 - 14</td>
<td>1 - 14</td>
<td>1 - 14</td>
</tr>
<tr>
<td>15 - 20</td>
<td>15 - 20</td>
<td>15 - 20</td>
</tr>
<tr>
<td>=&gt; 21</td>
<td>=&gt; 21</td>
<td>=&gt; 21</td>
</tr>
</tbody>
</table>

For how many years did you drink?

| <= 10 | <= 10 | <= 10 |
| 11 - 20 | 11 - 20 | 11 - 20 |
| >= 21 | >= 21 | >= 21 |

Former drinker, how many years ago did you quit?

Data collected by: ☐ Dental  ☐ RDH  ☐ Other
Clinical Exam

First exam:
- Extraoral exam (check symmetry, palpate lymph nodes and assess lips): Completed: [ ] Yes [ ] No
  Palpable lymph nodes? [ ] Yes [ ] No
  Comments:

Intraoral soft tissue exam (visualize and palpate oral tissues including tongue):
- Is there a lesion present? (If yes, please complete lesion grid) [ ] Yes [ ] No
  (Please exclude amalgam tattoo, Fossey's granules, vascularity and pigmentation)

If a lesion is present:
- Site: [ ] Right [ ] High risk sites: [ ] Low risk sites: [ ] Vestibule
  [ ] Left [ ] Floor of mouth [ ] Gingiva [ ] Buccal mucosa
  [ ] Upper [ ] Lateral or ventral tongue [ ] Retromolar [ ] Labial mucosa
  [ ] Lower [ ] Soft palate [ ] Hard palate [ ] Vermillion border

- Appearances: [ ] Homogeneous [ ] Nonhomogeneous
- Colour: [ ] White [ ] Red [ ] Brown [ ] Other:
- Texture: [ ] Smooth [ ] Rough [ ] Ulcerated [ ] Nodular [ ] Other:

Please illustrate your findings on the diagram:

![Diagram of oral structures]

FV Exam
- FV status: [ ] FVL (brown/black) [ ] FVR (green) [ ] Not Sure

Group 1 Low Risk
- Ulcer (< 2 weeks): [ ] Yes [ ] No [ ] Not Sure
- Trauma
- Candida (yeast)
- Geographic tongue
- Other: Please specify:

If clinically indicated, review in 2 - 3 weeks.
[ ] Review in 2 - 3 weeks
[ ] Yearly screening

Group 2 High Risk
- Non-healing ulcer (> 2 weeks)
- Lichen planus
- White patch
- Red patch
- Other: Please specify:

If clinical exam is yes or not sure and/or FVL then repeat exam in 2 - 3 weeks
[ ] Review in 2 - 3 weeks

2 - 3 Week Follow-Up Visit
- Date (YYYYMMDD):
- White light: Lesion still present: [ ] Yes [ ] No
- FV status: [ ] FVL [ ] FVR [ ] Not Sure
- An ulcer that is present longer than 2 weeks should be considered a group 2 lesion
- To be reviewed by BC OCPP staff: [ ] Yes [ ] No
- Date reviewed by BC OCPP staff (YYYYMMDD):
- BC OCPP staff comments:

Follow-up Visit

Date referred (YYYYMMDD):

Patients with Group 2 lesions which remains after recall and/or
are FVL and/or worsening - please fax the Oral Mucosal Disease Program at VGH via the BC OCPP (Fax 604-675-8079).

Date faxed to BC OCPP (YYYYMMDD):
CHAPTER 6 GENERAL DISCUSSION

6.1 Oral cancer background – need for early diagnosis

Oral cancer is a substantial, though often under-addressed issue globally, with close to 300,000 new cases reported annually. Early diagnosis is associated with significantly better prognosis. When the disease is diagnosed at a late stage, the chance of death is high and treatment can be disfiguring and devastating. Many factors would suggest that the diagnosis of oral cancer should be relatively easy: the major carcinogens and the major risk population are known: tobacco users, heavy alcohol consumers and individuals over the age of 40 years of age; oral cancer is most often preceded by premalignant lesions, and the high-risk premalignant lesions are known to present certain risk features, such as location at certain high-risk site, a non-homogeneous appearance and large size; the oral cavity is easy to access and the screening process is non-invasive, quick and pain-free; the process of malignant transformation for premalignant lesions is long, hence ample of time for detection and intervention; and the majority of people in developed countries have regular dental visits, again providing ample of chance for early detection and intervention.

The majority of oral cancers are diagnosed late in the developing countries. Surprisingly, even in developed countries, a significant percentage of oral cancers are still diagnosed at a late stage (40% in BC). The reasons for late diagnosis are multiple, varying from a patient problem (i.e. lack of knowledge and failure to have regular check ups), to a health professional problem (i.e., lack of knowledge in competent screening and the diagnosis of high-risk oral premalignant lesions and early cancer), to the current limitation of our ability in detecting early disease. Early diagnosis and intervention not only involves health professionals (proficient screening), and public education but also the development of new tools that could overcome our current limitations in early diagnosis. The overall goal of this thesis was to develop strategies for improving the detection of high-risk oral premalignant lesions (OPLs) through enhanced visualization of clinical lesions. This was addressed by both the development of the new diagnostic tools and effective education and triage systems through 4 research
projects. This chapter summarizes the problems the research projects were addressing, the main findings, limitations of the studies and future research.

6.1 Toluidine blue staining identifies high-risk primary oral premalignant lesions with poor outcome

Failure to recognize high-risk oral premalignant lesions and early oral squamous cell carcinoma is one major factor for late diagnosis and poor prognosis of the disease. Currently, the clinical diagnoses of high-risk lesions are based upon clinical recognition of oral premalignant lesions (OPLs). However we are severely limited in recognizing high-risk lesions due to the clinical similarity of oral premalignant lesions to benign reactive lesions and because high-risk lesions may not be clinically apparent or visible.

OPLs most commonly present as leukoplakia (white patches) and less frequently as erythroplakias (red patches). However, most white and red lesions in the oral cavity are benign reactive lesions (e.g., frictional keratosis) with no premalignant potential. Differentiation of leukoplakia and erythroplakia from reactive white and red lesions requires knowledge, expertise and experience. Yet even expert clinicians have difficulty determining the risk of a lesion by visual inspection alone. The difficulties in differentiating the very common reactive white and red lesions from high-risk lesions have a severe impact on the decision of whether a lesion should be biopsied, or where one should biopsy a lesion. To further compound the problem, many early lesions are not clinically apparent (subclinical), and some oral cancers seem to arise without visible preceding OPLs. Visual tools that could help clinicians in the differential diagnosis, in the identification of the area of highest risk for biopsy and in ‘seeing’ the subclinical lesions are urgently needed.

Toluidine blue (TB) is an acidophilic stain that has been used for more than 40 years in the detection of lesions at risk both in the cervix and in the oral cavity. TB has been found to be highly sensitive in the detection of oral squamous cell carcinoma (SCC), particularly when the patient returns in 2 weeks and the lesion is re-stained, allowing for the healing of traumatic or inflammatory lesions [1]. However results have been mixed in the detection of OPLs [2, 3] even when a 2 week re-staining occurs [4] since a significant proportion of OPLs are negative for TB.
In a longitudinal study (the first research project of this thesis), TB status was studied in 100 patients with primary OPLs and the association of TB staining with outcome as well as to conventional histopathological features and molecular risk patterns was investigated. TB was found to be predictive of low grade dysplasia progressing to carcinoma-in-situ (CIS) or SCC when used within the high-risk referral setting: a greater than 6-fold elevation in cancer risk was observed for TB-positive lesions, with positive retention of the dye present in 12 of the 15 lesions that later progressed to cancer (P = 0.0008), as compared to morphologically similar low-grade lesions that were TB negative. These lesions were also significantly associated with high-risk molecular patterns, and high-risk clinical patterns.

This is a significant study since TB dye can be easily applied in a dental office, and is inexpensive. Appropriate usage of the dye could help clinicians identify high-risk lesions, identify high risk areas to biopsy within a lesion, and better delineate the lesion area and margins.

Limitations of the study include: 1) the number of patients was small; 2) the study was set in a high-risk clinic and the patients all had histologically confirmed dysplasia; 3) it remains to be seen whether TB will have a similar value within the community setting; 4) the clinicians in the study all had expertise and extensive experience; and 5) TB will not replace clinical judgement but is an adjunctive tool. Continuing education of oral health professionals is required to ensure the correct application and interpretation of the tool.

Future studies needed include: 1) a multicentre trial of the dye with a larger number of patients; 2) a trial of the dye in a community setting where there are not only patients with known dysplasia history but also many with reactive lesions, and where the practitioners are general oral health professionals; and 3) the identification of the knowledge gaps regarding the diagnosis of high-risk lesions and model future education to fill the gaps.

6.2 Fluorescence visualization is associated with clinical and molecular markers of risk.

The need for a visual tool to facilitate clinical identification of high-risk lesions has also driven research in the development of optical or imaging devices. One area receiving attention is the use of imaging devices to detect cellular and tissue changes associated with
carcinogenesis. In the oral cavity, tissue fluorescence diminishes with the biochemical and tissue changes associated with carcinogenesis: breakdown of collagen in the stroma, alterations to scattering due to changes in the nucleus, alterations to flavin adenine dinucleotide (FAD), associated with increased metabolism, increased microvascularity associated with angiogenesis and thickening of the epithelium [5]. The development of a simple hand-held direct visualization tool to detect these changes has been made by the BC Cancer Agency. The second research project of my thesis is the first longitudinal study to validate the potential role of the device as an adjunctive tool for identification of the high-risk lesions as well as its association with other risk factors associated with oral cancer.

In this study, 170 patients with 192 lesions (100 primary low-grade dysplasias) were followed in an ongoing longitudinal study looking for an association between fluorescence visualization (FV) status and clinicopathological risk factors, molecular risk patterns and outcome (progression from low-grade OPLs to high-grade OPLs and SCC). FV was found to be significantly associated with clinical risk factors (site and appearance), with degree of dysplasia (98% of high-grade dysplasias and 95% of SCCs were FVL), and with high-risk molecular patterns.

When only the low-grade OPLs were looked at, FV showed no significant association with clinical or molecular risk factors, although there was a trend with lesions having a loss of FV (FVL) showing more loss of 3p and having more than one clinical risk factor. In addition, within the short follow-time, the only low-grade OPLs lesions progressing to high-grade OPLs or SCC were FVL.

We also compared TB and FV. These 2 adjunctive devices work with different mechanisms. TB is associated with nucleic acids; increasing concentration of DNA during carcinogenesis increases uptake of the stain, while FV targets biochemical and morphological changes in the tissue. FV identified almost all high-grade dysplastic lesions, 50/51 (98%) while TB identified 33/51 (65%) (P<0.001); however, TB identified a significantly less proportion of low-grade lesions as compared to FV: 23/66 (35%) TB+ vs. 49/66 (74%) FVL (P<0.001), Although FV identified more low-grade dysplasia lesions, in project 1, TB+ low-grade lesions contained significantly higher proportion of cases with high-risk clinical features and molecular features as well as 4 times increased relative cancer risk as compared to TB- lesions; whereas FVL lesions in study 2 did not show such differences. Of interest, 75% of high-grade dysplasia and SCC lesions
were both FVL and TB positive. If lesions which were negative for both tools showed no further progression within the high-risk clinic, this may provide a source of confirming the low-risk status of lesions. The combination of the 2 methods may lead to an increase in differentiating lesions at risk.

The limitations and future studies are similar to those of the Project 1 for TB study.

6.3 Community Screening Initiative Focus Groups

One major problem for the late diagnosis of oral cancer is a lack of knowledge and skill by health professionals in both educating the public (i.e., tobacco cessation) and in competent screening and detection of high-risk lesions. Even with the development of new visual tools, clinical knowledge and skill in the detection of disease are still of paramount importance. It is critical the problem be addressed by training health professionals not only with the appropriate knowledge and skills but in a manner that will enhance integration of the skill. The World Health Organization (WHO) Global Oral Health Programme has emphasized the importance of such training in oral cancer control by stating as its directive the importance of involving oral health professionals in detection, early diagnosis and treatment of oral cancer. In order to develop appropriate training, we need to find out what clinicians consider to be the barriers and facilitators for screening. Few studies have been done in this [6, 7].

Project 3 was aimed to understand why oral health professionals (OHPs) do not consistently screen for oral cancer. Two focus groups were held at the end of a pilot study which included a 1-day workshop that demonstrated procedures for conducting screening examinations including a hands-on clinical session and 3 months of community screening by the participants. A number of barriers were uncovered: 1) lack of motivation such as shortage of time and lack of financial incentive; and 2) lack of knowledge and skill including about how to communicate with patients regarding the disease, confusion regarding how, when and who to screen, and uncertainty regarding the dental professionals responsibility in cancer diagnosis. Suggestions to overcome lack of time included screening within the hygiene appointment, developing pamphlets and rehearsed short answers to potential questions. Increased public awareness was felt to be a significant facilitator for screening – if a patient demands screening then the practitioner will need to provide the service. Hands-on clinical training to aid in the
identification of both lesions of concern and benign or reactive lesions was also felt to be an important facilitator.

Limitations of this study include: holding only 2 focus groups; and study volunteers may not represent the beliefs of the majority of oral health professionals.

Future research should include 1) representatives from all health professions, including all oral health professionals, dental mechanics, physicians and community nurses partaking in focus groups on oral cancer screening; 2) more detailed evaluation of the need of health professionals regarding oral cancer screening within the scope of their daily clinical routine; and 3) education of health professionals through workshops, publications, guidelines and others.

In the development of project 4, we integrated feedback from the focus groups of project 3. More time was allotted to demonstrating and practicing screening, a session on how to discuss oral cancer screening and referral was included, participants were encouraged to integrate screening into the hygiene appointment, dentists were also encouraged to bring a staff member to the workshop, and academic detailing was available throughout the study. Finally, the information learned from the focus groups was integral in the formation of the oral cancer screening guidelines and referral pathways and an article on how to discuss screening with patients, both published in 2008 [8].

6.4 Community screening initiative

While focus group discussions shed some light on the barriers OHPs face, a more detailed investigation of these barriers could only be obtained through involvement with the OHPs in their community practices. There is a noticeable shortage of such studies. In addition, visual tools such as TB and direct FV found to be useful adjunctive tools in high-risk clinics need to be studied in the community setting. To date, no research has been completed within this setting.

The Cochrane Review [9], the American Cancer Society [10], the Surgeon General of the US [11], the Canadian Task Force on the Periodic Health Examination [12] and the UK working group on screening for oral cancer and precancer [13] all support opportunistic screening of high-risk patients and primary prevention as part of regular clinic activity. Unfortunately, past surveys of dentists have discovered that not only are all patients not being opportunistically
screened, but basic preventive screening in the form of enquiring about high-risk habit information (tobacco and alcohol use) is not universal and in the case of alcohol use, uncommon and a large proportion of OHPs do not feel adequately prepared to counsel their patients in tobacco cessation and alcohol moderation [14, 15].

Research project 4 evaluated 15 community dental practices using a standardized screening and triaging protocol over 11 months to: 1) determine the need of the OHPs related to oral cancer screening; 2) to train them in the screening of high-risk oral lesions through screening protocols; and 3) to establish a framework for both screening and evaluating adjunct tools in community settings through study of the tool of direct FV. The screening triage framework uncovered 3 key decision: 1) classification of lesions into risk groups; 2) identifying low-risk lesions with a known cause (i.e. trauma); and 3) request for facilitator follow-up or referral to a specialty clinic. The second point was greatly enhanced by a 3-week reassessment appointment which allowed time for reactive or inflammatory lesions to heal and hence prevent unnecessary referral. The use of a facilitator to reassess patient lesions within the community practice also prevented undo referrals and patient anxiety regarding referrals to the speciality clinic. The use of a facilitator was also a learning opportunity for the clinicians as they were able to have patients reviewed who they were hesitant to refer – they were able to confirm the negative.

The process for laying a framework for the introduction of new technology, FV, was also begun in this study. The 3-week reassessment appointment was very informative as the majority of FVL lesions were transient, likely to do to healing of a reactive lesion or the removal of trauma. Low-risk lesions which retained FVL were more apt to be referred or reviewed by the facilitator then lesions which retained fluorescence (FVR). Interestingly, all lesions in which FV status was uncertain (FVE) resolved and were FVR after 3 weeks. While clinicians may have regarded FVR status as low-risk, some FVR lesions were reviewed at the 3-week reassessment appointment and referred, thus implying that the clinicians still relied on their clinical judgement.

Another important outcome is the need for continued education during the career of the OHP. The value of a hands-on experience within the educational component of this study to build confidence was invaluable, both at the initial workshop and with the use of the community
facilitator (academic detailing). Hands-on screening education in study club settings might be an attractive method for disseminating screening information and building skills. It would appear that change is also required in dental school training as many participants found many aspects of their screening education lacking, particularly primary prevention.

Limitations of this study include: 1) require more emphasis and training on extraoral examinations; 2) lack of dental team involvement in screening education; and 3) a lack of a means to confirm negative screens within the dental office.

Future research should include evaluation of extraoral exams, quantitative cytological methods to confirm negative exams (ploidy), and development of standardized forms to test for increased documentation. Future research should also include an evaluation of decision making by the OHP regarding the risk of lesion and need for referral. Knowledge translation research into documentation weaknesses and whether the use of standardized screening forms aid in the following of screening guidelines. Further refinement of the triage system is required as well as research into the best means of disseminating this information to not only the dental community but other health care professionals, particularly those in underserved communities. Education into raising public awareness is also required as well as improvements of currently available oral cancer materials.

6.5 In summary

Oral health professionals share a responsibility in both primary and secondary prevention of oral cancer and OPLs. The early detection, referral and management of this disease must be an integral part of an OHP regular clinical behaviour. The development of an easy to follow triage framework and adjunctive tools which facilitate decision making will support this behaviour. Currently, many OHP are remiss in providing regular and consistent oral cancer screening exams, a fast and painless procedure, and a high proportion of oral cancers are diagnosed late when the effects of treatment can be extreme. The objective of this thesis was to develop strategies for improving the detection of high-risk OPLs through the development of the new diagnostic tools and effective education and triage systems through 4 research projects. Both diagnostic tools studied in this thesis (TB and FV) and the education and triage systems in the community dental clinics show promise in improving our ability for early
detection, diagnosis and intervention of the disease. The BCCA estimates a 21% increase in oral cancer cases in BC by 2023 and a 52% increase in deaths due this disease [16]. It is time to alter the trend of poor uptake of oral cancer screening within the community.

6.6 Other studies related to this thesis

Feedback from the research within both the high-risk referral and community clinics included in this thesis has led to the publication of the following papers, of which I am an author, not included within this document:


6.7 References


16. BCCA Population Oncology. *Projections (Incidence and Mortality) for years 2009 to 2023 by Health Authority, Cancer Type, Age at Diagnosis and Gender (VERSION BC.CAN11).* 2009 [cited 2009; Available from: www.bccancer.bc.ca.]