INTEGRATING FLUORESCENCE VISUALIZATION WITH CLINICAL MARKERS

to

PREDICT ORAL CANCER RECURRENCES

by

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Abstract

One reason for the poor survival rate of oral cancer is the high rate of recurrence (REC). The objective of this study is to investigate how fluorescence visualization (FV) may play in the prediction of oral cancer REC at a site previously treated for oral cancer. We will confirm previously identified clinical factors for REC such as lesion presence and TB status, and analyze if any combinations of these three factors at varying follow-up time intervals can suggest a higher risk for REC.

Information for this study will come from patients enrolled in the BC Oral Cancer Prediction Longitudinal study. Patients are eligible if: 1) they had a primary tumour diagnosis of SCC or CIS; 2) were treated with curative intent; and 3) had at least one recall visit within one year after completion of initial treatment. Data analyzed: 1) demographic and lifestyle habit information; 2) primary tumour information; 3) oral clinicopathological features during follow-up at 6, 12, 18, and 24 months.

For this thesis, 232 patients have been identified that fit the inclusion criteria. Of those, 34 patients developed recurrence, and 198 patients remained tumour free throughout their follow-up period. Demographic, smoking, alcohol and FV status were not found to be associated with a recurrence. Of significance, OPL status at all follow-up intervals (P<0.01), TB at 6, 12, 24 months (P<0.05), and TBFV at 6 and 12 months (P<0.05) were associated with REC.

There is a higher percentage of REC in patients with TB+FV+ status than other combinations of TBFV status, with significance found at 6 and 12 months follow-up post-treatment. With known risk factors for predicting REC, clinicians can recognize patients at increased risk, improving the chance of early detection. This can also drive clinician decision-making for patients deemed high-risk, increasing surveillance and improving patient care.
Preface

This thesis is original, unpublished, independent work by the author, K.Y. Wu.

This thesis is uses the data collected as part of the BC Oral Cancer Prevention Program’s Oral Cancer Prediction Longitudinal Study. The study data was collected from multiple institutions including the British Columbia Cancer Agency (BCCA), Vancouver General Hospital (VGH) and the University of British Columbia (UBC). This project was identified by Dr. Denise Laronde and modified by Dr. Miriam Rosina and Dr. Lewei Zhang. Data was collected by study personnel including the author. The author performed all the data analysis.

Ethics approval for this study was obtained from the University of British Columbia - British Columbia Cancer Agency Research Ethics Board (H98-61224; H05-60116).
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<th>Description</th>
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<tbody>
<tr>
<td>BCCA</td>
<td>British Columbia Cancer Agency</td>
</tr>
<tr>
<td>BC OCPP</td>
<td>British Columbia Oral Cancer Prevention Program</td>
</tr>
<tr>
<td>CIS</td>
<td>Carcinoma <em>in situ</em></td>
</tr>
<tr>
<td>CRF</td>
<td>Clinical research files</td>
</tr>
<tr>
<td>COE</td>
<td>Conventional oral examination</td>
</tr>
<tr>
<td>CS</td>
<td>Current smoker</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FV</td>
<td>Florescence visualization</td>
</tr>
<tr>
<td>FV-</td>
<td>Florescence visualization negative</td>
</tr>
<tr>
<td>FV+</td>
<td>Florescence visualization positive</td>
</tr>
<tr>
<td>FOM</td>
<td>Floor of mouth</td>
</tr>
<tr>
<td>FS</td>
<td>Former smoker</td>
</tr>
<tr>
<td>HGD</td>
<td>High-grade dysplasia</td>
</tr>
<tr>
<td>IMRT</td>
<td>Intensity modulated radiation therapy</td>
</tr>
<tr>
<td>LGD</td>
<td>Low-grade dysplasia</td>
</tr>
<tr>
<td>NONREC</td>
<td>No recurrence</td>
</tr>
<tr>
<td>OBS</td>
<td>Oral Biopsy Service</td>
</tr>
<tr>
<td>OCPL</td>
<td>Oral Cancer Prediction Longitudinal Study</td>
</tr>
<tr>
<td>OHS</td>
<td>Oral Health Study</td>
</tr>
<tr>
<td>OPL</td>
<td>Oral premalignant lesion</td>
</tr>
<tr>
<td>REC</td>
<td>Recurrence</td>
</tr>
<tr>
<td>SFT</td>
<td>Second field tumour</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SPT</td>
<td>Second primary tumour</td>
</tr>
<tr>
<td>TB</td>
<td>Toluidine blue</td>
</tr>
<tr>
<td>WLE</td>
<td>White light examination</td>
</tr>
</tbody>
</table>
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Mom and Dad: Thank you for all the love and support you gave me, not just during my Master’s education, but throughout my whole life. You raised me without pressure or expectations to be successful in the traditional sense, and only wished for me to be happy with whatever I choose to do with my life. You time and time again sacrificed your own comfort to make sure I had the tools to succeed in my endeavours. I hope this thesis and any future accomplishments of mine make you proud to be my parents.

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Chapter 1: Introduction

Defined as cancer of the lip and oral cavity (1), oral cancer (OC) is the sixth most common cancer in the world, with a five-year overall survival rate of 50% (2,3). Oral squamous cell carcinoma (OSCC) accounts for more than 90% of all oral cancers (1), with the remainder of cases being comprised of AIDS-associated Kaposi sarcoma, odontogenic neoplasms, bone and soft-tissue sarcomas, and malignant salivary tumours and lymphomas (4). Oral cancer has a poor prognosis and survival rate, often attributed to the advanced extent of the disease (large size of tumour and regional lymph node involvement) found at time of diagnosis and the high rate of recurrence (5). Despite the development of screening aids and improvements in treatment over the last three decades, there have been no significant increases in long-term prognosis of OC (6). This could be due to the lack of early detection of oral premalignant lesions (OPLs) and their associated risk factors. Early detection of OPLs can aid in preventing lesion progression, improving prognosis, and increasing overall patient survival (5).

Because of the low survival rates associated with late stage diagnosis, the best way to combat oral cancer is to educate oral health professionals and their patients on the importance of early detection of mucosal changes, the risk factors that predispose one to these malignancies, and the necessary steps one should take in managing a newly discovered lesion. To aid in early detection of these premalignant lesions, clinical and histopathological factors (3) (size, colour, texture), adjunctive aids (7,8) (toluidine blue and autofluorescence visualization), and molecular techniques such as identifying loss of
heterozygosity (LOH) have been developed to better predict malignant progression, guide individualized treatment, and aid in long-term follow-up (LOH).

To date, little is known on whether the clinicopathological risk factors associated with primary progression are also associated with recurrence (REC). OSCC has a high rate of secondary cancer formation, which is a major contributor to its poor prognosis, with up to 30% of previous oral cancer patients developing recurrences at the primary tumour site (9,10). Thus, the aim of the research in this thesis is to explore the relationship between clinical and histopathological risk factors and oral cancer recurrence, with an emphasis on the role of adjunctive screening devices. Hopefully, this information could aid in decision making for clinicians, and ultimately improve patient outcome and quality of life.

1.1 Epidemiology and Etiology

Of the 12.7 million cases of cancer worldwide, and 7.6 million cancer deaths occurring globally in 2008, oral cancer accounts for an estimated 263,900 new cases and 128,000 deaths annually (11). In Canada, 4,400 new cases of oral cancer (2,900 for men, 1,500 for women) are estimated to occur in 2015 (12). This equals to an estimated 8.8 of every 100,000 people will develop oral cancer. Specifically in British Columbia, 380 men and 180 women are estimated to develop new cases of oral cancer in 2015. There will be an estimated of 1,200 deaths (810 men, 390 women) in Canada in 2015, equaling 2.3 deaths per 100,000 oral cancer cases. Specifically in British Columbia, 110 men and 50 women will die from oral cancer in 2015 (12). Globally, OC is the sixth most common cancer in the world (13), being more prevalent in some countries than others. High
incidence rates for oral and pharyngeal cancer (OPC) can be found in South and Southeast Asia (Sri Lanka, India, Pakistan, Taiwan), Western and Eastern Europe (France, Hungary, Slovakia, Slovenia), parts of Latin America and the Caribbean (Brazil, Uruguay, Puerto Rico), and Pacific regions (Papua New Guinea and Melanesia)(13). Australia has a high incidence rate for cancers of the lip only, due to high exposure to UV-light and a large population of fair-skinned people(13). The difference in incidence rates of OPCs around the world can be explained by a number of factors, such as culture, diet, exercise, risk factors such as tobacco and alcohol use, development of the country, and the treatments available for patients(14). As a result, two thirds of all oral malignancies in the world are found in developing countries, due to socio-economic status disparities(13).

The etiology of OPCs is multifactorial; the different carcinogens one is exposed to can either act individually or synergistically to cause malignancy(13). Proven risk factors for OPCs include smoking tobacco, chewing tobacco, alcohol consumption, poor nutrition, and infection from the human-papillomavirus (HPV)(13).

Roughly 1.3 billion adults aged 15 or older smoke daily around the world, with each country having different prevalence of smokers due to different socio-economic factors(14). In Western cultures, smoking cigarettes, cigars, and pipes are common tobacco products(3) while chewing tobacco, areca nut, betel quid, pan masala, and gutka are all common in South/Southeast Asia. These smokeless products are highly addictive, and their use is on the rise due to increasing public policies that prohibit smoking in public areas(4). Tobacco use is a significant risk factor for OSCC, with current smokers at higher risk of developing OSCC than never or former smokers(15). Carcinogens
present in tobacco products (polycyclic aromatic hydrocarbons, nitrosamines, aromatic amines and acetaldehyde) come into contact with the oral mucosa, causing mutations in stem cells of the basal layer of the oral cavity(10). The relationship between tobacco and OSCC is also dose-dependent; the more one smokes, and the longer one smokes, the greater the risk of OSCC. This relationship is further highlighted when patients quit smoking. Former smokers who have abstained from smoking for more than 20 years show a lower risk of OSCC development than those who have abstained for less than 20 years(15).

Alcohol is also an important etiological factor for OSCC that works synergistically with tobacco to cause carcinogenesis(16). On its own, alcohol can be metabolized into the carcinogen acetaldehyde, which then comes into contact with the mucosal lining. Alcohol, like tobacco, has a dose-response relationship with oral cancer; the risk increases with the length of time and the amount of alcohol consumed. For non-smokers, there is a small increased risk for moderate drinkers (1 -2 drinks a day) but the risk increases to more than 2-fold for heavy drinkers (more than 4 drinks per day)(17,18). When used together, alcohol is thought to act as a solvent, increasing the permeability of the oral mucosal lining for carcinogens in tobacco to dissolve into the tissue(10). The risk of developing oral cancer has been shown to increase 3 to 9 times in people who smoke and drink, and can be much greater in people who smoke and drink heavily, when compared to those who do not(17,19).

Traditionally, those aged 50 to 70 are affected by OPC the most due the cumulative exposure to risk factors such as tobacco, alcohol, and poor nutrition throughout their lives(4). However, a new risk factor affecting the younger generation has
been identified for OPC development: the Human Papillomavirus (HPV)(20). HPV-related OPCs usually affect the tonsils, oropharynx, and posterior tongue(4,13), with an increasing trend seen in young adults in the United States and Europe due to changes in oral sexual behaviour(21). HPV-16 and HPV-18 are two subtypes of this virus that cause uncontrolled cell proliferation by inactivating tumour suppressor proteins(22). HPV-associated OPCs are usually seen in non-smoking, non-drinking, non-immunocompromised patients, lending evidence that this type of carcinogenesis is independent of other oral cancers(22).

1.2 The Oral Mucosa and its Changes

The following section will outline the differences between a healthy oral mucosa and the associated changes in oral malignancy.

1.2.1 The Normal Mucosa

The oral mucosa is an overarching term that includes the labial mucosa, buccal mucosa, tongue, soft and hard palate, floor of mouth, and gingiva of the oral cavity. The oral mucosa has two layers: the outermost layer or epidermis is composed of stratified squamous epithelium, and beneath, the lamina propria, which is composed of collagen and elastin. The epidermis is made of five layers: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. Of importance is the stratum basale, which contains basal cells along the basement membrane and the stratum corneum, which contain keratin cells. Stratum basale is the layer in which basal cells constantly divide and migrate superficially towards the stratum corneum. It is here where
carcinogens such as tobacco and alcohol have the greatest effect as basal cells are believed to host the stem cells of the epidermis. The stratum corneum is the outermost layer of the epidermis, and contains dead skin cells that serve as a physical barrier to the environment. This layer is composed of keratin cells, which determine the degree of keratinization of the oral mucosa depending on the site. Areas subjected to more trauma or forces such as the hard palate and gingiva are more heavily keratinized than the buccal mucosa (moderately keratinized). Tissue not generally subjected to trauma such as the ventral tongue and the floor of the mouth (FOM) are nonkeratinized.

1.2.2 Oral Premalignant Lesions

Prior to malignancy, morphological changes in the oral mucosa may be detected clinically through visual inspection or enhanced by adjunctive aids. These areas of change are called oral premalignant lesions (OPL), which are histologically altered oral tissue that are at a higher risk of becoming OSCC(23). OPLs may present with a combination of different clinical characteristics, such as colour, texture, size, and appearance. Many OPL are defined clinically by colour with white lesions being termed leukoplakia while red lesions are termed erythroplakia.

1.2.3 Leukoplakia

Leukoplakia is the clinical term to describe a white lesion that cannot be classified as any other definable lesion(23). Leukoplakia accounts for a large percentage of all OPLs at 85%(24), and is found more often in individuals who smoke and drink alcohol(24). However it can also be caused by irritation or abrasion, appearing anywhere
in the oral cavity, most commonly on the alveolar mucosa, buccal mucosa, tongue, and lower lip(3). The white appearance is due to thickening of the stratum corneum layer (hyperkeratosis) and/or thickening of the stratum spinosum (acanthosis)(3).

**1.2.4 Erythroplakia**

Erythroplakia is the clinical term to describe a red lesion that, like leukoplakia, cannot be classified as any other definable lesion(25). However, erythroplakias are generally considered to be at higher risk for OSCC development than leukoplakias(26) or other OPLs. Erythroplakia appears red due to any combination of epithelial thinning from trauma, increased inflammation, and neoplastic activity leading to more vascular tissue(10,25). If a lesion has both red and white coloured, it is called erythroleukoplakia(25).

**1.2.5 Dysplasia**

Dysplasia is a histopathological term used to describe cytological and architectural changes in the cells of the epithelium(10). Oral dysplasia is a well-established risk factor for OSCC, with increasing level of dysplasia severity increasing the risk of progression to invasive carcinoma(27). Severity of dysplasia is defined by the level of phenotypic and cellular change in the tissue, summarized in Table 1. Dysplasias are graded into mild, moderate, severe dysplasia or carcinoma in situ (CIS) by a pathologist. Mild dysplasia exhibits minimal architectural and cytological changes confined to the lower 1/3 of the epithelium(27). In moderate dysplasia, architectural and cytological changes are confined to the lower 2/3 of the epithelium, while severe
dysplasia exhibit these changes beyond 2/3 of the lower epithelium but has not reached the full epithelial layer(27).

Table 1.1 Architectural and Cytological Changes in Dysplasia

<table>
<thead>
<tr>
<th>Architectural Changes</th>
<th>Cytological Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cell hyperplasia</td>
<td>Increased nuclear-cytoplasmic ratio</td>
</tr>
<tr>
<td>Loss of polarity of basal cells</td>
<td>Cellular and nuclear pleomorphism (variation in shape)</td>
</tr>
<tr>
<td>Loss of intercellular adherence</td>
<td></td>
</tr>
<tr>
<td>Drop-shaped rete ridges</td>
<td>Cellular and nuclear aniso-cytosis and nucleosis</td>
</tr>
<tr>
<td>Irregular epithelial stratification</td>
<td>(variation in size)</td>
</tr>
<tr>
<td>Keratinization of one or more cells in the prickle cell layer</td>
<td>Nuclear hyperchromatism</td>
</tr>
<tr>
<td>Abnormally superficial mitoses</td>
<td>Enlarged nuclei</td>
</tr>
<tr>
<td>Increased amount of mitotic figures</td>
<td>Increased number and size of nucleoli</td>
</tr>
<tr>
<td></td>
<td>Atypical mitotic figures</td>
</tr>
</tbody>
</table>

Adapted from Warnakulasuriya et al., 2008

1.2.6 Carcinoma in situ and Squamous Cell Carcinoma

Once architectural and cytological changes extend throughout the epithelial layer without invasion of the basal layer, the lesion will be graded as carcinoma in situ(27). Squamous cell carcinoma is diagnosed once the changes break through the basement membrane (27).

1.3 Malignant Transformation

The clinical significance of monitoring patients with mucosal changes lies with the risk of OPLs undergoing malignant transformation into OSCC(28). Malignant transformation rates for primary progression range from 0.13%(29) to 17.5%(30), and
they differ due to different study methodologies(31), but a meta-analysis of this subject has suggested a malignant transformation rate of 12.1%(32). Many factors affect the differing rates seen in these studies, such as the follow-up study design, patient demographics, lesion histopathology, and patient lifestyle habits and geographical differences. For example, studies done in low-resource countries like India may have a higher incidence of malignant transformation than North American or European countries due to higher tobacco consumption(4). Differences between the studies’ inclusion criteria also contribute to different malignant transformation rates reported. Silverman et al. reported a low transformation rate of 0.13% in a study that included only benign leukoplakia(29), while in another study a 17.5% transformation rate was found when patients with only dysplastic lesions were included(30). That, with the addition of differing management and follow-up period of lesions makes it difficult for researchers to agree upon a common malignant transformation rate.

1.3.1  Predisposing Factors to Malignant Transformation

There are a multitude of factors that can influence malignant transformations of OPLs, including clinical characteristics (colour, texture, consistency, margins, size, and site) and presence of dysplasia. Each of these factors will be described below.

One of the first characteristics a clinician would note about any lesion is the colour, which can be indicative of an increased risk of malignant progression(26). As mentioned earlier, a white lesion (leukoplakia) is much more common than a red lesion (erythroplakia), but the malignant transformation rate of erythroplakias are much higher than leukoplakias(29,33). Silverman et al. found that erythroplakias had a four-fold
increased risk of having malignant transformation than leukoplakias(30). It is proposed that erythroplakias have a higher transformation rate because red lesions are often associated with lesions that already have high-grade dysplastic changes, while most leukoplakias can be benign, scar tissue, or trauma induced change(30,33). Speckled or erythroleukoplakia also exhibit an increased risk of malignant transformation.

The texture of an OPL can be described as smooth, rough, nodular, pedunculated or sessile, verrucous, granular, and/or ulcerated. It is not known if texture type of a lesion necessarily correlates with malignant transformation, but evidence has been found that two specific types of OPL, proliferative verrucous leukoplakia and erosive lesions, exhibit increased malignant transformation rates(29,31,34).

Other clinical risk factors found to be associated with increased risk of malignant transformation include appearance, the type of lesion margins, and size. The appearance of a lesion can be described as either homogenous (uniform in colour and texture) or non-homogenous (predominately red or a mixture of white and red colour and irregular texture)(23). Axell et al.(23) further classifies homogenous and non-homogenous lesions into four subtypes each. Homogenous lesions can be further categorized as flat, corrugated, wrinkled, or pumice type, while non-homogenous lesions can be grouped as verrucous, nodular (speckled), ulcerated, or erythroleukoplakia type(23). Generally, non-homogenous lesions show a greater risk of malignant transformation when compared to homogenous lesions, most likely due to a higher prevalence of dysplastic changes in the former(35). In one study, Holmstrup et al.(36) found a seven-fold increase in malignant transformation risk when the OPL was non-homogenous as opposed to homogenous. If the margins of a lesion are diffuse (ill-defined), that may also alert the clinician of a
higher risk lesion than if the lesion were discrete (well-demarcated boundaries)(26). In addition to this, Holmstrup et al.(36) have also shown that OPLs >200mm(2) in size have a 5.4-fold increase in malignant transformation risk as compared to those <200mm(2) in size.

The site of a lesion can also be indicative of malignant transformation. Because the ventrolateral side of the tongue and the floor of mouth can be constantly exposed to carcinogens (tobacco and alcohol) pooled in saliva(37), it is generally agreed in the western world that the anatomical site with the highest risk for malignant transformation in the oral cavity is the ventrolateral tongue and floor of mouth(37,38). These sites may also be more apt to progress because of the type of tissue present (non-keratinized or parakeratinized tissue) versus other sites (keratinized tissue)(30,39). However, this statistic changes as patient demographic and habits change, as seen in studies done in India, where the widespread use of chewing and smoking tobacco have caused the buccal mucosa and labial commissure to be the most high-risk sites identified(29,40).

OPLs that are dysplastic are considered to be at high risk for oral malignancy(30). Amagasa et al(39) found a statistical difference in malignant transformation rate between dysplastic OPLs versus OPLs without dysplasia (13.3% and 3.0% respectively), and other authors have found the similar conclusions (3,41). Ho et al.(28) also found an association with the degree of dysplasia and malignant transformation, stating that OPLs with severe dysplasia were more likely to progress than mild and moderate dysplasia combined. However, Holmstrup et al(36) disagrees with these findings as his results showed no relationship between dysplasia and malignant transformation. The authors speculated that this result may have been due to the subjectivity of diagnosing epithelial dysplasia(36).
Another reason why these study results are conflicting may be due to how the researchers grouped different degrees of dysplasias. Some analyze them individually, while some group mild and moderate dysplasia together, and severe and CIS grouped together.

1.4 Treatment of OSCC

Treatment for SCC in the oral cavity consists of one or a combination of three main treatment modalities: surgery, radiation, and chemotherapy. OSCC treatment planning is based on a variety of factors, such as the anatomical site, tumour stage, and weighing possible side effects and toxicities against quality of life(42,43). The goal of oral cancer treatment is to not only treat the primary tumour, but also preserve or restore the structure or function of the site, prevent metastasis and recurrence, and improve overall quality of life(43).

Since the oral cavity is easily accessible, surgery is usually the primary choice for treatment of early stage OSCC(43). It physically removes cancerous tissue until only healthy tissue margins are left, while maintaining the structure and function of the site(44,45). Radiation may be used as the primary choice of treatment if the anatomical site is not viable for surgery, if surgery severely hampers form and function of the site, or if the patient elects for the more conservative approach to treating their cancer(42,43). Radiation preserves a higher degree of oral tissue by targeting DNA in dividing cells in a localized area. However, radiation often leaves oral tissue difficult to resect(43), thus if these two modalities are combined (as they are in later staged OSCC), radiation will usually be performed after surgery as part of the post-operative therapy(43). Chemotherapy is usually reserved for tumours that are unresectable, and used
conjunctively with radiotherapy to manage tumour size and metastasis rather than with curative-intent\(^{(42,43)}\).

1.5 Follow-up

From the time that an OPL is detected and immediately after oral cancer treatment, patients are recalled into follow-up visits to allow the clinician to examine the lesion or treated site over time. These visits include updating relevant medical history, noting any changes in patient habits, and charting any changes clinically or histopathologically in the lesion presentation. However, clinicians may face some obstacles that hamper follow-up visualization of any new tissue changes. Depending on the severity of the disease and the type of treatment done, the clinician may have a hard time discerning between a regular healing wound from a suspicious change in the oral cavity. New tools have been developed to aid in the visualization of the OPL or former cancer site.

1.5.1 White Light Examination

The follow-up visit typically starts with a white light examination (WLE) with normal incandescent light to detect any noticeable abnormalities. While the WLE may be useful in detecting oral lesions, it may potentially miss OPLs that are not easily visible to the naked eye, often going undetected before progressing into oral cancer. A recent systematic review\(^{(46)}\) has shown varying results from studies determining the effectiveness of WLE in detecting OPLs. The lowest specificity found in these studies was 0.75, while the highest was 0.94. Sensitivity ranged from 0.60 to 0.97\(^{(46)}\). However,
none of these studies used the gold standard of histological biopsy to determine sensitivity and specificity. Their results were generated by a general dentist’s WLE findings compared with an experienced pathologist’s WLE, which was considered to be a “soft” standard. Therefore, the true sensitivity and specificity of WLEs is still unknown and can’t be used on its own to detect oral malignancies.

1.5.2 Adjunctive Devices

Adjunctive screening devices such as toluidine blue (TB) and fluorescence visualization (FV) may provide additional information for the clinician during follow-up. Generally speaking, TB is purported to be able to identify the most ‘high-risk’ spots within a lesion, while FV can identify the extent of a lesion showing a “field” of dysplastic or abnormal cells.

1.5.2.1 Toluidine Blue

A dye that stains nucleic acids and nucleophilic tissue components, TB has been used for decades to screen for mucosal abnormalities as an adjunct in detecting cancerous tissues(47,48). There are two purported ways in which TB works. With its high affinity for nucleic acids, the TB dye is more readily taken up by dysplastic or cancerous tissue because of the higher nucleic acid content present in neoplasms(49). Another reason how TB can differentially stain malignant epithelia from normal epithelia is because malignant epithelium may contain wider intracellular canals than normal epithelia, allowing for easier penetration of the dye(50). Upon staining, a dark royal blue colour should be regarded as positive nuclear staining, while no stain or a very pale blue colour
denotes negative nuclear staining(47). Studies testing TB and oral cancer have shown relatively high sensitivity, ranging from 0.92 to 1.00, but low specificity ranging from 0.42 to 0.62 (51). When used to screen for dysplasia, the sensitivity drops to roughly 0.74 with a specificity of 0.66(51). This shows that while TB is useful in detecting carcinomas, it may not be as effective in detecting dysplasias. Zhang et al.(7) have shown that positive TB staining may be predictive of high-risk molecular patterns that result in malignant transformation of oral primary lesions to squamous cell carcinoma. In this study, TB positive mild and moderate dysplasias had a 4-fold risk of malignant transformation(7).

1.5.2.2 Fluorescence Visualization

A fluorescence visualization device (FV) works to expose tissue to a specific wavelength of light, which results in excitation of cellular components that affect the scattering and absorption of light in the tissue(47). Specifically, a specific wavelength of light, in this case about 420nm), would excite fluorophores present in healthy tissues (NADH, FAD, collagen, and elastin) which then emits a green glow under the specific wavelength. In dysplastic or cancerous tissue, there is a breakdown of these fluorophores and their components, hence they no longer fluoresce and a dark colour is seen instead(52). VELscope (LED Medical, Burnaby, BC) is a device that utilizes FV, to test for precancerous or cancerous lesions by assessing tissue fluorescence and looking for tissue that appears darker than healthy tissues(47). A study has found 98% sensitivity and 100% specificity for finding dysplasia when compared to a gold standard of histopathology (52). Poh et al.(53) reflected upon three individual cases where they used
tissue fluorescence on otherwise clinically non-evident lesions that were previously excised and diagnosed as some form of dysplasia. FV in all three cases detected cytological changes that were later biopsied to confirm recurrence of dysplasia. This study, to some extent, demonstrates the ability of FV as a screening device capable of detecting dysplasias otherwise missed by conventional examination. Besides its use as a screening adjunct, FV has also been used to guide the surgical removal of dysplasias by detecting the tumour margins. In another study by Poh et al., 19 of 20 tumours demonstrated loss of fluorescence that extended as much as 25mm beyond clinically evident tumours(8). Similarly to TB, it may be beneficial to find out if FV can be used on lesions being followed up after oral cancer treatment, to see if there are any associations between loss of fluorescence and oral cancer recurrence.

1.5.2.3 Other Tissue Visualization Devices

Devices that aid in the visualization of suspicious lesions are constantly being developed and investigated to aid in follow-up and decision to biopsy like the VELscope. Some of these being discussed below are narrow band imaging, use of algorithms, optical coherence tomography, and a combination of fluorescence lifetime imaging and reflectance confocal microscopy.

1.5.2.3.1 Narrow Band Imaging

Originally developed to enhance the diagnostic power of standard white light endoscopy in the gastrointestinal, urinary, and upper aerodigestive tracts, narrow band imaging (NBI) has recently seen a rapid increase in its use for screening and detecting
SCC in the head and neck region (54). NBI works by filtering out all wavelengths of light except for the blue (between 400nm and 430nm) and green (between 525nm and 555nm) to be emitted on to tissue (54, 55). The blue wavelength light penetrates slightly into the mucosa to highlight the capillaries brown in colour due to hemoglobin’s absorption spectra, and the green wavelength penetrates deeper into the submucosa to highlight thicker blood vessels in a cyan colour (54, 55). Potentially malignant and malignant lesions have distinct microvasculature structures hence neo-angiogenesis is a good indicator for carcinogenesis (56), and it is this that the NBI detects. Use of NBI in the oral cavity is usually coupled with a high-definition television screen to provide excellent resolution and image quality. With this technique, Piazza et al. screened patients who were recently diagnosed with oral SCC, and found additional findings that could not be identified compared to standard WLE (57). Overall, they found a 27% diagnostic advantage when using NBI on patients who were recently diagnosed and patients who were treated for oral SCC (57). Much like Piazza et al.’s study, other researchers evaluating the use of NBI on the oral cavity used the same methods and patient population for their study (58, 59). This limits the clinical significance of NBI as an overall clinical tool as it has not been tested with the general population. Also, confounders such as hyperkeratinization and thick leukoplakias may obstruct vasculature visualization by NBI (55). Although NBI shows great promise as an adjunctive tool in screening for SCC, further research is required before it can be used as a “visual biopsy”.
1.5.2.3.2 Use of Algorithms

A severe limitation in using optical imaging devices for tissue visualization and diagnosis is the high level of interoperator variability. The interpretation of autofluorescence in tissue may vary between different clinicians depending on their experience. To remove this operator bias, algorithms based on quantitative data can be created using computer programs to analyze results such as tissue fluorescence(55). Roblyer et al. used a computer algorithm that took in the qualitative data red fluorescence and green fluorescence of tissue, and produced a red-to-green ratio that could then be used to identify if the tissue is malignant or not(60). With this algorithm, a picture of a lesion can be analyzed by the computer to create a “disease probability map” which would highlight areas that are deemed high risk to be dysplastic due to the abnormal red-to-green fluorescence ratio(60). However, the sample population used in this study was biased in that high-risk lesion sites were used and compared to healthy sites. Confounders like inflammation or benign lesions were not tested against this specific algorithm, and not representative of the general population. This is only one example of the use of an algorithm, where many other variables can be used in place of fluorescence to produce a new algorithm for a different use. The possibilities of this technology are still unknown, but future research could validate the results of Roblyer et al. such that producing a “disease probability map” in real-time chairside could prove useful in determining biopsy location and surgical margin delineation(60).
1.5.2.3.3 Optical Coherence Tomography

Optical coherence tomography (OCT) is similar to an ultrasound, where it uses back-scattered signals reflected from different layers within the tissue to reconstruct structural images on a computer. Low-powered infrared light (750nm to 1300nm) is used to produce high-resolution, cross-sectional, and subsurface tomographic images of tissue structure 1 to 3mm deep(61). OCT is a non-invasive tool that can produce 3D images consisting of keratin cell layer, epithelial layer, basement membrane, lamina propria, and rete pegs of oral mucosa(62). Although OCT is able to detect SCC by identifying the structural components above, it is not enough cellular information to grade differing levels of oral dysplasia(62). There has been controversy when determining the sensitivity and specificity of OCT, with some studies claiming a 93% sensitivity and 97% specificity(63) while others found much lower values, reporting that its ability to differentiate between different oral mucosal abnormalities were poor(64). Further research is needed to improve the repeatability of OCT studies before its use in visualizing and defining tumour margins.

1.5.2.3.4 Fluorescence Lifetime Imaging and Reflectance Confocal Microscopy

Often times, tissue visualization requires the combination of different devices or techniques, so that one device may perform a task that another may lack and vice versa. Fluorescence lifetime imaging (FLIM) assesses the decay of fluorescence rather than the intensity of fluorescence(65). Different fluorophores (NADH, FAD, collagen) have differing rates of fluorescence decay, and FLIM can measure the fluorescence intensity of that decay from each individual fluorophore by their corresponding peak emission
wavelength(66). How this becomes useful is its ability to distinguish between benign lesion (where inflammation is the main contributor of loss of fluorescence) and malignant lesions (where loss of collagen is the main contributor of loss of fluorescence) by analyzing the metabolic activity in lesions over fluorescence intensity alone(66). FLIM also has a large field of view, so that the whole oral cavity can be imaged easily. The combination of large field of view imaging (such as FLIM) with high resolution imaging or point measurement techniques has the potential to improve visualization and diagnosis by guiding the clinician to the lesion site with the most advanced state of disease in the oral cavity(66). Reflectance confocal microscopy (RCM) proves to be the point measurement of choice for Jabbour et al. RCM uses spatial filtering through a confocal pinhole, generating high resolution “optical sections” of tissue(66). Variation in RCM image contrast is determined by a tissue refractive index, which accounts for nuclear-to-cytoplasmic ratio, large and prominent nucleoli, increased mitotic activity, abnormal mitoses, and cellular and nuclear pleomorphism(66). RCM can then use these morphological changes to distinguish between normal, precancerous, and cancerous oral tissue(67). This technique shows the most promise as an “optical biopsy”, where FLIM would scout the oral cavity for abnormalities, followed by specific examination by RCM. However, Jabbour et al.(66) performed the study on hamster cheeks and human oral biopsies, although with promising results. This technique is still in its infant stages, and its next step is to repeat its experimental methods on humans in vivo.
1.6 Second Oral Malignancies

Second oral malignancies (SOM) is an overarching term used that refers to any tumour, regardless of location in the mouth, that occurs in OSCC patients after the treatment of their primary cancer. Researchers have further defined SOM into different branches: local recurrences, second primary tumours (SPT), and second field tumours (SFT). This section first discusses the prevailing theory behind how SOM occur, followed by the definitions of the different classifications of SOM.

1.6.1 Field Cancerization

Field cancerization is a term first coined by Slaughter et al. in the 1950s to describe the abnormal tissue adjacent to an oral tumour site, as a possible explanation for the development of second primary tumours or local recurrences(68). With their early histological research, they found that all epithelium surrounding the boundaries of oral tumours showed abnormal histological changes. This expanded “field” of abnormal mucosa is thought to be due to prolonged exposure to carcinogens, causing an altered field of mucosa more susceptible to malignant transformation(69,70). From this, Slaughter et al. proposed that cancer does not arise as a single, isolated malignancy, but rather as a multifocal field consisting of many anaplastic cells that progress to malignancy at differing rates(68). With advances in scientific methods and a better understanding of cell processes, researchers have identified molecular analyses that better identify and describe field cancerization with the use of DNA amplification techniques, immunohistochemistry, and in situ hybridization(69,70). With the introduction of molecular assays that assess loss of heterozygosity (LOH), microsatellite alternations,
chromosomal instability, and TP53 gene mutations, the definition of field cancerization can now be expanded to describe the presence of one or more areas consisting of epithelial cells that have genetic alterations adjacent to the primary oral tumour(2). Thus, the following concepts about oral field cancerization can be said(69,70):

1. Oral cancer develops in multifocal areas of precancerous change.
2. Abnormal tissue surrounds the tumour.
3. Multiple independent lesions can coalesce into one tumour.
4. Persistence of abnormal tissue after removal of the oral tumour may explain occurrence of local recurrences and second primary tumours.

1.6.2 Monoclonality Theory

One theory that attributes field cancerization to an increased risk to developing local recurrences or second primary tumours is the monoclonality theory(70). Before the development of an altered mucosal field, a stem cell first acquires the genetic alteration(s) needed to escape normal growth control, gain growth advantage, and produce more genetically altered daughter cells. The genetically altered daughter cells grow to become “clusters” or “patches” (<200 cells diameter) that all share the same common progenitor and genotype, with a mutation in the TP53 gene(69,70). As the patch grows laterally, it displaces normal epithelium and now becomes a field(69,70). At this point, the field may be large enough to be seen clinically. Over time, additional genetic alterations are added onto the continuously dividing cells at various locations of the field(69). As a result, different “subclones” are found within the field that can differ in genotype and phenotype, but still share the common progenitor(69). Thus when an oral tumour is
removed, a field is left behind. A subclone, through clonal divergence and selection, may eventually evolve within that field into recurrent or second primary cancer(69).

The significance of field cancerization is great in that it provides a possible explanation for the occurrence of recurrent cancers. As mentioned above, the presence of a “remaining field” of genetically altered cells even after surgical removal of the primary tumour presents as a significant risk factor for oral cancer recurrence. Thus, clinical detection of these possible altered fields must be improved by further exploring how adjunctive aids, such as TB and FV, can be used with clinicopathological features to characterize risk of recurrence(69,71). Hopefully with new advances in visualization, clinically detectable risk factors can be identified to better guide decision-making by clinicians and predicting risks of developing recurrences(69,71).

### 1.6.3 Local Recurrences and SPTs

Warren and Gates first laid out the definition of SPTs, setting the criteria as: 1) both primary and secondary tumour are malignant; 2) the primary tumour site is geographically separate from the secondary tumour site; and 3) the SPT must not be a metastasis of the first primary tumour(72). Later, authors would expand on Warren and Gates’ traditional method of definition and include that a SPT must occur at least 1.5-2cm away from the initial site, while a local recurrence would then be classified as occurring within 2cm of the original tumour diagnosis(73). However, there is no consensus between different authors regarding the maximum distance allowed between two tumours before a REC becomes a SPT, with distances varying from 1.5cm-3cm being used(74–76). Another factor to distinguish REC from SPT is the time of occurrence after
the primary tumour. If a REC develops 3 or more years after primary tumour diagnosis, then it is considered a SPT. It is often problematic to use time of diagnosis as a criterion for distinguishing REC and SPT, since timetables for cancer treatments vary per patient. Instead, time from end of treatment may be a better time criterion, measuring the “disease free” time until REC or SPT. If time from diagnosis was used, and a patient who underwent lengthy radiotherapy treatments experienced a SOM, a REC that occurred may be mislabelled as a SPT by clinicians.

Moving away from REC and SPTs, Brakkhuis et al. (77) proposed a new term for SOMs that did not require the criterion of distance or time from the primary tumour. Brakkhuis et al. coined the term “second field tumours” (SFTs), which uses molecular markers to assess if the SOM originated from the primary tumour. The SFT theory states that a new tumour arising from a preconditioned field will share similar genetic characteristics as the primary tumour during its early stages of development, but may acquire additional genetic mutations as it grows (69,73). SFT describes a tumour with similar genetic alterations as the primary tumour, based on the molecular comparisons between microsatellite analysis of LOH and p53 mutation analysis (69,73,78). However, this theory is difficult to incorporate clinically, as not all tumours are tested with genetic analysis. The different SOM classifications are outlined in the following table:
Table 1.2 SOM Classifications.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Second Oral Malignancy</strong></td>
<td>An umbrella term that encompasses tumour REC, SFTs and SPTs. In literature this definition has been used interchangeably with any of the listed terms.</td>
</tr>
<tr>
<td><strong>Recurrent Tumour</strong></td>
<td>A second tumour that is located in the same or contiguous anatomical site and all genetic alterations are similar to the primary tumour. For the purposes of this thesis a REC occurs within 3cm of the original tumour site.</td>
</tr>
<tr>
<td><strong>Second Field Tumour</strong></td>
<td>A second tumour that has developed from the same genetically altered field as the first tumour, regardless of the time or distance. Some molecular markers are similar and others differ.</td>
</tr>
<tr>
<td><strong>Second Primary Tumour</strong></td>
<td>A true second tumour that develops independently from the primary tumour. Molecular profiles are completely different from the primary tumour. For the purposes of this proposal, SPT occurs at least 3cm away from the primary site.</td>
</tr>
<tr>
<td><strong>Metastasis</strong></td>
<td>A second tumour that develops in a different and distant anatomical site and all genetic aberrations are alike to the initial tumour.</td>
</tr>
</tbody>
</table>

Adapted from Braakhuis *et al.*, 2002 and Rosin *et al.*, 2002. (73,78)

### 1.6.4 Incidence and Time to REC

The rate of recurrence for oral cancer has been found to be 15-30% (79). Most recurrences occur within the first 2 years of follow-up after the completion of treatment.
1.6.5 Determining Risk of REC

Treatment of the primary oral cancer can result in tissue changes at the former cancer site. Scars as a result of surgery and radiation changes to the tissue may increase the difficulty of visualizing tissue changes in the area. To add to the difficulty, these sites may be subject to further trauma or reactive changes due to the treatment and potentially more friable tissue. A challenge faced by clinicians is the ability to discriminate between treatment effects and new lesions.

Recurrence may be a result of a small number of abnormal cells remaining at the site that can’t be detected with histology or it may be due to field cancerization. The primary tumour was part of a larger field of altered cells that did not progress until after the first tumour had been treated(69,76).

To date most markers for recurrence are based on the primary tumour, such as disease at the margins, stage of disease and treatment. Positive margins, later stage and treatment with radiation or radiation and chemotherapy have been associated with an increased risk of REC(80,81). While important indicators of risk, none of these will help the clinician assess risk of REC during surveillance. Clinical indicator are needed and adjunctive devices need investigation to determine if they can aid the clinician to distinguish tissue at risk from benign reactive change or treatment effects and whether these indicators maintain their efficacy throughout follow-up.
Chapter 2: The Problem

As the sixth most common cancer in the world with a global 5-year survival rate of 50% (2,3), clinicians and researchers are looking for ways to improve survival of those diagnosed with oral cancer. One way purported to improve patient survival is to monitor the oral cancer survivor closely for early signs of REC, which has been reported to occur in up to 30% of patients (9,10,82–86). Thus, there is a need to identify clinical markers present at the former tumour site of oral cancer survivors that may help clinicians better detect and treat a REC or potential REC sooner, improving the patient’s chance of survival and quality of life. Some clinical markers such as presence of an OPL and TB have been found to be associated with primary progression of oral cancer (7,26,47), but it is unknown how well these factors, along with FV may guide a clinician to assess risk of a REC in an oral cancer survivor. It is also unknown whether these clinicopathological risk factors may vary over time in surveillance. Ultimately, the goal of this research is to increase in the survival rate of oral cancer by early detection of oral cancer REC.
Chapter 3: Objectives

1. To determine the demographic, clinical, and histopathological risk factors associated with local recurrences in patients previously treated for primary oral cancer.

2. To determine if FV is associated with oral cancer recurrence.

3. To determine if the clinical risk factors associated with local recurrences are similar at different time points in follow-up.
Chapter 4: Hypotheses

1. Demographic, clinical, and histopathological risk factors associated with local recurrences will be similar to those associated with oral premalignant lesion progression to primary tumours.

2. Loss of fluorescence is associated with recurrence in patients with a history of oral cancer.

3. Clinical risk factors will be the same regardless of the amount of time passed since the end of treatment.
Chapter 5: Materials and Methods

5.1 The BC Oral Cancer Prediction Longitudinal Study

The source of patients and data for this study comes from the Oral Cancer Prevention Longitudinal Study (OCPL), an ongoing prospective study supported by the National Institute of Dental Craniofacial Research and the British Columbia Cancer Foundation. The OCPL study began in British Columbia, Canada in 1997, with the goal of improving detection, risk assessment, and management of patients with oral malignant or premalignant disease. The study has two arms in follow-up: the first arm includes patients diagnosed with SCC or CIS and assesses risk of recurrence (REC) or second primary tumours; while the second arm include patients with oral premalignant lesions (OPL) (mild, moderate, or severe oral dysplasia) and assesses risk of progression to primary oral cancer. Patients are identified and referred to the OCPL study through the British Columbia Oral Biopsy Service. Directed by Dr. Lewei Zhang, the Oral Biopsy Service is a provincial pathology referral service for general physicians and dentists in BC to use for histopathological diagnosis of oral disease. Once identified, the patients are referred to one of the five Oral Dysplasia Clinics for evaluation and potential recruitment to the study.

Patients participating in the OCPL attend any of the five Oral Dysplasia/Oral Oncology Clinics (Vancouver Cancer Centre (VCC), Fraser Valley Cancer Centre (FVCC), Vancouver General Hospital (VGH), University of British Columbia Specialty Clinic, and Southern Interior Cancer Centre (SICC), where they obtain treatment and follow-up.
Ethics approval, confidentiality, and the process of obtaining consent in the OCPL are described later under Patient Consent and Confidentiality.

5.2 Eligibility

The current study utilizes patients from the existing OCPL database who enrolled in the study from January 1, 1999 to December 17, 2014. Patients are eligible for this study if they: 1) are aged 18 years or older; 2) had a primary tumour diagnosis of SCC or CIS; 3) treated with curative intent with non-laser surgery and/or radiation therapy; and 4) were accrued to the study and had at least one follow-up visit within one year after completion of primary oral tumour treatment. Patients were excluded if their medical history precludes them from participating in standard diagnostic tests or regular follow-up. Patients were also excluded if they were diagnosed with severe dysplasia, CIS or SCC at a follow-up visit within 6 months of the completion of cancer treatment. This exclusion removed patients whose initial treatment may not have been curative and were left with residual disease. Outcome for this study is defined as a recurrence (REC) or death. A recurrence is defined as a second oral malignancy at a site ≤ 3cm away from the initial site and occurring ≥6 months after the primary tumour treatment, determined from biopsy. An oral malignancy diagnosed at a site greater than 3cm from the initial site, it is identified as a second primary tumour and the patient is excluded from the study. Curative intent refers to treatment provided to completely remove all malignant tissue.

A total of 232 patients that fit the inclusion criteria, 34 developed a REC and 198 did not develop a REC prior to the last follow-up date.
5.3 Patient Consent and Confidentiality

Ethics approval for the OCPL study was obtained through the University of British Columbia and BC Cancer Agency Institutional Research Board (H98-61224; H05-60116).

Patients are first made aware of the study when they visit one of the Oral Dysplasia Clinics for their initial consult with the oral medicine specialist. If the patient shows interest, the study’s aim and goals are described to the patient by a collaborating clinician and study coordinator. Patients are advised that participation in the OCPL study is strictly voluntary, and they are able to withdraw from the study at any time. Interested patients then sign a written informed consent form.

A unique study identification number is assigned to each consenting patient at the point of accrual. All patient information, health history, clinical and molecular data collected is stored in a password protected secured server with access limited to select OCPL staff. Data required for this thesis was provided in coded form to King Yin Wu with the only identifiable information being the unique study identification numbers.

5.4 Data Collection

Each patient accrued to the study has a clinical research file (CRF) which holds their clinical and pathology documents. Study personnel, including graduate students collect and document data. The clinical examination is completed by qualified study personnel and an oral medicine specialist. Demographic and risk habit information is collected by questionnaire. Digital images of all lesions being followed for the study are uploaded and stored on a secure server. All data is uploaded to the study database.
5.4.1 Data Collection at Entry

On accrual to the study, the patient first completes a standardized study questionnaire (Appendix A) detailing their demographic information (age, gender, ethnicity), medical and family history, and risk habits associated with oral cancer. Further detail regarding risk habit information collected is found below. Medical history includes all current medical conditions, prescription and non-prescription medications, allergies, personal and family history of cancer, and recent hospital visits.

5.4.2 Initial Visit

The initial visit includes the review of pathology reports, a clinical examination and the collection of study samples. The clinical examination includes the palpation and visualization of the extraoral tissue of the head and neck (extraoral exam) and the oral cavity (intraoral exam). The former cancer site is assessed for the presence of a lesion. All lesions present at the time of the visit are assessed and each lesion is assigned a code: lesion site A (LSA), lesion site B (LSB) and so on, as necessary. Lesions within 3cm of the treated oral cancer site are part of the original lesion field and receive the same code. Lesions are further documented on an illustrated mouth map (Appendix B). Initial study samples collected include a saline wash, and cytology brushings of the lesions and a normal site. The following data is collected and recorded in the CRF:

1) Health history collection/update
2) Extra-oral and Intra-oral (white light) examination
3) Lesion site (Appendix B)
4) Lesion presence/absence and characteristics (colour, texture, appearance, size, and margins) (Appendix B)
5) Fluorescence visualization (FV) (VELscope®, Burnaby, BC) results
6) Toluidine Blue (TB) staining results
7) Exfoliative cytology samples taken
8) Digital photographs taken at white light examination, FV, and TB steps
9) Document interim therapy
10) Additional comments on lesion by specialist or study staff

The patient CRF is separate from their medical chart, and is available only to study personnel. All data and information are then uploaded into the OCPL database and sorted by patient identification number and date of visit.

5.4.3 Data Collection at Follow-up

Patients enrolled in the study are seen at regular recall intervals. A certified oral medicine specialist and study coordinator oversees all regular follow-up visits of the patients. The interval at which the patient is followed-up can be three, six, or 12 months depending on lesion condition and the specialist’s recommendation. Patients with a history of oral cancer are seen every 3 months for the first 2 years and then every 6 months for the duration of the study. Tobacco and alcohol exposure and usage are updated yearly using an annual questionnaire (Appendix C). The follow-up visit includes a medical history update, an extra-oral, intra-oral, TB and FV exam and lesion brushings. All clinical findings are documented in the CRF, and digital photographs are taken and stored in the OHS database.
5.4.4 Tobacco and Alcohol Data

Risk habit information collected include the type, amount, and the frequency of tobacco and alcohol consumption and exposure, and betel nut usage. Alcohol usage is classified into ever drinkers or never drinkers. Never drinkers are defined as having less than one alcoholic beverage per month. For ever drinkers, the type and amount of alcohol consumed is collected. Type is listed as beer, wine and spirits and quantified by the number of drinks consumed per week. One drink is defined as 8 oz. beer, 4 oz. wine, or 1 oz. spirits.

Regarding tobacco use, patients are categorized as an ever smoker or a never smoker. A never smoker is someone who has smoked less than 100 cigarettes (or equivalent) in their lifetime. Ever smoker is further divided into current smoker and former smoker. For the purpose of this study, former smoker (FS) is further broken down into FS quit smoking more than one year prior to primary oral cancer diagnosis and FS quit less than one year prior to or at diagnosis. A current smoker is as an individual who continues to smoke or use tobacco products after their oral cancer diagnosis. The amount smoked is recorded in pack-years, which is defined as the number of packs of cigarettes smoker per day multiplied by the number of years smoked. For patients with a history of smoking pipes and/or cigars, one cigar and one pipe are equivalent to 2 and 3 cigarettes, respectively (as per BCCA standards). Patients are also asked about second hand smoke exposure at home, work, or public places per week. Second hand tobacco exposure will not be reviewed in this thesis.
5.4.5 Primary Tumour Information

Primary tumour (primary CIS or SCC) is collected, including histological diagnosis of tumour, date of diagnosis, TNM staging, tumour grade, treatment modality and the start and end date of treatment. This data is obtained from the pathology reports (diagnosis) and the BCCA database (stage, grade and treatment type and end dates). The treatment end date is the last date of all treatment related to the primary tumour.

5.4.6 Extra-oral Examination

The extra-oral examination consists of the clinician inspecting and palpating the patient’s head and neck and surrounding structures, including the forehead, temporomandibular joint (TMJ), mandible, bridge and ala of the nose, lips, chin, and the neck. The clinician also inspect the masseter and sternocleidomastoid muscle, inquiring the patient if they are tender upon inspection. The purpose of this exam is to assess for any lymph nodes or extraoral skin lesions. Specific lymph nodes assessed include the periauricular, submental, buccal, cervical, occipital and supraclavicular nodes.

5.4.7 Intra-oral Examination

The intraoral examination is also known as the white light examination (WLE). For this exam, the clinician visualizes and palpatates all soft and hard tissue of the oral cavity (buccal mucosa, labial mucosa, upper and lower gingiva, tongue, hard and soft palate, and tonsils). The former tumour site is inspected for any irregularities. The clinician assesses the presence (OPL+) or absence (OPL-) of a lesion at the former tumour site, noting its position on the mouth map (Appendix B) and clinical
characteristics on the lesion-tracking sheet (Appendix D). The characteristics of a lesion are explained below:
Table 2.3 Characteristics of a Lesion Included in the Patient CRF

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence</td>
<td>An oral premalignant lesion is identified (OPL+) or absent (OPL-)</td>
</tr>
<tr>
<td>Location</td>
<td>The location of the lesion is documented on the mouth map on a grid system</td>
</tr>
<tr>
<td>Outline</td>
<td>The lesion can be <strong>discrete</strong> (well defined borders) or <strong>non-discrete</strong> (ill-defined borders)</td>
</tr>
<tr>
<td>Size</td>
<td>The size of the lesion in length, width and thickness, measured in millimeters using a standard colour-coded dental probe</td>
</tr>
<tr>
<td>Colour</td>
<td>The colour of a lesion, being red, white, or a mix of both</td>
</tr>
<tr>
<td>Appearance</td>
<td>The lesion can be <strong>homogenous</strong> (of same colour and texture throughout), or <strong>non-homogenous</strong> (different colour and texture throughout)</td>
</tr>
<tr>
<td>Texture</td>
<td>The lesion can be ulcerated, nodular, sessile, smooth, velvety, verrucous, fissured, or others</td>
</tr>
<tr>
<td>Fluorescence Visualization Results</td>
<td>Fluorescence Visualization <strong>positive</strong>, <strong>negative</strong>, or <strong>masking/equivocal</strong></td>
</tr>
<tr>
<td>Toluidine Blue Stain Results</td>
<td>Toluidine Blue stain <strong>positive</strong> uptake, <strong>negative</strong> uptake, or <strong>equivocal</strong> uptake</td>
</tr>
<tr>
<td>Biopsy Taken</td>
<td>Yes or No</td>
</tr>
<tr>
<td>Interim Therapy</td>
<td>Details of any treatment or therapy the patient is scheduled to receive between the present appointment to the next</td>
</tr>
</tbody>
</table>

5.4.8 Toluidine Blue (TB) Staining

TB staining is completed at each site and at each study visit. The solution is prepared in the hospital (BCCA) pharmacy by mixing 1 gram of TB with 10 ml of acetic
acid, 4.9 ml of absolute alcohol, and 86 ml of distilled water yielding a 1% TB solution. The pH of the solution is adjusted to 4.5 using 2M NaOH.

The clinician applies the TB stain to the former tumour site and any other suspect areas found in the oral cavity. To prepare the site for staining, it is first cleared of any debris with a cotton tip swab soaked in 1% acetic acid solution. The site is then painted using another cotton tip swab soaked in 1% TB solution. After 45 seconds, a third cotton tip swab is used with 1% acetic acid to take away excess dye at the site followed by a rinse with water. Notes are taken of all stained sites, but the TB status of interest to this study is that of the former tumour site.

The attending clinician identifies a positive uptake (TB+) if the site is stained with a royal blue colour. If there is weak stain uptake or if the clinician is unsure of the result, the stain is considered equivocal (TBE). If there is no uptake at the site, it is negative (TB-).

5.4.9 Fluorescent Visualization (FV)

FV status is determined using the VELscope, (VELscope®, Burnaby, BC) in a dark or dimly lit setting. The room light is turned off to carry out this exam. The FV light is shone on all intraoral surfaces similar to the intraoral examination with an emphasis on the former tumour site and any other suspicious areas. Results are recorded for all sites, however for this study the FV status of interest for this study is that of the former tumour site.

FV status is assessed by the amount of tissue fluorescence at the site. Tissue that has no loss of fluorescence appears fluorescent green (FV-). A loss of fluorescence (FV+)
is identified if the area observed emits no fluorescence from the area; it appears noticeably darker shade than the surrounding area. Results are considered equivocal (FVE) when then there is uncertainty regarding a loss of fluorescence. There are confounders to FV status that would yield a masking result. The gingiva and hard palate are areas in the oral cavity with high levels of keratinization that would appear as FV+, but in reality the result is “masked” by keratin. In the presence of an ulcer or trauma not related to oral premalignancy, the area may also appear to be FV+ confounded by the increased level of blood flow and inflammation at the site.

5.4.10 Digital Photos

Photographs of all lesions, including the former tumour site documenting the results from WLE, FV, and TB are taken by the clinician using a Nikon D7100 digital camera at each visit. Images prior to digital technology were taken on slides and later scanned into an image database. At the end of each clinic, the study coordinator transports the digital files from the camera memory card into the OCPL database, where they are sorted by study ID and date of appointment visit. Study personnel are able to access these photos through the OCPL database for study purposes and quality control.

5.4.11 Biopsy

The OCPL study protocol calls for a biopsy every two years, but this period can be shorter if the attending clinician deems it necessary. If the clinician identifies a suspicious lesion at the former tumour site based on WLE, FV, and TB findings, he/she would suggest a biopsy be taken.
The biopsy procedure involves the following:

1) **Site Selection:** The attending clinician identifies the site within a suspicious lesion to be biopsied. The clinician will most often choose to biopsy the area where he/she notices the most change, guided by clinical observations, FV, and TB findings. In the case of large lesions, multiple biopsies may be taken.

2) **Biopsy Procedure:** Topical anesthetic is applied to the biopsy site to minimize discomfort. Local anesthetic is then injected into the oral tissue adjacent to the biopsy site to avoid possible artefacts in histological diagnosis from needle penetration. Ideally, biopsies are 5mm in diameter with a depth of at least 2mm. Hemostasis is achieved with AgNO$_3$ cauterization and/or sutures. Smokers are advised to refrain from smoking until the wound is healed, and all patients are asked to be cautious when eating or speaking so as to not risk reopening the wound.

3) **Biopsy Submission:** The biopsy sample is stored in a container of 10% neutral buffered formalin fixative solution and submitted to the BC Oral Biopsy Service for histological assessment.

### 5.4.12 Statistical Analysis

Data will be compared between two main groups: those who developed an oral cancer recurrence (REC) and those who remained oral cancer free after primary treatment (Non-REC). Demographic, lifestyle, tumour characteristics and clinical risk factors between the two groups are analyzed to determine variables associated with recurrence or non-recurrence.
Statistical tests are performed to determine any differences or associations between the recurring and non-groups. Categorical data such as gender, tobacco and alcohol consumption, presence of tumour, clinical appearance, site, tumour stage, histological diagnosis grade, TB and FV results are analyzed using the Pearson’s chi-squared test, while Fishers exact test will be applied when the sample size is small or for 2x2 contingency tables. The Student’s t-test is used for continuous parametric data such as: age, tumour size, and mean follow-up time of patients. For continuous data that has a non-Gaussian distribution, a Mann-Whitney test is applied.

The clinical characteristics OPL, TB, and FV were also compared between REC and Non-REC groups with the Chi-squared test. These three characteristics were analyzed further by comparing each status at different time periods in follow-up (6m, 12m, 18m and 24m from treatment completion). In the case where no follow-up visit was scheduled at 6, 12, 18, or 24 months after treatment completion, data from the closest follow-up visit available (±3 months from intended date after treatment) was used. The relative risk of OPL, TB, and FV status with REC are expressed as odds ratios with 95% confidence intervals. The worst clinicopathological risk factors ever (“ever”) in follow-up and their association with REC and NONREC are also compared using the Chi-squared test.

For survival analysis, the time-to-recurrence or time-to-last follow-up curves are calculated with the Kaplan-Meier (KM) estimator, and to compare any survival distribution significances the log-rank test is applied. For example, for the KM curve for the presence of an OPL at 6 months and REC, the patients are categorized into 2 groups, those with an OPL at 6 months and those without and the presence or absence of a REC.
in follow-up. The HR and corresponding 95% confidence intervals are revealed using the cox-regression analysis. All the tests are two-sided and the results are considered statistically significant $P < 0.05$. Statistical analyses are done with SPSS software (Version 24, 2016).
Chapter 6: Results

This study investigated clinicopathological indicators of REC at different time periods during follow-up to determine association with that outcome. The data will be presented in the following order: demographic data, tumour data, clinical data ever in follow-up, clinical data at different time points in follow-up, and biopsy data. Overall, 34 patients (15%) developed a REC while 198 (85%) remained tumour free for the duration of their time in the study. Of the REC patients, 8 (24%) developed within their first year of follow-up, another 8 (24%) developed within the second year of follow up, 3 (9%) developed within the third year of follow up, 6 (18%) developed within 4 years of follow up, and 9 (26%) developed after 4 years of follow up. Median time to REC was 26.5 months and median time to last day of follow-up for non-REC patients was 62 months.

6.1 Demographics and Lifestyle Habits of the Study Population and REC

Patient characteristics collected include demographic data (age at primary diagnosis, sex, ethnicity), and lifestyle habits (frequency and duration of tobacco and alcohol usage). Table 6.1 shows this data for the 232 patients who met the eligibility criteria for this study.

There was no significant association between demographic or risk habit data and oral cancer recurrence (Table 6.1). The majority of the study population was over 40 years old (92%) at the time of their primary diagnosis; 85% and 93% were over 40 years of age for the REC and NONREC groups, respectively. Overall, the median age of all patients was 60 years old. REC patients had a median age of 58 years, and NONREC had
a median age of 60 years. Although not statistically significant, a greater proportion of patients whose primary tumour was diagnosed under 40 years of age (26%) developed a REC compared to 14% of patients over 40 years of age (P=0.17). Almost two-thirds of the patients were male (N=148) and of these, 22 (15%) developed a REC. A similar proportion of the 84 females (14%) developed a REC in follow-up. More than 80% of the study population were Caucasian (N=187), and the remaining 43 patients are listed as “Other” (Asian, First Nations, and mixed decent). No association was found between ethnicity and REC (P=0.87). Two patients in the study were missing information on their ethnicity.

There were no significant differences in risk habits and REC. All but one patient in the study population had smoking data available for analysis; of these, 81 (35%) patients had never smoked, 92 (40%) patients were former smokers who quit at least one year prior to their primary diagnosis, 14 (6%) patients were former smokers who quit at or within one year of diagnosis of oral cancer, and 44 (19%) were current smokers. Of interest, the highest proportion of REC occurred in the group who continued to smoke after their primary cancer diagnosis (21%), followed by never smokers (16%), former smokers who quit more than one year prior to their primary diagnosis (13%). None of the 14 patients who quit at diagnosis or within the year prior to diagnosis developed a REC. One hundred seventy-four patients had data on their alcohol usage, and of these 144 patients (83%) admitted to having ever consumed alcohol in their lifetime. Although not significant, a higher proportion of never drinkers developed a REC (23%) than patients with a history of alcohol use (17%). When comparing “heavy drinkers” to “light or never
drinkers”, roughly the same proportions from both these groups developed a REC (18% and 19% for light/never and heavy drinkers, respectively).

Table 6.4 Demographics of Study Population

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)</th>
<th>Non-REC (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>232</td>
<td>34 (15)</td>
<td>198 (85)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (N=232)</strong> 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40 years</td>
<td>19 (8)</td>
<td>5 (26)</td>
<td>14 (74)</td>
<td>0.17</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>213 (92)</td>
<td>29 (14)</td>
<td>184 (86)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex (N=232)</strong> 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>148 (64)</td>
<td>22 (15)</td>
<td>126 (85)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>84 (36)</td>
<td>12 (14)</td>
<td>72 (86)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity (N=230)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>187 (81)</td>
<td>28 (15)</td>
<td>159 (85)</td>
<td>0.87</td>
</tr>
<tr>
<td>Other 4</td>
<td>43 (19)</td>
<td>6 (15)</td>
<td>37 (85)</td>
<td></td>
</tr>
<tr>
<td><strong>Tobacco (N=231)</strong> 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoker</td>
<td>81 (35)</td>
<td>13 (16/38)</td>
<td>68 (84/35)</td>
<td></td>
</tr>
<tr>
<td>Former Smoker &gt;1yr</td>
<td>92 (40)</td>
<td>12 (13/36)</td>
<td>80 (87/40)</td>
<td>0.53</td>
</tr>
<tr>
<td>Former Smoker ≤1yr</td>
<td>14 (6)</td>
<td>0 (0)</td>
<td>14 (100/7)</td>
<td></td>
</tr>
<tr>
<td>Current Smoker</td>
<td>44 (19)</td>
<td>9 (21/26)</td>
<td>35 (79/18)</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol (N=173)</strong> 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol Ever</td>
<td>144 (83)</td>
<td>25 (17)</td>
<td>119 (83)</td>
<td>0.44</td>
</tr>
<tr>
<td>Alcohol Never</td>
<td>29 (17)</td>
<td>6 (23)</td>
<td>23 (77)</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol (N=173)</strong> 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light/Never Drinker</td>
<td>131 (75)</td>
<td>23 (18)</td>
<td>108 (82)</td>
<td>0.82</td>
</tr>
<tr>
<td>Heavy Drinker</td>
<td>42 (25)</td>
<td>8 (19)</td>
<td>34 (81)</td>
<td></td>
</tr>
</tbody>
</table>

1. All percentages for all tables are given for rows. Percentage are listed in row/column for categories with more than 2 rows.
2. Median overall age of diagnosis was 60 years old, median age of diagnosis was 58 for REC patients, 60 for NONREC patients.
3. Ethnicity data missing for 2 patients.
4. “Other” ethnicity includes Asian, First Nations, and Mixed race.
5. Never smokers are those who have smoked less than 100 cigarettes in their lifetime.
6. Former smokers are divided into those who have quit greater or less than 1 year after primary diagnosis.
7. Consumption of 1 drink is defined as consumption of 8 oz of beer, 4 oz of wine, or 1 oz of spirits; heavy drinkers consume more than 14 and 21 drinks per week for women and men, respectively.
6.2 Primary Tumour Characteristics of the Study Population and REC

The characteristics of the primary tumour of the patient population evaluated in this study include the site, stage at diagnosis, and the method of treatment. Table 6.2 shows these data.

None of the primary tumour characteristics were associated with REC although stage at diagnosis showed a trend. High-risk sites in the western world are the ventral and lateral tongue and the floor of the mouth (see section 1.1 in introduction). The majority of the primary tumours in this study were found at high-risk sites (81%), were early stage (58%) and were treated with surgery only (77%). “Other” sites included the buccal mucosa, gingiva, hard and soft palate, and upper and lower lip. A greater proportion of patients whose primary tumour site was located in “other” sites developed a REC compared to high risk sites (22% vs 13%, P=0.16).

One hundred and sixty-eight of the primary tumours had staging information available. Of those with staging data available 41 were CIS (24%), 98 were early stage SCC (58%) and 29 were late stage SCC (17%). A larger proportion of CIS patients developed a REC (29%) than either early stage (15%) or late stage SCC (17%), although the difference was not found to be significant (P=0.06). Collapsing invasive cancers together and comparing to CIS, a larger portion of CIS patients developed a REC (24%) than SCC patients (12%), and surprisingly this difference was found to be significant (P=0.04).

All treatment was completed with the intent to cure. The majority of patients (N=178, 77%) had surgery only as their mode of treatment, in addition a further 19% (N=45) had surgery alone or in combination with radiation and/or chemotherapy, with the
remaining 4% (N=9) having been treated with radiation and/or chemotherapy only. There was no significant difference between the type of treatment modality and recurrence, however of the three patients who received surgery and chemotherapy, two developed a REC (67%) and of the 2 who had received all three treatment modalities one developed a REC (50%). All 5 of these patients had been diagnosed with late stage SCC. Overall, 14% with surgery only, 15% with surgery and radiation, and 12% of radiation only developed a REC.
Table 6.5 Primary Tumour Characteristics

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Non-REC (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>232</td>
<td>34 (15)</td>
<td>198 (85)</td>
<td></td>
</tr>
<tr>
<td><strong>Site (N=232)&lt;sup&gt;2&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tongue/FOM</td>
<td>187 (81)</td>
<td>24 (13)</td>
<td>163 (87)</td>
<td>0.16</td>
</tr>
<tr>
<td>Other&lt;sup&gt;3&lt;/sup&gt;</td>
<td>45 (19)</td>
<td>10 (22)</td>
<td>35 (78)</td>
<td></td>
</tr>
<tr>
<td><strong>Stage at diagnosis (N=168)&lt;sup&gt;3&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>41 (24)</td>
<td>12 (29/38)</td>
<td>29 (71/21)</td>
<td>0.06</td>
</tr>
<tr>
<td>Stage 1 and 2</td>
<td>98 (58)</td>
<td>15 (15/47)</td>
<td>83 (85/61)</td>
<td></td>
</tr>
<tr>
<td>Stage 3 and 4</td>
<td>29 (17)</td>
<td>5 (17/16)</td>
<td>24 (83/18)</td>
<td></td>
</tr>
<tr>
<td><strong>Stage at diagnosis (N=232)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>CIS</td>
<td>50 (22)</td>
<td>12 (24)</td>
<td>38 (76)</td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>182 (78)</td>
<td>22 (12)</td>
<td>160 (88)</td>
<td></td>
</tr>
<tr>
<td><strong>Treatment (N=232)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>Surgery only</td>
<td>178 (77)</td>
<td>24 (14/71)</td>
<td>154 (86/78)</td>
<td></td>
</tr>
<tr>
<td>Surgery and Radiation</td>
<td>40 (17)</td>
<td>6 (15/17)</td>
<td>34 (85/17)</td>
<td></td>
</tr>
<tr>
<td>Surgery and Chemotherapy</td>
<td>3 (1)</td>
<td>2 (67/6)</td>
<td>1 (33/&lt;1)</td>
<td></td>
</tr>
<tr>
<td>Radiation only</td>
<td>9 (4)</td>
<td>1 (12/3)</td>
<td>8 (89/4)</td>
<td></td>
</tr>
<tr>
<td>All 3 treatments</td>
<td>2 (1)</td>
<td>1 (50/3)</td>
<td>1 (50/&lt;1)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages in rows and rows/columns for categories with more than 2 rows.
2. "Other" sites include buccal mucosa, buccal gingiva, hard and soft palate, and upper and lower lip.
3. Stage at diagnosis data for 64 patients unavailable.

6.2.1 Relationship between Treatment Type and Primary Tumour Stage and REC

Table 6.3 shows a significant association between primary tumour stage and the type of treatment performed on the patient. A greater percentage of patients diagnosed with CIS had surgery only (94%) compared to patients with SCC (72%). Overall, 28% of patients diagnosed with SCC had either a combination of treatment modalities or radiation and/or chemotherapy in the absence of surgery. A significant association was found between surgery only versus other modalities alone or in combination and primary tumour stage.
Table 6.6 Primary Tumour Stage and Treatment Type

<table>
<thead>
<tr>
<th>Treatment Type (N=232)</th>
<th>ALL (%)</th>
<th>CIS (%)</th>
<th>SCC (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>232</td>
<td>50 (22)</td>
<td>182 (78)</td>
<td></td>
</tr>
<tr>
<td>Surgery only</td>
<td>178 (77)</td>
<td>47 (26/94) (^1)</td>
<td>131 (74/72)</td>
<td></td>
</tr>
<tr>
<td>Surgery and Radiation</td>
<td>40 (17)</td>
<td>0 (0)</td>
<td>40 (100/22)</td>
<td>0.006</td>
</tr>
<tr>
<td>Surgery and Chemotherapy</td>
<td>3 (1)</td>
<td>1 (33/2)</td>
<td>2 (67/1)</td>
<td></td>
</tr>
<tr>
<td>Radiation only</td>
<td>9 (4)</td>
<td>2 (22/4)</td>
<td>7 (78/4)</td>
<td></td>
</tr>
<tr>
<td>All 3 treatments</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>2 (100/1)</td>
<td></td>
</tr>
</tbody>
</table>

Surgery effect (N=232)

| Surgery only           | 178 (77)| 47 (26/94) | 131 (74/72)|         |
| Radiation and/or Chemotherapy involved | 54 (23)| 3 (6/6) | 51 (94/28)| 0.001   |

\(^1\) Percentage given in row/column.

6.2.2 Demographic and Risk Habit Characteristics and Primary Tumour Stage

There were no differences found when comparing the demographic and risk habit characteristics between invasive cancer (SCC) and CIS. Although not significant, a greater percentage of patients with diagnosis of CIS were over 40 years of age, had consumed alcohol and were heavy drinkers, as seen in Table 6.4.
### Table 6.7 Comparison of Characteristics of CIS and SCC Patients

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>CIS (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SCC (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>232 (100)</td>
<td>50 (22)</td>
<td>182 (78)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (N=232)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt;40 years</td>
<td>19 (8)</td>
<td>3 (16)</td>
<td>16 (84)</td>
<td>0.77</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>213 (92)</td>
<td>47 (22)</td>
<td>166 (78)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex (N=232)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>148 (64)</td>
<td>34 (23)</td>
<td>114 (77)</td>
<td>0.51</td>
</tr>
<tr>
<td>Female</td>
<td>84 (36)</td>
<td>16 (19)</td>
<td>68 (81)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity (N=230)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Caucasian</td>
<td>187 (81)</td>
<td>37 (20)</td>
<td>150 (80)</td>
<td></td>
</tr>
<tr>
<td>Other&lt;sup&gt;2&lt;/sup&gt;</td>
<td>43 (19)</td>
<td>12 (28)</td>
<td>31 (72)</td>
<td></td>
</tr>
<tr>
<td><strong>Tobacco (N=231)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Never Smoker&lt;sup&gt;3&lt;/sup&gt;</td>
<td>81 (35)</td>
<td>12 (15/25)</td>
<td>69 (85/38)</td>
<td></td>
</tr>
<tr>
<td>Former Smoker&lt;sup&gt;4&lt;/sup&gt; &gt;1yr</td>
<td>92 (40)</td>
<td>21(23/43)</td>
<td>71 (77/39)</td>
<td></td>
</tr>
<tr>
<td>Former Smoker&lt;sup&gt;4&lt;/sup&gt; &lt;1yr</td>
<td>14 (6)</td>
<td>5 (36/10)</td>
<td>9 (64/5)</td>
<td></td>
</tr>
<tr>
<td>Current Smoker</td>
<td>44 (19)</td>
<td>11 (25/22)</td>
<td>33 (75/18)</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol (N=158)&lt;sup&gt;5,6&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Light/Never Drinker</td>
<td>121 (77)</td>
<td>27 (22)</td>
<td>94 (78)</td>
<td></td>
</tr>
<tr>
<td>Heavy Drinker</td>
<td>37 (23)</td>
<td>13 (35)</td>
<td>24 (65)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages in rows and rows/columns for categories with more than 2 rows.
2. “Other” ethnicity includes Asian, First Nations, and Mixed race.
3. Never smokers are those who have smoked less than 100 cigarettes in their lifetime.
4. Former smokers are divided into those who have quit greater or less than 1 year after primary diagnosis.
5. Consumption of 1 drink is defined as consumption of 8 oz of beer, 4 oz of wine, or 1 oz of spirits; heavy drinkers consume more than 14 and 21 drinks per week for women and men, respectively.
6. Amount of alcohol information missing for 10 patients.

### 6.3 Clinical Characteristics of OPLs and REC

#### 6.3.1 Clinical Characteristics of “Ever” OPLs in Follow-up and REC

Clinical characteristics of OPLs that were investigated in this study include lesion size, appearance, margin definition, texture, and colour. Table 6.5 shows these data for patients that developed an OPL in follow-up at any time (“ever”). The OPL was
evaluated as positive for a feature in this analysis, if it was present at any time during follow-up.

There were no significant associations between any of these clinical features of OPL and the development of a REC, when evaluated using “ever” status. Although more lesions showed characteristics associated with risk in the REC group than in the NonREC group (e.g. more were non-homogenous (56% REC vs. 27% NonREC), not smooth in texture (41% REC vs. 30% NonREC), and of red (15% vs. 9%) or mixed colour (56% vs 49%)), these differences were not significantly different. Collapsing colour into 2 categories, red and mixed red and white versus white was also not significant (70% vs 58%, P=0.32, data not shown). Lesion size did not affect REC with 36% of lesions greater than or equal to 12mm REC compared with 64% of similar size in the non-REC lesions (P=0.81). A greater proportion of OPLs with discrete margins recurred than those with diffuse margins but the results were also not significant (56% vs. 56%, P=0.31). OPLs that were rough, verrucous, or ulcerated in texture were categorized as “other” in the texture category.
Table 6.8 Clinical Characteristics of OPLs Ever within Follow-up

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)$^1$</th>
<th>Non-REC (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (N=71)</strong></td>
<td>71</td>
<td>27 (38)</td>
<td>44 (62)</td>
<td></td>
</tr>
<tr>
<td><strong>Size</strong> (N=71)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥12mm</td>
<td>36 (51)</td>
<td>13 (36/48)</td>
<td>23 (64/52)</td>
<td>0.81</td>
</tr>
<tr>
<td>&lt;12mm</td>
<td>35 (49)</td>
<td>14 (40/52)</td>
<td>21 (60/48)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean size (± SD)</strong></td>
<td>71 (100)</td>
<td>14.8 ± 11.4</td>
<td>15.3 ± 12.4</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Median size</strong></td>
<td>71 (100)</td>
<td>12 ± 11.4</td>
<td>12 ± 12.4</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Appearance (N=70)$^3$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogenous</td>
<td>39 (56)</td>
<td>12 (31/44)</td>
<td>27 (69/63)</td>
<td>0.15</td>
</tr>
<tr>
<td>Non-Homogenous</td>
<td>31 (44)</td>
<td>15 (48/56)</td>
<td>16 (52/27)</td>
<td></td>
</tr>
<tr>
<td><strong>Margins (N=64)$^4$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse</td>
<td>35 (55)</td>
<td>11 (31/46)</td>
<td>24 (69/60)</td>
<td>0.31</td>
</tr>
<tr>
<td>Discrete</td>
<td>29 (45)</td>
<td>13 (45/54)</td>
<td>16 (55/40)</td>
<td></td>
</tr>
<tr>
<td><strong>Texture (N=71)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth</td>
<td>47 (66)</td>
<td>16 (34/59)</td>
<td>31 (66/70)</td>
<td>0.44</td>
</tr>
<tr>
<td>Other$^5$</td>
<td>24 (34)</td>
<td>11 (46/41)</td>
<td>13 (54/30)</td>
<td></td>
</tr>
<tr>
<td><strong>Colour (N=70)$^6$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>26 (37)</td>
<td>8 (31/30)</td>
<td>18 (69/42)</td>
<td>0.53</td>
</tr>
<tr>
<td>Mixed</td>
<td>36 (51)</td>
<td>15 (42/56)</td>
<td>21 (58/49)</td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>8 (11)</td>
<td>4 (50/15)</td>
<td>4 (50/9)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages are given for row/column.
2. 12mm was the median length of all OPLs, thus used as the cut-off.
3. Data for appearance was missing for one patient.
4. Data for margins were missing for 7 patients.
5. “Other” textures include rough, verrucous and ulcerated.
6. Data for colour was missing for one patient.

6.3.2 Clinical Status of Patients at Follow-up Visits

Patients enrolled in the study were recalled at 3 month intervals for the first two years following the completion of their treatment. At each follow-up appointment, the presence or absence of a lesion at the former cancer site was determined as well as clinical status of the former cancer site utilizing white light examination (WLE), fluorescence visualization (FV), and toluidine blue (TB) stain. Tables 6.6, 6.7, 6.8 and 6.9 show these data at 6, 12, 18, and 24 months follow-up intervals.
6.3.2.1 Clinical Status at 6 Months

The presence of an OPL, TB staining and the combination of TB staining and FV loss at the 6 month visit were associated with REC. At that visit, 18% of the former cancer sites had an OPL, 67% of the sites were FV+, 7% were TB+, and 6% were both TB+ and FV+. The presence of an OPL at 6 months follow-up was found to be strongly associated with a REC (p<0.001); 18 out of the 40 (46%) patients who had an OPL at 6 months later developed a REC, compared to 14 out of 181 (8%) patients who did not have an OPL. Having an OPL presented a 9.8-fold increase in risk of developing a REC (4.3-22.3 95% CI).

In contrast, FV status at 6 months was not found to be associated with REC: 3 out of 58 (5%) FV- patients and 11 out of 118 (9%) FV+ patients later developed a REC. However, 95% of FV- sites did not develop a REC during follow-up, suggesting a negative predictive value for this indicator at that time interval.

TB positivity was found to be associated with REC (p=0.046). Five out of 15 (33%) TB+ patients were later diagnosed with a REC, compared to 26 out of 201 (13%) TB- patients (P=0.046). TB positivity showed a 3.4-fold increase in risk of developing a REC compared to TB- (1.1-10.6 95% CI).

The combination of TB and FV status was investigated next. No patients were found to be TB+ and FV- at this time period. The concurrent presence of TB+ and FV+ status at the former cancer sites at 6 months was found to be significantly associated with REC (p=0.034) with a 6.8-fold increase in risk (1.2-39.4 95% CI) compared to sites that were TB- and FV-. Three out of 11 (27%) patients with TB+FV+ developed a REC while
only 3 out of 57 (5\%) and 8 out of 107 (7\%) developed a REC in the TB-FV- and TB-FV+ status groups respectively.

### Table 6.9 Clinical Status at 6 Months Follow-up

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)</th>
<th>Non-REC (%)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPL (N=221)(^3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPL-</td>
<td>182 (82)</td>
<td>15 (8)</td>
<td>167 (92)</td>
<td>1</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OPL+</td>
<td>39 (18)</td>
<td>17 (44)</td>
<td>22 (56)</td>
<td>8.6</td>
<td>(3.8-19.6)</td>
<td></td>
</tr>
<tr>
<td><strong>FV (N=176)(^3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FV-</td>
<td>58 (33)</td>
<td>3 (5)</td>
<td>55 (95)</td>
<td>1</td>
<td>-</td>
<td>0.40</td>
</tr>
<tr>
<td>FV+</td>
<td>118 (67)</td>
<td>11 (9)</td>
<td>107 (91)</td>
<td>1.9</td>
<td>(0.5 – 7.0)</td>
<td></td>
</tr>
<tr>
<td><strong>TB (N=216)(^4)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-</td>
<td>201 (93)</td>
<td>26 (13)</td>
<td>175 (87)</td>
<td>1</td>
<td>-</td>
<td>0.046</td>
</tr>
<tr>
<td>TB+</td>
<td>15 (7)</td>
<td>5 (33)</td>
<td>10 (67)</td>
<td>3.4</td>
<td>(1.1-10.6)</td>
<td></td>
</tr>
<tr>
<td><strong>TB/FV (N=175)(^5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-FV-</td>
<td>57 (33)</td>
<td>3 (5/21)</td>
<td>54 (95/34)</td>
<td>1</td>
<td>-</td>
<td>0.034</td>
</tr>
<tr>
<td>TB-FV+</td>
<td>107 (61)</td>
<td>8 (7/57)</td>
<td>99 (93/61)</td>
<td>1.5</td>
<td>(0.4-5.7)</td>
<td></td>
</tr>
<tr>
<td>TB+FV+</td>
<td>11 (6)</td>
<td>3 (27/21)</td>
<td>8 (73/5)</td>
<td>6.8</td>
<td>(1.2-39.4)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages in rows and rows/columns for categories with more than 2 rows.
2. Out of 232 patients in the study, 11 were not seen at 6 months follow-up, thus had no OPL data.
3. Forty-five patients had no FV data at 6 months follow-up.
4. Five patients had no TB data at 6 months follow-up.
5. Forty-six patients had no TB and FV data at 6 months follow-up.

### 6.3.2.2 Clinical Status at 12 Months

Table 6.7 shows the clinical characteristics of the former cancer sites at the one year follow-up appointment. Only 16\% had an OPL at the former cancer site; 76\% were FV+, 13\% were TB+ and 11\% were both TB+ and FV+. Similar to the 6 month visit, FV status alone was found not to have a significant association with REC. Other associations were positive.
The presence of an OPL at 12 months after the completion of treatment was strongly associated with a REC (p<0.001). More than half of the sites with an OPL (57%) later developed a REC, compared to only 5% of patients who did not have an OPL at this time frame. Having an OPL showed a 24.7-fold increase in risk of developing a REC (9.7-63.1 95% CI).

FV status was not found to be associated with REC, with 3 out of 46 (7%) FV- patients, and 16 out of 148 (11%) FV+ patients, developing a REC. In contrast, TB positivity was again found to be associated with REC (p<0.001). Almost half (46%) of the 28 TB+ sites developed a REC, compared to 9% of the 192 TB- patients. TB positivity showed an 8.9-fold increase in risk of developing a REC compared to TB- (3.6-21.8 95% CI). Concurrent presence of TB+ and FV+ status at 12 months was strongly associated with REC (p=0.004) with a 11.4-fold increase in risk (2.1-60.7 95% CI) compared to sites which were TB-FV-. Eight out of 21 (38%) patients with TB+FV+ developed a REC while 2 out of 39 (5%), 7 out of 125 (6%), and 1 out of 3 (33%) developed a REC in the TB-FV-, TB-FV+, and TB+FV- status groups respectively. Ninety-five percent of the former cancer sites that were TB-FV- remained REC free.
### Table 6.10 Clinical Status at 12 Months Follow-up

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)</th>
<th>Non-REC (%)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPL (N=223)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPL-</td>
<td>188 (84)</td>
<td>9 (5)</td>
<td>179 (95)</td>
<td>1</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OPL+</td>
<td>35 (16)</td>
<td>20 (57)</td>
<td>15 (43)</td>
<td>26.5</td>
<td>(10.3-68.4)</td>
<td></td>
</tr>
<tr>
<td><strong>FV (N=194)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FV-</td>
<td>46 (24)</td>
<td>3 (7)</td>
<td>43 (93)</td>
<td>1</td>
<td>-</td>
<td>0.57</td>
</tr>
<tr>
<td>FV+</td>
<td>148 (76)</td>
<td>16 (11)</td>
<td>132 (89)</td>
<td>1.7</td>
<td>(0.5 – 6.3)</td>
<td></td>
</tr>
<tr>
<td><strong>TB (N=220)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-</td>
<td>192 (87)</td>
<td>17 (9)</td>
<td>175 (91)</td>
<td>1</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TB+</td>
<td>28 (13)</td>
<td>13 (46)</td>
<td>15 (54)</td>
<td>8.9</td>
<td>(3.6-21.8)</td>
<td></td>
</tr>
<tr>
<td><strong>TB/FV (N=188)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-FV-</td>
<td>39 (21)</td>
<td>2 (5/11)</td>
<td>37 (95/22)</td>
<td>1</td>
<td>-</td>
<td>0.004</td>
</tr>
<tr>
<td>TB-FV+</td>
<td>125 (66)</td>
<td>7 (6/39)</td>
<td>118 (94/69)</td>
<td>1.1</td>
<td>(0.2-5.5)</td>
<td></td>
</tr>
<tr>
<td>TB+V-</td>
<td>3 (2)</td>
<td>33 (6)</td>
<td>2 (67/1)</td>
<td>9.3</td>
<td>(0.6-150.7)</td>
<td></td>
</tr>
<tr>
<td>TB+V+</td>
<td>21 (11)</td>
<td>8 (38/44)</td>
<td>13 (62/8)</td>
<td>11.4</td>
<td>(2.1-60.7)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages in rows and rows/columns for categories with more than 2 rows.
2. FV data was not available for 29 patients at the 12 month visit.
3. TB data was not available for 3 patients at the 12 month visit.
4. Both TB and FV data were missing for 35 patients at the 12 month visit.

### 6.3.2.3 Clinical Status at 18 Months

At the 18 month visit the adjunctive devices had less impact on REC prediction than at earlier visits. Fifteen percent of the former cancer sites had an OPL, 55% were FV+, only 3% were TB+ and only 2% were both TB+ and FV+. As at both the 6 and 12 month visits, the presence of an OPL at 18 months was found to be associated with later development of a REC (p=0.003). A smaller proportion of patients with lesions developed REC at 18 months compare to the earlier time frames. Eight out of 29 (28%) patients who had an OPL developed a REC, compared to 12 out of 171 (7%) patients who did not have an OPL. There was a 5-fold increased risk of developing a REC if an OPL was present at 18 months (1.9-13.8 95% CI). FV status was again not found to be associated with REC, with 3 out of 74 (4%) FV- patients developing a REC versus 7 out
of 91 (8%) that were FV+. TB was not found to be associated with REC, although there was a trend toward significance, and only 3% of all the cases were TB+ at 18 months. A much greater proportion of TB+ patients developed a REC versus TB- but the result was not significant (40% vs 9%, P=0.074) although there was an almost 7-fold risk of REC (1.1-43.9 95% CI). Although 25% of the sites that were TB+ and FV+ developed a REC compared to only 5% of TB-FV- and 7% of TB- FV+ sites, the results were not significant.

Table 6.11 Clinical Status at 18 Months Follow-up

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)</th>
<th>Non-REC (%)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPL (N=200)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>OPL-</td>
<td>172 (86)</td>
<td>12 (7)</td>
<td>160 (93)</td>
<td>1</td>
<td>-</td>
<td>0.002</td>
</tr>
<tr>
<td>OPL+</td>
<td>28 (15)</td>
<td>8 (29)</td>
<td>20 (71)</td>
<td>5.3</td>
<td>(1.9 - 14.6)</td>
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</tr>
<tr>
<td>**FV (N=165)**²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FV-</td>
<td>74 (45)</td>
<td>3 (4)</td>
<td>71 (96)</td>
<td>1</td>
<td>-</td>
<td>0.51</td>
</tr>
<tr>
<td>FV+</td>
<td>91 (55)</td>
<td>7 (8)</td>
<td>84 (92)</td>
<td>2.0</td>
<td>(0.5 – 7.9)</td>
<td></td>
</tr>
<tr>
<td>**TB (N=197)**³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-</td>
<td>192 (97)</td>
<td>17 (9)</td>
<td>175 (91)</td>
<td>1</td>
<td>-</td>
<td>0.074</td>
</tr>
<tr>
<td>TB+</td>
<td>5 (3)</td>
<td>2 (40)</td>
<td>3 (60)</td>
<td>6.9</td>
<td>(1.1 - 43.9)</td>
<td></td>
</tr>
<tr>
<td>**TB/FV (N=166)**⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-FV-</td>
<td>75 (45)</td>
<td>4 (5/36)</td>
<td>71 (95/46)</td>
<td>1</td>
<td>-</td>
<td>0.16</td>
</tr>
<tr>
<td>TB-FV+</td>
<td>87 (52)</td>
<td>6 (7/55)</td>
<td>81 (93/52)</td>
<td>1.3</td>
<td>(0.4 - 4.8)</td>
<td></td>
</tr>
<tr>
<td>TB+FV+</td>
<td>4 (2)</td>
<td>1 (25/9)</td>
<td>3 (75/2)</td>
<td>4.7</td>
<td>(0.5 - 70.45)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages in rows and rows/columns for categories with more than 2 rows.
2. FV data was not available for 35 patients at the 18 month visit.
3. TB data was not available for 3 patients at the 18 month visit.
4. Both TB and FV data were missing for 34 patients at the 18 month visit.

6.3.2.4 Clinical Status at 24 Months

The clinical status at 24 months appeared to more similar to the 6 and 12 month clinical results with the presence of an OPL and TB+ being significantly associated with REC. Twelve percent of the patients at 24 months had OPLs at their former cancer sites,
63% were FV+, 6% were TB+ and only 5% were both TB+ and FV+. The presence of an OPL at 24 months was again strongly associated with a REC (p<0.001); 10 of the 22 (46%) patients who had an OPL developed a REC, compared to 9 out of 171 (5%) patients who did not have an OPL. The presence of an OPL at 24 months was associated with a 15.0-fold increase in risk of developing a REC (5.1-43.9 95% CI). FV status was not found to be associated with REC, with 5 out of 69 (7%) FV- patients and 10 out of 115 (9%) FV+ patients developing a REC. TB positivity was found not to be associated with REC (p=0.06) with 27% of TB+ patients later developing a REC, compared to 7% of TB- patients. While a greater proportion of patients who were both TB+ and FV+ (13%) developed a REC the results were not statistically significant (P=0.40).
Table 6.12 Clinical Status at 24 Months Follow-up

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)</th>
<th>Non-REC (%)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPL (N=193)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPL-</td>
<td>171 (89)</td>
<td>9 (5)</td>
<td>162 (95)</td>
<td>1</td>
<td>-</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>OPL+</td>
<td>22 (12)</td>
<td>10 (46)</td>
<td>12 (54)</td>
<td>15.0</td>
<td>(5.1-43.9)</td>
<td></td>
</tr>
<tr>
<td><strong>FV (N=184)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td>FV-</td>
<td>69 (38)</td>
<td>5 (7)</td>
<td>64 (93)</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FV+</td>
<td>115 (63)</td>
<td>10 (9)</td>
<td>105 (91)</td>
<td>1.2</td>
<td>(0.4-3.7)</td>
<td></td>
</tr>
<tr>
<td><strong>TB (N=186)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>TB-</td>
<td>175 (94)</td>
<td>13 (7)</td>
<td>162 (93)</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>TB+</td>
<td>11 (6)</td>
<td>3 (27)</td>
<td>8 (73)</td>
<td>4.8</td>
<td>(1.1-19.8)</td>
<td></td>
</tr>
<tr>
<td><strong>TB/FV (N=171)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>TB-FV-</td>
<td>62 (36)</td>
<td>3 (5/27)</td>
<td>59 (95/37)</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>TB-FV+</td>
<td>101 (59)</td>
<td>7 (7/64)</td>
<td>94 (93/59)</td>
<td>1.5</td>
<td>(0.4-5.9)</td>
<td></td>
</tr>
<tr>
<td>TB+FV+</td>
<td>8 (5)</td>
<td>1 (13/9)</td>
<td>7 (87/4)</td>
<td>2.8</td>
<td>(0.3-30.8)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages in rows and rows/columns for categories with more than 2 rows.
2. FV data was not available for 9 patients at the 24 month visit.
3. TB data was not available for 7 patients at the 24 month visit.
4. Both TB and FV data were missing for 22 patients at the 24 month visit.

6.4 Lesion Persistence

Table 6.10 shows persistence of lesions, TB and FV status over follow-up. Less than one quarter of the patients had an OPL for more than 1 follow-up visit, 11% were TB+ at least twice but almost 80% were FV+ more than once. Of the persisting OPLs, 65% developed a REC compared to 17% remaining REC free (P<0.001). Similarly, sites that were TB+ at more than one visit were more likely to REC than those that were positive only once or not at all (P=0.005). In contrast, the presence of FV positivity at two or more visits did not seem to influence risk of REC, with only 17 or 158 (11%) of such lesions developing a REC. Interestingly, although not significant (P=0.13), 95% of lesions that had FV- status at least twice would remain tumour free.
Table 6.13 Occurrence of Clinical Status Twice or More During Follow-up

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)</th>
<th>Non-REC (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>232</td>
<td>34 (15)</td>
<td>198 (85)</td>
<td></td>
</tr>
<tr>
<td><strong>OPL (N=228)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2x Positive</td>
<td>175 (77)</td>
<td>11 (6/35)</td>
<td>164 (94/84)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>≥ 2x Positive</td>
<td>53 (23 )</td>
<td>20 (38/65)</td>
<td>33 (62/16)</td>
<td></td>
</tr>
<tr>
<td><strong>TB (N=230)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2x Positive</td>
<td>204 (89)</td>
<td>24 (12/73)</td>
<td>180 (88/91)</td>
<td>0.005</td>
</tr>
<tr>
<td>≥ 2x Positive</td>
<td>26 (11)</td>
<td>9 (35/27)</td>
<td>17 (65/9)</td>
<td></td>
</tr>
<tr>
<td><strong>FV (N=201)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2x Positive</td>
<td>43 (21 )</td>
<td>4 (9/19)</td>
<td>39 (91/22)</td>
<td>1.00</td>
</tr>
<tr>
<td>≥ 2x Positive</td>
<td>158 (79)</td>
<td>17 (11/81)</td>
<td>141 (89/78)</td>
<td></td>
</tr>
<tr>
<td><strong>FV (N=197)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2x Negative</td>
<td>115 (58)</td>
<td>13 (11/76)</td>
<td>102 (89/57)</td>
<td>0.13</td>
</tr>
<tr>
<td>≥ 2x Negative</td>
<td>82 (42 )</td>
<td>4 (5/24)</td>
<td>78 (95/43)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages displayed for rows/columns.
2. Multiple visit data was not available for 4 patients.
3. TB data was not available for 2 patients.
4. FV data was not available for 31 patients.
5. Both TB and FV data were missing for 35 patients.

6.5 Median and Mean Time to REC or Last Day of Follow-up

Table 6.11 shows the mean and median time to REC for REC patients, and the mean and median time to the last day of follow-up for Non-REC patients. Patients who developed a REC did so in a median time of 26.5 months or a mean of 28.6 months. Those who remained tumour free remained in follow-up for a median time of 61.4 months or mean of 64.3 months. There was a significant difference (P<0.001) between time to REC for REC patients and time to last day of follow-up for Non-REC patients.
Table 6.14 Median and Mean Time (months) to REC or Last follow-up

<table>
<thead>
<tr>
<th></th>
<th>Recurrence (%)</th>
<th>Non-Recurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (N=232)</strong></td>
<td>34 (15)</td>
<td>198 (85)</td>
</tr>
<tr>
<td><strong>Mean ± SD</strong></td>
<td>38.6 ± 34.3</td>
<td>64.3 ± 32.4</td>
</tr>
<tr>
<td><strong>Median (range)</strong></td>
<td>26.5 (7-150)</td>
<td>61.4 (6-168)</td>
</tr>
</tbody>
</table>

6.5.1 Time from First OPL and TB to REC

Table 6.12 shows the median and mean time from the first OPL or first TB+ to a REC. The median time from the first OPL during follow-up to REC was 15 months, with a mean of 23 months. The median time from the first TB+ during follow-up to REC was 19 months, with a mean of 21.9 months.

Table 6.15 Median and Mean Time (months) from First OPL and TB to REC

<table>
<thead>
<tr>
<th></th>
<th>All (months)(^1)</th>
<th>Median (range)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>REC (N=34)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPL+</td>
<td>27</td>
<td>15.0 (1-93)</td>
<td>23.0 ± 22.6</td>
</tr>
<tr>
<td>TB+</td>
<td>21</td>
<td>19.0 (1-65)</td>
<td>21.9 ± 21.2</td>
</tr>
</tbody>
</table>

\(^1\) Number will not equal 34 since some were both OPL and TB+ at the same visit.

6.6 OPL Survival Curve at 6, 12, 18, and 24 Months Follow-up

The following figures illustrate the probability of developing a REC dependent on the presence or absence of an OPL at 6, 12, 18, and 24 months of follow-up. Patients were placed into OPL status categories at each of the indicated time periods, then followed for outcome thereafter. Figure 6.1 shows OPL status of patients at different
times during follow-up (OPL+ or OPL-) plotted against their time to REC or time of last follow-up visit for NONREC. At all four time frames the presence of an OPL was associated with an increased risk of developing a REC. The presence of an OPL at 12 months was associated with the greatest risk of the four time periods (OR 26.5 (95% CI: 10.3-68.4; P<0.001) compared to OPL- patients. Of interest is the steep drop in the curve for the OPL+ curves at 6 and 12 months, and the drop in the OPL- curves at approximately 100 months.

6.6.1 TB Survival Curve at 6, 12, 18, and 24 Months Follow-up

Figure 6.2 illustrates the probability of developing a REC dependent on the presence or absence of a TB at 6, 12, 18, and 24 months of follow-up. Patients were placed into TB status categories at each of the indicated time periods, then followed for outcome thereafter. Figure 2 shows TB status of patients at different times during follow-up (TB+ or TB-) plotted against their time to REC or time of last follow-up visit for NONREC. At 6 and 12 month time frames the presence of a TB+ was associated with an increased risk of developing a REC. The TB results at 12 months was associated with the greatest risk (OR 8.9 (95% CI: 3.6-21.8; P<0.001) of developing a REC compared to TB- patients.

6.6.2 FV Survival Curve at 6, 12, 18, and 24 Months Follow-up

Figure 6.3 illustrates the probability of developing a REC dependent on the presence or absence of a FV at 6, 12, 18, and 24 months of follow-up. Patients were placed into FV status categories at each of the indicated time periods, then followed for
outcome thereafter. Figure 3 shows FV status of patients at different times during follow-up (FV+ or FV-) plotted against their time to REC or time of last follow-up visit for NONREC. Figure 3 demonstrates the lack of association between the FV status of the former tumour site at 6, 12, 18 and 24 months, respectively, and the development of a REC. Of interest is the relatively flat FV- curve.

6.6.3 TBFV Survival Curve at 6, 12, 18, and 24 Months Follow-up

Finally, figure 6.4 demonstrates the combination of TB and FV results and the probability of developing a REC. Figure 4 illustrates the probability of developing a REC dependent on the presence or absence of a TBFV at 6, 12, 18, and 24 months of follow-up. Patients were placed into status categories at each of the indicated time periods, then followed for outcome thereafter. Figure 3 shows TBFV status of patients at different times during follow-up (TB-FV-, TB-FV+, TB+FV-, or TB+FV+) plotted against their time to REC or time of last follow-up visit for NONREC. Only the combination of the TB+FV+ at the 6 and 12 month visit was associated with a significantly increased risk of REC than TB-FV- sites.
Figure 6.1. Probability of developing a REC according to the presence or absence of an OPL at the former cancer site at 6, 12, 18 and 24 months. The blue line represents the OPL-negative former cancer sites while the green line represents the patients with an OPL at the former cancer site at the follow-up visit at A) 6 months; B) 12 months; C) 18 months; and D) 24 months.
Figure 6.2 Probability of developing a REC according to the uptake or lack of uptake of toluidine blue (TB) stain at the former cancer site at 6, 12, 18 and 24 months. The blue line represents the TB- negative former cancer sites while the green line represents the patients who stained TB+ at the follow-up visits at A) 6 months; B) 12 months; C) 18 months; and D) 24 months.
Figure 6.3 Probability of developing a REC according to the presence or absence of fluorescence visualization (FV) at the former cancer site at 6, 12, 18 and 24 months. The blue line represents the FV- former cancer sites while the green line represents the patients who were FV+ at the follow-up visits at A) 6 months; B) 12 months; C) 18 months; and D) 24 months.
Figure 6.4 Probability of developing a REC according to the TB and FV status at the former cancer site at 6, 12, 18 and 24 months. The blue line represents the TBFV- former cancer sites, the green line represents the patients who were TB-FV+, the purple line is TB+FV-, and the beige line represents patients were both TB+ and FV+ at the follow-up visits at A) 6 months; B) 12 months; C) 18 months; and D) 24 months.
6.7 Clinical Lesion Characteristics and Biopsy

The next factor examined was whether biopsied sites were more likely to recur, and whether the presence of OPL, TB+, or FV+ affected the clinicians’ decision to biopsy during follow-up.

6.7.1 Recurrence at a Former Biopsy Site

Table 6.13 shows the relationship between biopsying at different follow up periods and the development of a REC. For example, at 6 months, a total of 48 patients were biopsied with 10 (21%) later developing a REC; in comparison, of 175 patients without biopsy, 23 (13%) later developed REC. Data are also shown for 12, 18 and 24 months. All of these comparisons were found to be not significant (P<0.05).

Table 6.16 Biopsy Effect on REC

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>REC (%)</th>
<th>Non-REC (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>232</td>
<td>34 (15)</td>
<td>198 (85)</td>
<td></td>
</tr>
<tr>
<td>Biopsy at 6 months (N=223)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-</td>
<td>175 (78)</td>
<td>23 (13)</td>
<td>152 (87)</td>
<td>0.25</td>
</tr>
<tr>
<td>Biopsy+</td>
<td>48 (22)</td>
<td>10 (21)</td>
<td>38 (79)</td>
<td></td>
</tr>
<tr>
<td>Biopsy at 12 months (N=213)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-</td>
<td>202 (95)</td>
<td>24 (12)</td>
<td>178 (88)</td>
<td>0.15</td>
</tr>
<tr>
<td>Biopsy+</td>
<td>11 (5)</td>
<td>3 (27)</td>
<td>8 (73)</td>
<td></td>
</tr>
<tr>
<td>Biopsy at 18 months (N=192)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-</td>
<td>173 (90)</td>
<td>15 (9)</td>
<td>158 (91)</td>
<td>0.10</td>
</tr>
<tr>
<td>Biopsy+</td>
<td>19 (10)</td>
<td>4 (21)</td>
<td>15 (79)</td>
<td></td>
</tr>
<tr>
<td>Biopsy at 24 months (N=182)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy-</td>
<td>169 (93)</td>
<td>15 (9)</td>
<td>154 (91)</td>
<td>0.12</td>
</tr>
<tr>
<td>Biopsy+</td>
<td>13 (7)</td>
<td>3 (23)</td>
<td>10 (77)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages by rows.

6.7.2 The Presence of an OPL and Biopsy

Table 6.14 examines whether the presence of an OPL at different follow-up intervals and overall influenced the clinician’s decision to biopsy the former cancer site. Not surprisingly, the
presence of an OPL ever in follow-up was associated with an increased proportion of biopsies (P<0.03). This effect is confounded by the tendency of clinicians to also biopsy the former cancer site for comparison purposes only, for example, every 2 years, and not for clinical reasons. Interestingly, only the presence of an OPL at 6 months follow-up was significantly associated with biopsying the former cancer site (P=0.02). Although not significant at 12, 18, or 24 months, a larger proportion of those who were OPL+ at 18 (20%) and 24 months (16%) were biopsied than those who were OPL- (9% and 7%, respectively).

Table 6.17 OPL Influence on Biopsy at 6, 12, 18 and 24 Months

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>Biopsy- (%)</th>
<th>Biopsy+ (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (N=232)</strong></td>
<td>232</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>OPL Ever (N=232)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>162 (70)</td>
<td>89 (55)</td>
<td>73 (45)</td>
<td>0.03</td>
</tr>
<tr>
<td>Positive</td>
<td>70 (30)</td>
<td>23 (33)</td>
<td>47 (67)</td>
<td></td>
</tr>
<tr>
<td><strong>OPL at 6 mo (N=214)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>177 (83)</td>
<td>144 (81)</td>
<td>33 (19)</td>
<td>0.02</td>
</tr>
<tr>
<td>Positive</td>
<td>37 (17)</td>
<td>23 (62)</td>
<td>14 (38)</td>
<td></td>
</tr>
<tr>
<td><strong>OPL at 12 mo (N=208)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>175 (84)</td>
<td>166 (95)</td>
<td>9 (5)</td>
<td>0.69</td>
</tr>
<tr>
<td>Positive</td>
<td>33 (16)</td>
<td>31 (94)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td><strong>OPL at 18 mo (N=183)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>158 (86)</td>
<td>144 (91)</td>
<td>14 (9)</td>
<td>0.15</td>
</tr>
<tr>
<td>Positive</td>
<td>25 (14)</td>
<td>20 (80)</td>
<td>5 (20)</td>
<td></td>
</tr>
<tr>
<td><strong>OPL at 24 mo (N=173)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>154 (89)</td>
<td>144 (93)</td>
<td>10 (7)</td>
<td>0.16</td>
</tr>
<tr>
<td>Positive</td>
<td>19 (11)</td>
<td>16 (84)</td>
<td>3 (16)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages are calculated by row.

6.7.3 The Effect of Toluidine Blue Status Biopsy

Surprisingly, the presence of a TB+ staining result appeared to have only a limited effect on the decision to biopsy in the first 2 years of follow-up. Throughout follow-up the presence of a TB+ OPL was associated with an increased proportion of biopsies occurring (P=0.01).
However, within the first two years only TB+ results at the one year mark was significantly associated with the biopsy (P=0.04). At all other follow-up intervals, although not significant, a larger proportion of TB+ patients were biopsied (33% at 6 months, 20% at 18 months, 18% at 24 months) than those who were TB- and biopsied (21% at 6 months, 10% at 18 months, 6% at 24 months).

Table 6.18 TB Influence on Biopsy at 6, 12, 18 and 24 Months

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>Biopsy- (%)</th>
<th>Biopsy+ (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (N=232)</td>
<td>232</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB Ever (N=232)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>176 (76)</td>
<td>98 (56)</td>
<td>78 (44)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Positive</td>
<td>56 (24)</td>
<td>14 (25)</td>
<td>42 (75)</td>
<td></td>
</tr>
<tr>
<td>TB at 6 mo (N=209)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>194 (93)</td>
<td>153 (79)</td>
<td>42 (21)</td>
<td>0.33</td>
</tr>
<tr>
<td>Positive</td>
<td>15 (7)</td>
<td>10 (67)</td>
<td>5 (33)</td>
<td></td>
</tr>
<tr>
<td>TB at 12 mo (N=205)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>179 (87)</td>
<td>172 (96)</td>
<td>7 (4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Positive</td>
<td>26 (13)</td>
<td>22 (85)</td>
<td>4 (15)</td>
<td></td>
</tr>
<tr>
<td>TB at 18 mo (N=181)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>176 (97)</td>
<td>158 (90)</td>
<td>18 (10)</td>
<td>0.43</td>
</tr>
<tr>
<td>Positive</td>
<td>5 (3)</td>
<td>4 (80)</td>
<td>1 (20)</td>
<td></td>
</tr>
<tr>
<td>TB at 24 mo (N=173)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>162 (94)</td>
<td>152 (94)</td>
<td>10 (6)</td>
<td>0.17</td>
</tr>
<tr>
<td>Positive</td>
<td>11 (6)</td>
<td>9 (82)</td>
<td>2 (18)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages are calculated by row.

6.7.4 Fluorescence Visualization Status and Biopsy

The loss of fluorescence was found to be associated with biopsy ever in follow-up and at the 6 and 18 month follow-up visit. FV+ is significantly associated with the decision to biopsy ever (P<0.01), 6 months (P=0.01), and 18 months (P<0.01). At 12 and 24 months, although not significant, a larger proportion of FV+ patients were biopsied at 12 months (6%) and 24 months.
(8%) compared to FV- patients at 12 months (0%) and 24 months (3%). These results may be
influenced by the large number of FV+ former cancer sites.

Table 6.19 FV Influence on Biopsy at 6, 12, 18 and 24 Months

<table>
<thead>
<tr>
<th></th>
<th>All (%)</th>
<th>Biopsy- (%)</th>
<th>Biopsy+ (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (N=204)</strong></td>
<td>204</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FV Ever (N=204)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>26 (13)</td>
<td>22 (85)</td>
<td>4 (15)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Positive</td>
<td>178 (87)</td>
<td>72 (40)</td>
<td>106 (60)</td>
<td></td>
</tr>
<tr>
<td><strong>FV at 6mo (N=176)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Negative</td>
<td>58 (33)</td>
<td>51 (88)</td>
<td>7 (12)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>118 (67)</td>
<td>84 (71)</td>
<td>34 (29)</td>
<td></td>
</tr>
<tr>
<td><strong>FV at 12mo (N=185)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Negative</td>
<td>43 (77)</td>
<td>43 (100)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>142 (23)</td>
<td>134 (94)</td>
<td>8 (6)</td>
<td></td>
</tr>
<tr>
<td><strong>FV at 18mo (N=159)</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Negative</td>
<td>71 (45)</td>
<td>69 (97)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>88 (55)</td>
<td>72 (82)</td>
<td>16 (18)</td>
<td></td>
</tr>
<tr>
<td><strong>FV at 24mo (N=164)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Negative</td>
<td>57 (35)</td>
<td>55 (97)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>107 (65)</td>
<td>99 (92)</td>
<td>8 (8)</td>
<td></td>
</tr>
</tbody>
</table>

1. Percentages are calculated by row.
Chapter 7: Discussion

Oral cancer has a high rate of recurrence which contributes to its poor 5-year survival rate (87). As a result oral cancer survivors undergo routine follow-up examinations in order to detect early signs of REC. Routine follow-up is important; the main objective is to allow the early detection of recurrences that would lead to earlier and potentially more effective treatment (88). The first few years of follow-up is particularly important as the majority of REC are believed to occur within the first 2 years after completion of the primary treatment, although REC can occur many years after treatment (89,90). Clinicians have had to rely on the clinical risk factors associated with primary OPL progression due to the lack of clinical evidence of REC. Previous theses have found that most of the clinical factors associated with progression are not associated with oral cancer REC (10,91). This reliance on clinical risk factors that lack evidence in oral cancer survivors may negatively affect the clinician’s ability to detect a patient at high-risk of recurrence and hence have little impact on overall survival. The presence of an OPL at the former tumour site has been found to be associated with REC and there is mixed evidence on the presence of TB positive stain and REC (91,92). To date, it is unknown if clinical risk factors and their association with REC may vary with time in follow-up, nor is their evidence on the ability of fluorescence visualization (FV) to predict a REC. It is also unknown if the presence of an OPL, TB and FV status affect the clinician’s decision to biopsy. The focus of this thesis is to determine if FV is associated with REC, how FV, TB, and OPL statuses can be used to predict REC at different time points in follow-up, and how these clinical risk factors may influence a clinician’s decision to biopsy.
The incidence of an oral cancer recurrence at the former cancer site for this research project was 15%, which is at the low end of the range found in the literature (15-30%)(83,85,86,89). Geographical, treatment, age of study, and risk habits differences may account for the broad range of REC. Studies performed in countries with higher rates of risk habits such as tobacco and alcohol may lead to more REC; treatment may differ amongst different centres and may change and improve over time leading to variation amongst the rate of REC.

In this chapter, key findings from the previous chapter will be discussed in detail to show the implications (if significant) for follow-up care of oral cancer survivors. Findings on the influence of biopsy effect from clinical markers may help clinicians understand when they should biopsy. Limitations of the study and future directions will also be discussed.

7.1 Demographics, Risk Habits and REC

In this study, none of the demographic variables nor the risk habits investigated were associated with REC. Only 8% of the patients accrued to this study were under the age of 40 years, however a greater proportion developed a REC. Although not a significant finding, it should be noted that patients diagnosed at a younger age have had a worse outcome in some studies(93). Patients who develop an oral cancer at a younger age may have different genetic risks than older patients(93). The median age at diagnosis of the primary oral cancer was 58 years for patients who developed a REC and 60 for those who did not; the majority of oral cancers are diagnosed after the age of 60(94).

Tobacco use has been strongly associated with the development of primary oral cancer and REC(95). Past research has suggested that continued use of tobacco after the primary diagnosis
was associated with a REC(96). In contrast, the results of this study found no difference between smoking and REC. Of interest, in this study, none of the former smokers who had quit less than one year prior to their primary diagnosis developed a REC, although it should be noted that the number of patients in this category was small. Although not found to be significant, the largest proportion of REC occurred in patients who continued to smoke after their primary tumour diagnosis. The lack of significance may be due to the smaller number of current smokers at primary diagnosis.

The use of alcohol has also been strongly linked to both primary oral cancer and REC(17,96). A meta-analysis found that non-smokers who drank alcohol had a 32% greater risk of developing an oral and pharyngeal cancer while heavy drinkers had a 2.54 greater risk than non-drinkers. In an older study looking at tobacco and alcohol use and risk of second oral cancers, patients who drank more than 15 beer a week had an almost 4-fold increased risk. However, alcohol was also not a factor found to be associated with a REC in this study.

7.2 Primary Tumour Characteristics

In the past, research related to REC has focused mainly on the characteristics of the primary tumour and treatment. Characteristics such as tumour site, stage and treatment modality have all been investigated. The site of primary tumour development (tongue and FOM) and the method of treatment of the primary cancer (radiation and/or chemotherapy without surgery) was found to be associated with REC by Hong et al.(97). The stage at which the primary tumour was diagnosed was also found to be associated with REC by Antoniades et al.(98), where over 60% of SCC patients developed a REC compared to none in the CIS group in their study.
In this thesis, primary lesion site, stage at diagnosis and treatment modality were studied. Although there were no statistical differences found between these criteria and REC, there was a trend for a greater proportion of CIS to REC than SCC. Almost 93% of CIS were treated with surgery only compared to 64% of the SCC. Since the cases in this thesis date back more than 15 years, some of the earlier surgeries for CIS may not have taken as wide a margin as is take for SCC. Considering field cancerization, there may have been patches of undetected abnormal cells left at the margin. Not surprisingly, SCC was more often treated with multiple modalities of treatment. This study included mostly CIS and early stage tumours with only 17% of cases being late stage (stage III and IV). Late stage oral cancers may have been difficult to treat and this thesis only dealt with primary tumours that were treated with curative intent. Patients with late stage primary tumours may have also passed away due to their oral cancer prior to the development of a REC or developed a second cancer at another site, particularly if they used tobacco and alcohol. This can be seen in the limited cases treated with any type of chemotherapy (although not common in oral cancer treatment in British Columbia) or multiple treatment modalities.

The most common site for the diagnosis of oral cancer in the western world is the ventral and lateral tongue(13). Along with the floor of the mouth (FOM) these sites are considered high-risk(13,91). Not surprisingly, the majority of the cases in this study, 81%, were on the tongue and FOM. It is interesting, that although not significant, more REC occurred at sites other than the tongue and FOM. This finding is different than Kernohan et al. who found that the tongue, followed by the floor of mouth were the most common site for REC(90). The difference in these results may be due to risk factors associated with other sites such as chewing tobacco or betel
quid, both of which have been found to be associated with oral cancer in South Asians(13). In South Asia, the most common risk site is the buccal mucosa(13).

7.3 Demographic and Risk Habit Characteristics and Primary Tumour Stage

In this demographics and risk habits were not found to be associated with the primary tumour diagnosis. None of the demographic characteristics, age, gender, and ethnicity were associated with stage at primary diagnosis.

Risk habits were also not found to be associated with stage in this study. Of interest, all the former smokers, who had quit less than one year prior to their diagnosis were SCC. Neither the use of alcohol ever nor the amount consumed was associated with stage.

7.4 Clinical Characteristics of OPLs and REC

7.4.1 Clinical Characteristics of OPLs Ever in Follow-up and REC

The presence of an OPL is a major risk factor for the development of a primary oral cancer(41). OPL characteristics such as a non-homogenous appearance, large in size, non-smooth texture, red or mixed red and white, and having diffuse margins have all been found to be associated with an increased risk of progression to a primary oral cancer(31,33,41). This thesis looks at these characteristics and the development of a REC at the former cancer site.

Tissue at the former cancer site may be less resistant to trauma or risk habits as a result of the initial diagnosis and the subsequent treatment, particularly radiation. Anatomical changes to the site may lead to an increase in the amount of trauma subjected to the site. These changes may
be difficult to differentiate from true neoplastic changes. Unlike in primary progression, none of the clinical characteristics were found to be associated with REC in this cohort. In a thesis by Park (2013), none of appearance, margin, size or OPL thickness during the first four years of follow-up were associated with REC(10). It is important to note that of the 232 cases followed in this study, only 71 developed an OPL in follow-up so there was limited power within this subset. Similar to primary progression, a greater proportion of non-homogenous, non-smooth, or non-white OPLs, progressed to REC than homogenous, smooth or white OPLs, although the results were not significant. In contrast, more OPLs with discrete margins developed a REC than diffuse lesions. Further research once this subgroup increases will see if these results hold and reach significance.

7.5 Clinical Status of Patients at Follow-up Visits

7.5.1 Presence of an OPL in Follow-up

As mentioned, the presence of an OPL is associated with increased risk of primary progression(7). In a study of clinical indicators of REC in a small cohort of 84 former oral cancer patients, Laronde found that the presence of an OPL was strongly associated with a REC and that the rate of malignant transformation was greater than in primary progression(91). Park, 2013, found supporting results in his thesis and found the presence of an OPL within the first year was associated with the highest proportion of OPLs developing a REC; an OPL within the first year of follow-up was associated with a 6.1 fold increased risk of REC than patients who developed an OPL later in follow-up(10). Park also found that patients who had an OPL always in follow-up had a 67.8-fold increased risk of a REC than patients who never had an OPL in follow-up.
The OR for patients who ‘sometimes’ had an OPL was 5.2(10). Similar results were found in this study; at all 4 time frames the presence of an OPL was strongly associated with the development of REC. While Park found a high risk of REC for OPL within the first year, in this study the highest risk of REC was for the presence of an OPL at the 12 month visit. The OR was lower at 18 months but increased again at the 24 month follow-up visit. An OPL at a former oral cancer site in follow-up should be considered a significant risk factor for REC regardless of its clinical characteristics. This risk is greatest in lesions which persist for more than one follow-up visit.

7.5.2 Toluidine Blue Positive Staining in Follow-up

Toluidine blue is an acidophilic tissue stain that has been used to aid in the identification of oral SCC(7). Its high affinity for acidic tissue allows it to stain tissues rich in DNA and RNA, found in excess in malignant tissue(49). By highlighting areas of potentially malignant oral epithelium that the naked eye otherwise may not detect, it is useful in also selecting the biopsy sample site in the premalignant lesion(49).

Laronde (2005) found that OPL that stained positive for TB at one year or ever in follow-up was associated with an increased risk of REC(91). Similar results were found in this study where TB+ staining of OPLs at 6, 12 and 24 months were significantly associated with REC, and at 18 months there was a trend towards TB and REC. It is difficult to predict why the TB results at 18 months were not significant but it may be due to only 5 OPLs being TB+ at this point in follow-up. Only a small proportion of lesions stained TB+ at the four time points (5-13%). It may be that some TB+ results were believed to be mechanical pick-up due to treatment effects, which may account for why some TB- lesions developed a REC (13% at 6 month visit) or that
the TB+ did not occur within the first year. Less than 10% of TB- OPLs at 12, 18, and 24 months progressed. TB+ sites that persisted for 2 or more visits were also associated with REC. These sites may have shown ‘true’ TB results versus mechanical or trauma related pick-up. Park did not find an association with TB and REC within any time frame(10).

7.5.3 Fluorescence Visualization and REC in Follow-up

For the first time, this study investigates the role of FV and its association with REC in patients with a history of oral cancer. FV is a light in the ultraviolet spectrum (530nm) that can highlight changes in the oral mucosa that may otherwise appear as occult in WLE(8). Specifically, a loss of fluorescence (FV+) results from alterations in the tissue matrix, such as breakdown of collagen and a decrease of FAD+ molecule due to tissue growth and increased metabolism associated with malignancy(8). One of the main confounders for FV is the presence of inflammation and infection which may decrease its sensitivity and sensitivity(47).

FV was not found to be associated with REC at any of the 4 time points in follow-up. There was a high proportion of FV+ lesions that did not develop a REC (89-93%). This may be due to the ongoing healing processes at the former tumour site, friable, fragile tissue at the former tumour site due to treatment effects, and ongoing biochemical changes in the tissue due to the original diagnosis and treatment. Of interest, only a small proportion (4-7%) of FV- sites developed a REC. Perhaps the lack of FV loss might be a negative predictor of REC, providing some level of reassurance to the clinician. Further research into this area is required. FV persistence over multiple follow-up visits was not associated with REC. This result is not surprising due to the high proportion of FV+ results over all time points.
7.5.4 Toluidine Blue and Fluorescence Visualization and REC in Follow-up

The combination of TB and FV was also investigated. Former cancer sites that were both TB+ and FV+ at 6 and 12 months were associated with REC although this association did not hold in the second year of follow-up. Further research needs to be done to investigate the importance of the interactions (if any) between TB, FV, and even the presence of an OPL. It is interesting that only 3 cases were TB+FV- and all 3 occurred at the 12 month mark. The high rate of FV in this cohort makes this combination rare.

7.6 Time to REC

Past research has determined that the majority of REC occur within the first two years following the treatment of the primary oral cancer(85,90). In this study, we excluded REC that occurred within the first 6 months as they were likely to be residual primary tumour. Almost half of the REC (47%) occurred within the first two years of follow-up. The quick time to REC is potentially due to established genetic alterations in the tissue surrounding the former tumour site(77).

In this study, the median time to REC was 26 months and the mean time to REC was about 39 months. An outlier that developed a REC at 150 months after the completion of treatment affected the mean time. The median results are similar to Laronde’s and Park’s results of 23 and 25 months, respectively(10,91). It was also found that the development of a REC occurred in less than 2 years after the first OPL or TB+ results.
7.7 Summary of Differences between REC and SPT

As mentioned before, for the purpose of this thesis, a REC is defined as a recurrence of SCC, CIS, or severe dysplasia in the oral cavity within 3cm of the original cancer site while a SPT is a recurrence outside 3cm of the original site. While the stage of diagnosis was the only factor of significance associated with REC in my study, tobacco, alcohol, and site of primary tumour were all significantly associated with a SPT(99). This difference of risk factors seen between REC and SPT may be explained by the field cancerization theory. If the presence of a separate genetically altered field or a field distant to the primary site, is still present after the removal of the primary tumour, further genetic hits from tobacco and alcohol consumption, as well as the location being at a high risk site, may increase the risk of a SPT developing.

7.8 Clinical Lesion Characteristics and Biopsy

The decision to biopsy is a difficult one. The presence, duration, size, appearance, site, colour and texture of a lesion as well as TB status factor into the decision to biopsy a primary lesion. Factors such as the confidence of the clinician and patient anxiety must also be considered. In patients with a history of oral cancer, the decision to biopsy a former cancer site may be even more challenging due to the effects of treatment, fear of a second cancer, and recommendations regarding the regular biopsy of a site for comparison purposes(100).

In this study, it was found that the presence of an OPL at the former cancer site early in follow-up at the 6-month visit was associated with the completion of a biopsy. This may be due to the clinician suspecting residual disease present in the outer margins of the original site, missed during the original treatment. The proportion of biopsies completed at 12, 18 and 24
months drops off dramatically. Fewer biopsies at 12 months may be a result of an increase of biopsies at 6 months; clinicians may not want to biopsy again so soon. The rate of biopsies increased at 18 and 24 months versus 12 months. It is also study protocol to biopsy every 2 years, however, this decision is left to the clinician and is often influenced by the patient’s concerns and anxiety.

TB is often used in primary lesions to aid the clinician in determining where to biopsy a lesion. TB has a high sensitivity for oral SCC in primary lesions but is less reliable in dysplasia. Following up a TB+ result in 2-3 weeks to confirm the result increases the sensitivity and specificity. Zhang et al, found that TB was associated with a 4-fold increased risk of progression for low grade dysplastic lesions(7). In this study, TB did not appear to influence the decision to biopsy except at the one year mark. Overall, less than a third of TB+ lesions were biopsied at 6 months, perhaps the clinician considered the result to be due to treatment effects. Fewer TB+ OPLs were biopsied at 12, 18 and 24 months, giving the appearance that the clinician did not give much credence to the TB results in the follow-up of former cancer patients.

FV has not been studied in its association with the decision to biopsy primary OPLs. FV was associated with biopsy at the 6 and 12 month visit; very few FV- OPLs were biopsied. In fact at 12 months no lesions that were FV- were biopsied. The clinicians may consider it the lack of FV loss to be associated with a better outcome.

7.9 Limitations

Some limitations to this study is identified below, so that future studies with similar objectives may better model their methods of study. Since this study started gathering data back
in 1997, many changes have occurred since then until current day. For example, there were
different clinicians examining and gathering the data back in the beginning of the study than
those working the study present day. There is a certain subjectivity to some of the clinical data
recorded, as often times it is not as easy as labeling a clinical factor as “positive” or “negative”.
There may be some gradient of result or confounders that would cause one clinician to disagree
with another.

Different techniques and devices were also used throughout the time of the entire OCPL.
FV was not introduced until 2003, so there were no FV data to work with prior to that date.
Surgery of primary cancers also started incorporating FV use to guide surgical margins, which
may have altered the REC rate from the beginning. There was also a lot of missing data scattered
throughout the entirety of the study, some from patients missing visits, or clinicians simply not
performing the screening tests or recording the clinical marker data. Radiation treatment has also
improved over the course of the OCPL study, with the introduction of intensity-modulated
radiation therapy (IMRT). IMRT allows for higher doses of radiation administered to a localized
area, while decreasing damage to surrounding healthy tissues compared to traditional radiation
therapies, thus potentially decreasing the REC rates of oral cancer patients. Data was also found
to be recorded much more consistently and accurately in later years.

This study had only one criteria when determining a REC; occurrence of a second cancer
3cm within the former cancer site. However, a second criterion in addition to classifying REC
may be to limit REC to only those that recur within 5 years of completion of treatment, and any
REC after 5 years as a SPT. This additional criterion would have excluded a number of REC
patients in the patient pool, possibly changing some REC results.
Tobacco and alcohol use largely relied on patient self-reporting through an annual survey, where some patients may downplay their tobacco and/or alcohol use. It may be due to this fact that we did not find any significance between any level of smoking status or alcohol use with REC.

7.10 Future Directions

Over time, the sample size of the cohort will increase with more OPLs present in follow-up, providing more power to the statistical analysis looking at TB, FV, biopsy trends, and other clinical factors that may play a role. There also comes a time when one can compare the differences between a REC that occurred within the first two years and one that occurred after, determining if at that point it is still truly a REC, or if it is now a SPT. The results from this thesis can also serve as a template for future studies that aim to create a statistical model for predicting REC, using different combinations of clinical risk factors to predict the likelihood of a REC happening.
Chapter 8: Conclusion

Around 15-30% of primary oral cancer survivors will develop a REC, but it is uncertain which 15 to 30 patients out of 100 will be the ones to recur. Clinicians must be able to identify those who are at a higher risk than others, to increase follow-up intervals or provide management of the second OPL. There are documented studies showing certain clinical risk factors are significantly associated with primary progression of oral cancer, but little research has been done into identifying clinical predictors for REC that can be easily used by the clinician.

The results of this study provide evidence that the presence of an OPL was a significant indicator of risk of REC, regardless of the clinical characteristics of the OPL or the time interval during follow-up. Risk factors that are associated with primary progression such as appearance, size, and colour, are not predictive for REC. Toluidine blue positivity was also found to be associated with REC early in follow-up, with the highest risk at 6 and 12 months. For the first time, FV was studied for associations with REC, and this study found that regardless of follow-up interval, FV positivity was not predictive of REC. However, although not significant, FV negativity may have some potential as a negative predictive value, but this area requires further research. In the clinical setting, this thesis pay provide some guidance to clinicians when determining which patients are considered higher risk for REC, and should be followed-up more closely. Those patients whose OPL are also TB+ and FV+ at 6 or 12 months should be watched more carefully, as they were found in this study to be at a higher risk of developing a REC compared to if they only have TB+ alone. There is also the risk of patients with a history of oral cancer developing a SPT, thus the importance of follow-up and surveillance is not limited to just detecting signs of REC. With this information in hand, clinicians may begin developing a
framework or model to help better manage OPL at former tumour sites, catching early signs of clinical indicators and reducing the risk of REC.
References


combined narrow band imaging and autofluorescence mucosal assessment of patients with

detection and delineation of oral neoplasia using autofluorescence imaging. Cancer Prev


validation of oral mucosal tissue using optical coherence tomography. Head Neck Oncol

dysplasia and malignancy using optical coherence tomography: Preliminary studies in 50


Fluorescence lifetime imaging distinguishes basal cell carcinoma from surrounding

imaging and reflectance confocal microscopy for multiscale imaging of oral precancer. J
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ndertype=abstract

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73. Available from: http://dx.doi.org/10.1016/j.clon.2010.05.016


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Appendices

Appendix A  Initial Study Questionnaire

**ORAL STUDY QUESTIONNAIRE**

1. In addition to being Canadian or a landed immigrant, what is your ethnic or cultural heritage?
   (Check one box only):
   - White
   - East or South-east Asian (eg. China, Japan, Indonesia, Philippines, Vietnam)
   - South Asian (eg. India Pakistan, Sri Lanka)
   - First Nations
   - Black
   - Other (Please Specify)__________________________

2. a) What is the highest grade (or year) of high school or elementary school that you have completed?
   Grade __________ Never attended school ______

   b) How many years of post-secondary school have you completed (college, university)?
   Years ______ None ______

3. a) Have you ever used chewing tobacco?
   Yes ______ No ______

   b) Have you ever used betel nut?
   Yes ______ No ______

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

   If Yes, please specify:
   a) At what age did you begin smoking:
      Cigarettes? ______
      Cigars? ______
      Pipes? ______

   b) Do you currently smoke:
      Cigarettes? Yes ______ No ______
      Cigars? Yes ______ No ______
      Pipes? Yes ______ No ______

   c) If you have quit smoking, at what age did you permanently stop:
      Cigarettes? ______
      Cigars? ______
      Pipes? ______
d) Looking back over your entire life, on average, how many did you usually smoke per day?

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

Are you regularly exposed to smoke of others:

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

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

How often are you regularly exposed to smoke of others:

<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>
<tbody>
<tr>
<td>Cigarettes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigars</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pipes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</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 
   Wine 
   Spirits (liquor) 

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

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

Parents
Brothers/sisters
Daughters/sons
Grandparents
Aunts/uncles related by birth not marriage
Appendix B  Lesion Mouth Map
Appendix C  Annual Study Questionnaire

ORAL STUDY
ANNUAL QUESTIONNAIRE

1. During the last year, did you regularly smoke cigarettes, cigars or pipes more than once per week? Yes No

If Yes, please specify:

a) Type:
   - Cigarettes?
   - Cigars?
   - Pipes?

b) Did you quit smoking:
   - Cigarettes?
   - Cigars?
   - Pipes?

 c) Looking back over the last year, how many did you usually smoke per day?

   - Cigarettes
   - Cigars
   - Pipes

2. 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).

   Were you regularly exposed to smoke of others:
   - At home? Yes No
   - At work? Yes No
   - In public places? Yes No
If Yes, to any of the above, please specify:

During the **last year**, how often were you regularly exposed to smoke of others:

<table>
<thead>
<tr>
<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>
</table>

At home?

At work?

In Public Places?

3. During the **last year**, did you regularly consume alcoholic beverages more than once per month over the last year?  
   Yes  No

   If Yes, please specify:

   a) Type:
      Beer?  
      Wine?  
      Spirits (liquor)?

   c) Did you quit drinking?
      Beer?  
      Wine?  
      Spirits (liquor)?

   d) On average, how much did you usually drink per week:
      Beer  bottles
      Wine  glasses
      Spirits (liquor)  (shots – 1 oz.)

4. Over the **last year** 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: ________
# Appendix D  Lesion Tracking Sheet/Clinical Research Form

## Lesion Tracking Sheet

**Complete at initial and each follow-up visit.**

Use one tracking sheet for each lesion

### Patient Name: __________________________ Site: __________________________

#### Visit Number (L, v1, v2, etc)

#### Date (yyyy/mm/dd)

### Lesion Grid Location

- Specify grid site
- N/C = no change

### Lesion Currently Present

- * If no, do not enter lesion details
- **Lesion** 1: scar or graft = 0

### Clinical Description of Site

Use code sheet to describe site – Record all that apply

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>1=diffuse 2= discrete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (mm)</td>
<td></td>
</tr>
<tr>
<td>Width (mm)</td>
<td></td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>0=Normal 1=White 2=More than 50% white 3=More than 50% red 4=Re 5=Other - specify in memo</td>
</tr>
<tr>
<td>Appearance</td>
<td>1=Homogenous 2=Nonhomogenous</td>
</tr>
<tr>
<td>Texture</td>
<td>1=Ulcerated 2=Smooth 3=Velvety/Grainy 4=Nodular 5=Vernacious 6=Fissure 7=Other n/c=No Change</td>
</tr>
</tbody>
</table>

### FV Results

* If 0 do not enter FV details

0=Neg 1=Pos 2=Equivocal 3=Not done 4=masking - gingiva

### FV Positive Details

- *only if FV=1 or 2*
- 5.1=scar within 6 months of surgery;
- 5.2=scar greater than 6 months after surgery;
- 5.3=pigmentation at soft palate and FOM;
- 5.4=infection/inflammation;
- 5.5=other – to be reviewed

### FV Grid Location

(Specify where on grid)

### FV Length (mm)

### FV Width (mm)

### Orange Fluorescence

1=Yes 0=No

### Toluidine Blue Results

0=Neg 1=Pos 2=Equivocal 3=Not done

### LS (Lesion Brush) Done

1=Yes 0=No

### GEO Done

1=Yes 0=No

### Biopsy

1=Yes 0=No

If yes, then use the Biopsy Tracking Sheet

### Digital Images Taken

1=Yes 0=No

### Interim Therapy

1=Surgery 2=Laser Surgery 3=Radiation 6=Local Chemo 7=Systemic Chemo 9=Systemic Steroid 10=Other 11=Incisional Bx 13=Antitumor Agent 14=Topical Pain Med 18=Topical Steroid 88= None

### Date of Interim Therapy

If available (yyyy/mm/dd)