

Original Investigation

Fluorescence Visualization–Guided Surgery for Early-Stage Oral Cancer

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IMPORTANCE The prevalence of genetically altered cells in oral cancers has a negative influence on the locoregional recurrence rate and lowers survival. Fluorescence visualization (FV) can identify clinically occult, high-risk oral lesions by allowing health care professionals and surgeons to visualize and map occult disease. This process may improve overall survival by decreasing rates of locoregional recurrence.

OBJECTIVE To assess the efficacy of FV-guided surgery in reducing locoregional recurrence and improving overall survival.

DESIGN, SETTING, AND PARTICIPANTS A retrospective, case-control observational study was conducted on patients registered at a single oral oncology clinic from September 1, 2004, to August 31, 2009. The study included 246 patients 18 years or older with a diagnosis of a high-grade lesion (severe dysplasia or carcinoma in situ) or squamous cell carcinoma of less than 4 cm who underwent curative surgical treatment with at least 1 follow-up visit. Among these patients, 154 underwent surgery with FV guidance (FV group) and the other 92 underwent conventional surgery (control group). Demographic and lesional characteristics and outcomes were collected, and the key factors for the efficacy of FV-guided surgery were examined. Follow-up was completed on December 31, 2011, and data were analyzed from May 1 to November 30, 2013.

MAIN OUTCOMES AND MEASURES Local recurrence of oral lesions with a histologic grade of severe dysplasia or higher, the presence of regional failure (ie, a metastatic lesion in the cervical lymph nodes), or disease-free survival after surgery.

RESULTS Among the 246 patients included in the study (mean [SD] age, 60 [12] years; 108 women and 138 men), 156 had squamous cell carcinoma and 90 had high-grade lesions. There were no significant differences between the FV (n = 154) and control (n = 92) groups in age, smoking history, anatomical site of the lesion, tumor size, and previous oral cancer. Among the 156 patients with squamous cell carcinoma, the 92 patients in the FV group showed significant reduction in the 3-year local recurrence rate, from 40.6% (26 of 64 patients) to 6.5% (6 of 92 patients) ($P < .001$). Among the 90 patients with high-grade lesions, the 62 patients in the FV group showed a reduction in local recurrence rate from 11 of 28 patients (39.3%) to 5 of 62 patients (8.1%) ($P < .001$). The data also indicated that, compared with conventional surgery, the FV-guided approach for squamous cell carcinoma was associated with less regional failure (14 of 92 patients [15.2%] vs 16 of 64 [25.0%]; $P = .08$) and death (12 of 92 patients [13.0%] vs 13 of 64 [20.3%]; $P = .22$), although these differences were not statistically significant.

CONCLUSIONS AND RELEVANCE In this study, the use of FV as part of the surgical margin decision process significantly reduced the rate of local recurrence in preinvasive high-grade and early-stage oral cancers. An ongoing multicenter, phase 3, randomized surgical trial has completed accrual, and the data will be used to validate the results of this study.

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Oral cancer is a major global health issue with high rates of morbidity and mortality.¹ Most troubling, however, is the lack of significant improvement in the prognosis of this disease during the past 5 decades, even in nations with high levels of resources.² This failure is attributed to the late stage at diagnosis of the disease and the difficulty in capturing all of the cancer at treatment.

Genetically altered cells are often widespread across the oral mucosa of patients with squamous cell carcinoma (SCC), which presents as low-grade disease or normal tissue clinically and histologically.^{3,4} This extension laterally and in depth and the difficulty in identifying such residual cells can be responsible for the high rate of recurrence at the primary site (10%-30% of SCC cases).⁵⁻⁷ Such recurrence rates of aggressive disease remain a concern. In recognition of this risk for recurrence, surgeons try to remove oral cancer with a significant additional margin of surrounding normal-appearing oral mucosa. However, the occult disease can vary in size, and a wealth of evidence suggests that occult disease frequently extends beyond the clinical tumor clearance area.⁸⁻¹¹ The cases of high-grade precancerous lesions (HGLs) (ie, severe dysplasia and carcinoma in situ) have less developed consensus about their management. High rates of persistence, recurrence, and eventual progression of such stages to invasive SCC¹² have led to a general agreement that the lesions need to be removed. However, no agreement exists on the width of surgical clearance. This uncertainty results in high rates of recurrence and progression after surgery.^{13,14} Development of new approaches that can be adopted easily in clinical settings to facilitate the detection of clinically occult fields with a high risk for oral cancer remains a pressing need.

One such promising approach involves the use of tissue autofluorescence. The association of cancer development with a loss of normal tissue autofluorescence has been well established in a number of tissues and organs.^{1,15-19} More recently, visual aids using optical methods to detect such loss have been shown to reveal premalignant and malignant lesions that are not detected by the unaided eye.^{12,20,21} The interaction of light with tissue has generally been found to highlight changes in the structure and metabolic activity of the areas optically sampled. Specifically, the loss of tissue autofluorescence is believed to reflect a complex mixture of alterations to intrinsic tissue fluorophore distribution, such as the breakdown of the collagen matrix, a decrease in flavin adenine dinucleotide concentration owing to tissue remodeling, and increased metabolism associated with neoplastic development and angiogenesis.^{12,20,22,23}

A clinical research team from Vancouver, British Columbia,^{24,25} has been on the leading edge of using fluorescence visualization (FV) technology to detect the change within and around the clinically visible oral lesions. The published pilot study²⁴ showed that the loss of autofluorescence in clinically occult margins of oral cancers was associated not only with high-grade histologic change but also with the presence of high-risk molecular clones. The objective of the present study was to assess the efficacy of intraoperative FV-guided surgery in the improvement of disease-free survival in the surgical site and regional lymph nodes and in oral cancer-related mortality.

Methods

Patients

In British Columbia, approximately 75% of patients diagnosed as having T1 or T2 (<4 cm in the largest dimension) SCC or HGL were referred to the BC (British Columbia) Cancer Agency Oral Oncology clinic at the Vancouver site for assessment before intent-to-cure surgery. From September 1, 2004, to August 31, 2009, 246 eligible patients were recruited. The patients were 18 years or older, able to provide informed consent, and able to attend at least 1 postsurgical follow-up visit. Among these patients, 154 had the surgery performed under FV guidance (FV group); the other 92 were treated with conventional surgery (control group) (Figure 1). The decision to use FV-guided surgery was based on the availability of one of us who is an FV specialist (C.F.P.). This study was approved by the ethics board of the BC Cancer Agency. All patients provided written informed consent.

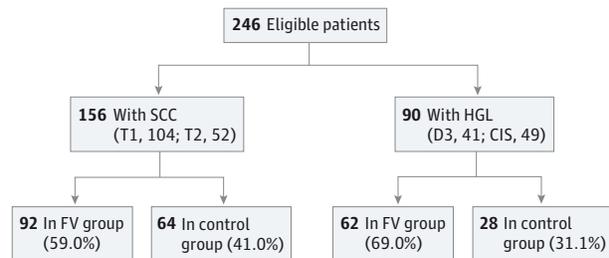
FV Device

A description of the FV device (VELscope; LED Medical Diagnostics, Inc) and its use was given in Lane et al.²⁶ Briefly, the device consists of a bench-top light source coupled to a handheld unit for direct visualization. Lesions were illuminated by this blue-violet light source and then directly visualized through long-pass and notch filters that allowed passage of green and red autofluorescence. Under direct FV, the normal oral mucosa emitted various shades of pale green autofluorescence. Clinical lesions that retained the normal green autofluorescence under FV were defined as *FV retained* (Figure 2). Tissue with a reduction in the normal pale green that appeared as dark patches was classified as *FV loss*.²⁴

Surgical Procedure

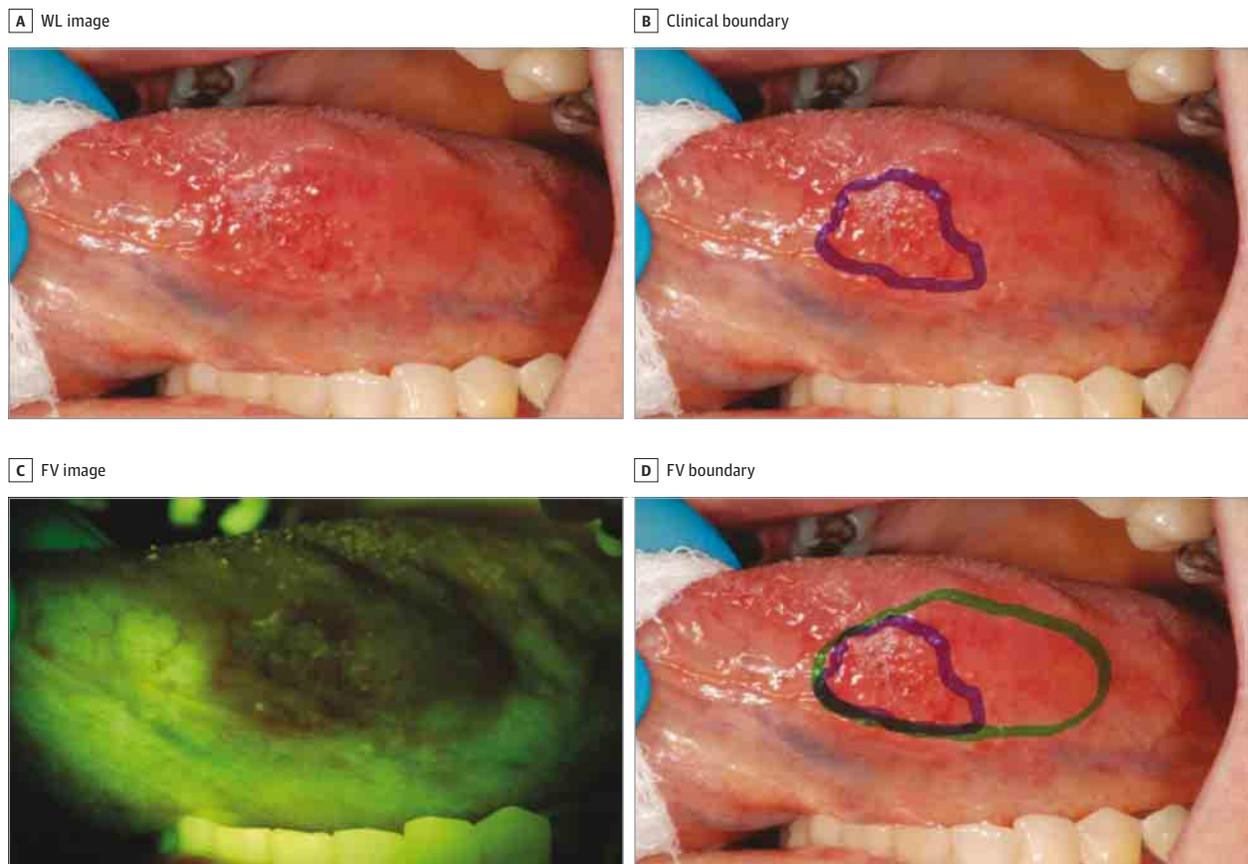
The protocol for the FV group involved examination of the surgical site of each patient under regular operating room illumination (white light) and with direct FV in a stepwise fashion as described previously.²⁴ All procedures were performed in the operating room while the patient was under general anesthesia, and each step was photographed for documentation. The steps included an initial assessment under regular

Figure 1. Study Schema



The numbers of patients, their diagnoses, and the types of treatment they received are shown. CIS indicates carcinoma in situ; D, dysplasia; D3, severe dysplasia; FV, fluorescence visualization; HGL, high-grade lesions; and SCC, squamous cell carcinoma.

Figure 2. Representative Clinical Lesion Assessment Using White Light (WL) and Fluorescence Visualization (FV)



A, A WL image of an ill-defined red lesion at the left lateral tongue. B, The clinical tumor boundary is outlined using a blue skin marker. C, FV image at the same area. D, The FV boundary is outlined using a green marker.

operating room light with demarcation of the boundary of the clinical tumor using a blue marker (Devon skin marker; Ludlow Company) (Figure 2B) as judged by the surgeon (D.W.A. or J.S.D.) followed by assessment of the site for altered fluorescence using direct FV (by C.F.P.). The latter examination was performed with the regular light turned off, and the areas showing loss of normal green fluorescence were outlined, demarcating FV loss boundaries (Sharpie green marker; Sanford) (Figure 2D). The regular light was turned back on, and the final surgical boundary with an additional 10 mm of normal-appearing mucosa around the FV loss boundaries was demarcated using a blue marker. For the control group, the surgeons used the conventional white-light approach to judge the clinically visible lesion with an additional 10 mm of normal-appearing mucosa around the lesional boundary.

Statistical Analysis

Data were analyzed from May 1 to November 30, 2013. Demographics and potential prognostic factors were compared between the FV and control groups using the Fisher exact test for categorical variables and the unpaired 2-tailed *t* test for continuous variables. The time-to-event outcomes after surgery, including pathologically proved local recurrence to severe dysplasia or higher, regional treatment failure for positive cervi-

cal nodes, and disease-free survival, were calculated from the date of surgery to the date of the event or to the last follow-up date before December 31, 2011. The outcome data were analyzed using several statistical tools. Marginal survival functions for the FV and control groups were estimated using Kaplan-Meier methods. We performed log-rank tests to assess the significance of the observed differential survival rates in the 2 groups. Potential joint and interaction effects of FV and other prognostic factors were investigated through fitting a multivariate Cox proportional hazards regression model guided by the Lasso variable model selection procedure²⁷ and the Akaike information criterion. We performed 2-sided tests of their hazard ratios, with $P < .05$ considered to be statistically significant, and the corresponding 95% CIs were determined. Statistical analyses were performed using SPSS (SPSS, Inc) and the R (<https://www.r-project.org/>) software packages.

Results

Patient Demographic and Lesion Characteristics

Among the 246 patients, 156 had SCC and 90 had HGL (Table 1). Among patients with SCC, 92 were in the FV group and 64 in the control group; among patients with HGL, 62 were in the

Table 1. Patient and Lesion Characteristics^a

Variables	Total (N = 246)	SCC Group (n = 156)		P Value	HGL Group (n = 90) ^b		P Value
		FV Subgroup (n = 92)	Control Subgroup (n = 64)		FV Subgroup (n = 62)	Control Subgroup (n = 28)	
Sex, No. (%)							
Male	138 (56.1)	49 (31.4)	46 (29.5)	.02	28 (31.1)	15 (16.7)	.50
Female	108 (43.9)	43 (27.6)	18 (11.5)		34 (37.8)	13 (14.4)	
Race, No. (%)							
White	196 (79.7)	72 (46.2)	56 (35.9)	.20	45 (50.0)	13 (14.4)	>.99
Nonwhite	50 (20.3)	20 (12.8)	8 (5.1)		17 (18.9)	5 (5.6)	
Age, mean (SD), y	60 (12)	61 (12)	60 (14)	.77	57 (12)	61 (10)	.09
Tobacco use, No. (%)							
Nonsmoker	89 (36.2)	39 (25.0)	19 (12.1)	.13	22 (24.4)	9 (10.0)	.81
Ever smoker	157 (63.8)	53 (34.0)	45 (28.8)		40 (44.4)	19 (21.1)	
Anatomical site ^c							
High risk	190 (77.2)	70 (44.9)	43 (27.6)	.27	56 (62.2)	21 (23.3)	.10
Medium to low risk	56 (22.8)	22 (14.1)	21 (13.5)		6 (6.7)	7 (7.8)	
History of oral cancer							
Yes	64 (26.0)	11 (7.1)	12 (7.7)	.26	24 (26.7)	17 (18.9)	.07
No	182 (74.0)	81 (51.9)	52 (33.3)		38 (42.2)	11 (12.2)	
Tumor size, cm ^d							
CIS (T0)	49 (23.9)				NA	NA	
T1 (<2)	104 (50.7)	66 (42.3)	38 (24.4)	.12	NA	NA	NA
T2 (2-4)	52 (25.4)	26 (16.7)	26 (16.7)		NA	NA	
TNM stage ^e							
I-II	138 (88.5)	84 (53.8)	54 (34.6)	.21	NA	NA	NA
III-IV	18 (11.5)	8 (5.1)	10 (6.4)		NA	NA	
Follow-up, mean (SD), mo	38 (20)	38 (20)	33 (19)	.03	44 (21)	41 (20)	.55

Abbreviations: CIS, carcinoma in situ; FV, fluorescence visualization; HGL, high-grade lesion; NA, not applicable; SCC, squamous cell carcinoma.

^a Percentages have been rounded and may not total 100.

^b Includes severe dysplasia and/or CIS.

^c High risk indicates tongue and floor of mouth; medium to low risk, soft palate complex, gingiva, and buccal mucosa.

^d Includes 205 patients: 156 with SCC and 49 with CIS.

^e Includes SCC only (n = 156).

FV group and 28 in the control group. For the SCC and HGL groups, we found no significant difference between the FV and control subgroups in age, smoking history, ethnicity (white or nonwhite), lesion anatomical site, and history of treatment for oral cancer or HGLs. The only difference for the SCC group was more women in the FV subgroup (43 of 156 [27.6%]) than in the control subgroup (18 of 156 [11.5%]) ($P = .02$). For the SCC group, we found no differences in tumor size (T1 vs T2) and TNM stage (stages I-II vs III-IV). The overall mean (SD) follow-up time was 38 (20) months. Patients undergoing the FV-guided approach in the SCC and HGL groups had a slightly longer follow-up time (38 [20] and 44 [21] months, respectively) compared with those undergoing conventional surgery (33 [19] and 41 [20] months, respectively) ($P = .03$ in the SCC group and $P = .55$ in the HGL group). This comparison ensured that the favorable outcomes observed in the FV group did not result from the difference in the length of follow-up.

Outcome Analysis

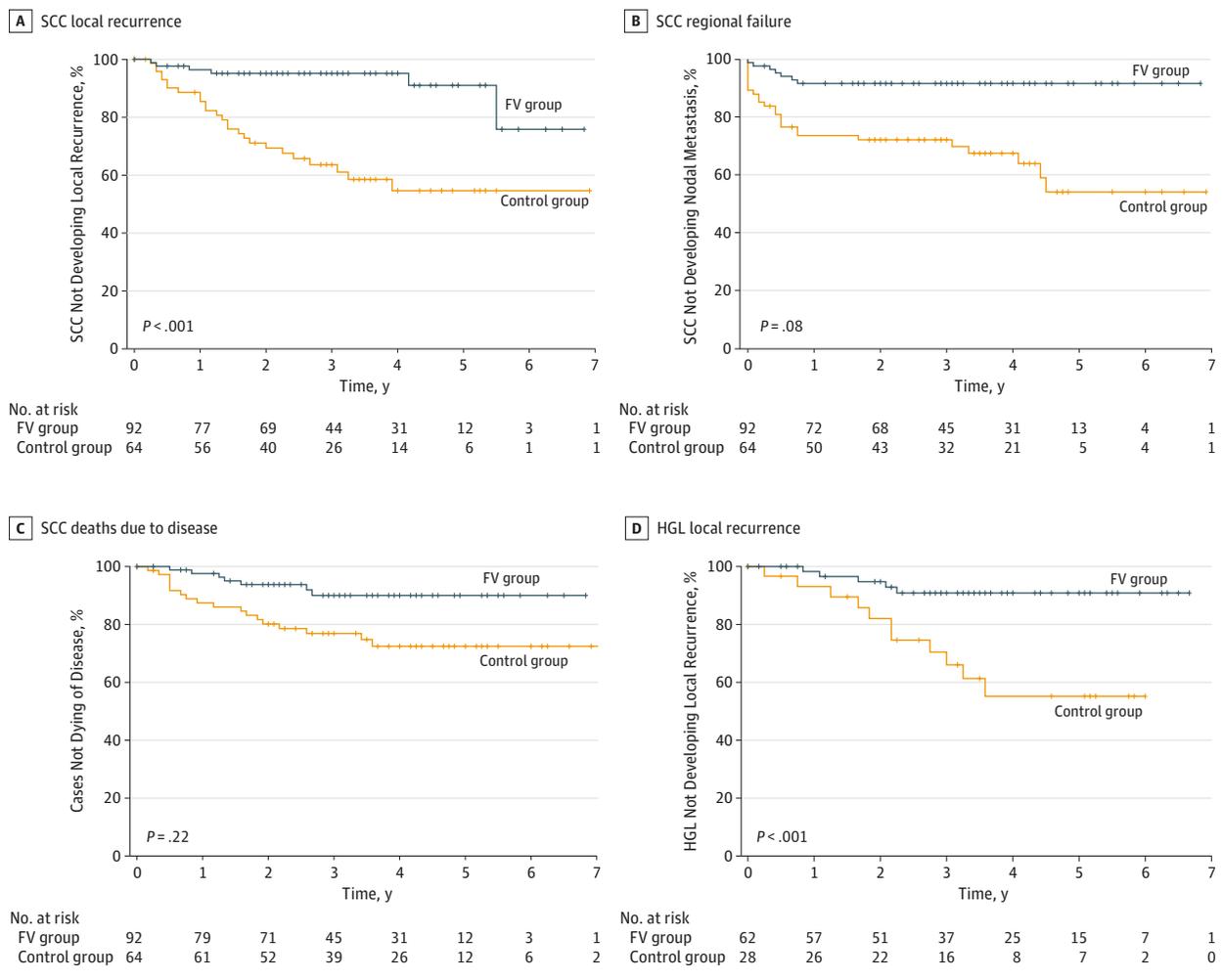
The rates of local recurrence, regional treatment failure, and disease-free survival in the SCC group were 20.5% (32 of 156 patients), 19.2% (30 of 156 patients), and 84.0% (131 of 156 pa-

tients), respectively; the local recurrence rate of the HGL group was 17.8% (16 of 90 patients). Using the Fisher exact test, patients undergoing FV-guided surgery showed significantly lower local recurrence in the SCC (6 of 92 patients [6.5%] vs 26 of 64 [40.6%]; $P < .001$) and HGL (5 of 62 [8.1%] vs 11 of 28 [39.3%]; $P < .001$) groups. The data also indicated that the FV-guided approach had less regional failure (14 of 92 patients [15.2%] vs 16 of 64 [25.0%]) and death (12 of 92 [13.0%] vs 13 of 64 [20.3%]) in the SCC group, although these differences were not statistically significant ($P = .08$ and $P = .22$, respectively).

Time to Outcome Analysis Examining the FV-Only Effect

The Kaplan-Meier plots and log-rank test for the time-to-outcome data (Figure 3A) revealed a significant difference within the SCC group between the 2 interventions in local recurrence ($P < .001$). When we compared the FV and control subgroups, the 2- and 3-year local recurrence rates showed significant reductions from 30.0% (19 of 64 patients) to 4.3% (4 of 92 patients) and from 35.9% (23 of 64 patients) to 4.3% (4 of 92 patients), respectively ($P < .001$). The rate of regional treatment failure for the patients with SCC was reduced in the

Figure 3. Kaplan-Meier Plot of Time to Outcomes



Kaplan-Meier plots represent probabilities of not developing the outcome of interest. FV indicates fluorescence visualization; HGL, high-grade lesion; and SCC, squamous cell carcinoma.

FV subgroup (15.2% [14 of 92 patients] vs 25.0% [16 of 64 patients]), although the difference was not significant ($P = .08$) (Figure 3B). We found no difference in the rates of disease-free survival between the FV and control subgroups (13.0% [12 of 92 patients] vs 20.3% [13 of 64 patients]; $P = .22$) (Figure 3C).

The same analysis methods were applied to patients with HGL and revealed a significant difference in local recurrence rates ($P < .001$) (Figure 3D). Based on Kaplan-Meier estimates for 2- and 3-year local recurrence rates, the FV subgroup showed significant reductions to 5% and from 30% to 9%, respectively, compared with 18% and 30% in the control subgroup. No patients in the HGL group developed regional failure. Hence, a meaningful comparison between the FV and non-FV groups was not possible.

Multivariate Analysis

The multivariate Cox proportional hazards regression model with the Lasso variable model selection procedure and the Akaike information criterion was used to examine the joint ef-

fects on local recurrence due to the FV approach and other factors, including age, sex, history of ever smoking, race (white vs nonwhite), previous cancer, anatomical site of the lesion, tumor size (T1 vs T2), and TNM stage (I-II vs III-IV). In the SCC group, the FV approach and age were identified as having a significant effect on local recurrence, with hazard ratios of 0.16 (95% CI, 0.06-0.39; $P < .001$) and 1.05 (95% CI, 1.02-1.08; $P = .002$) vs baseline, respectively. In other words, the FV-guided approach was estimated to reduce the hazard by a ratio of 0.16, whereas age inflated the hazard (Table 2).

Using the same method of analysis, the FV approach and previous cancer were shown to have a prediction effect for regional failure, with hazard ratios of 0.79 (95% CI, 0.36-1.74; $P = .56$) and 5.51 (95% CI, 1.79-17.0; $P < .001$), respectively. The beneficial effect of FV was observed but not significant for disease-free survival, whereas age and TNM stage (III-IV) had negative effects on survival. In the HGL group, the same analysis revealed that the FV approach had a significant beneficial effect in reducing the recurrence hazard against the baseline

Table 2. Significant Variables With Joint Effect to Outcomes

Variable	HR (95% CI)	P Value
Patients with SCC (n = 156)		
Local recurrence ^a		
FV guidance	0.16 (0.06-0.39)	<.001
Age	1.05 (1.02-1.08)	.002
Regional treatment failure ^b		
FV guidance	0.79 (0.36-1.74)	.56
Previous HGL	5.51 (1.79-16.99)	<.001
Death due to SCC ^c		
FV guidance	1.05 (0.34-1.67)	.48
Age	0.75 (1.02-1.10)	<.001
TNM stage III-IV	3.20 (1.06-9.62)	.39
Patients with HGL (n = 90)		
Local recurrence ^d		
FV guidance	0.16 (0.05-0.52)	.002
Previous HGL	2.99 (1.05-8.53)	.04

Abbreviations: FV, fluorescence visualization; HGL, high-grade lesion; HR, hazard ratio; SCC, squamous cell carcinoma.

^a Six of 92 patients (6.5%) in the FV subgroup and 26 of 64 patients (40.6%) in the control subgroup had local recurrence ($P < .001$).

^b Fourteen of 92 patients (15.2%) in the FV subgroup and 16 of 64 patients (25.0%) in the control subgroup had regional failure ($P = .08$).

^c Twelve of 92 patients (13.0%) in the FV subgroup and 13 of 64 patients (20.3%) in the control subgroup died of SCC ($P = .22$).

^d Five of 62 patients (8.1%) in the FV subgroup and 11 of 28 patients (39.3%) in the control subgroup had local recurrence ($P < .001$). No patients in either subgroup had regional failure ($P > .99$); 1 patient (3.6%) in the control subgroup died of HGL ($P > .99$).

by a factor of 0.16 (95% CI, 0.05-0.52; $P = .002$), and previous cancer had a significant harmful effect in inflating the hazard by a factor of 2.99 (95% CI, 1.05-8.52; $P = .04$).

For the SCC and HGL groups, the FV-guided approach was a strong and independent factor in the prediction of local recurrence. The beneficial effect of FV-guided surgery remained strong and significant after taking other potential confounding factors into consideration. In general, age and previous cancer inflated the recurrence hazards.

Discussion

This study is the first, to our knowledge, to present outcome data using a novel optical approach at the point of care to treat early-stage oral cancer. Moreover, we used the standard approach of a 10-mm clearance margin for HGL surgical treatment and examined the outcome. Owing to the lack of consensus in management and a high rate of local recurrence,^{28,29} the success of this approach in controlling the local recurrence of HGLs can have important clinical implications.

Extensive research on the importance of examining the field surrounding oral cancers for risk assessment and management of this disease has been performed.³⁰ Local recurrence remains a common problem, largely because of the presence of clinical occult disease spreading across the mucosal surface that is not apparent at the time of surgery.

One may argue the benefit of including a wider normal-appearing mucosa in the surgical margin. However, a previous study²⁴ has demonstrated that this lateral occult extension varies in size and, more important, in a nonuniform manner (ie, not even expansion) around the clinically apparent lesion. Thus, the strategy of arbitrarily setting a standard 10-mm normal-appearing surgical margin will not be effective in capturing all the disease. Overcutting without any guidance will not improve control of local recurrence and inevitably will cause further cosmetic and functional morbidity, leading to a failure of best practice. In this study, the use of the FV-guided approach demonstrated a significant reduction in local recurrence rates of SCC (from 40.6% to 6.5%) and HGL (from 39.3% to 8.1%) in 3 years. Owing to the length of mean follow-up time in this study cohort, we did not discuss the 4- and 5-year outcomes.

A wealth of literature supports the use of tissue autofluorescence in the detection of precancers in the lung, uterine cervix, skin, and oral cavity^{19,31-39}; this approach is already in clinical use in screening for lung cancer. The basic mechanism of action of tissue autofluorescence has been well characterized.⁴⁰ The FV device used in this study is a simple handheld field-of-view device for direct visualization of tissue fluorescence in the oral cavity. The tool serves as a research device and is the only such tool approved for commercial use by the US Food and Drug Administration and Health Canada. If validated in a randomized clinical study, the device and its approach can be translated into real-world use, with an immediate effect on oral lesion assessment before surgery or in the operating room. We are currently completing an interim assessment of results by monitoring the reappearance of this change at treated sites (C.F.P., D.W.A., J.S.D., et al; unpublished data; September 2015). This tool also has been used to follow up clinical changes to the oral mucosa of all patients in the Oral Cancer Prediction and Longitudinal Study, originally funded by the National Institute of Dental and Craniofacial Research, National Institutes of Health.⁴¹

In addition to demonstrating improved local recurrence, the results show a slightly improved regional control from 25.0% to 15.2%. This finding can be explained thusly: the removal of a bulk of diseased tissue during a single procedure may reduce the chance of progression and development of nodal metastasis. Local recurrence caused by incomplete excision of clinically occult disease at the margins remains the primary concern; however, residual disease at the surgical bed (ie, the deep surgical margin) also can cause later local recurrence or regional failure. Although one may argue that the device has its limitation in the assessment of the deep resection margin, this limitation does not seem to be an issue in our study because of the extensive experience of our study surgeons. We agree that device limitations can be an issue with residual disease (ie, tumor cells or tumor-associated fibroblast resulting from epithelial connective tissue crosstalk) at the surgical bed. A collaboration is under way with a study group led by Zhang et al⁴² in which investigators plan to use the fibroblast activation protein beacon approach to capture the residual disease at the surgical bed. The approach may impose a potential solution to this question. Our present study is the first step in

using a novel optical technology to examine this long overlooked question. A stepwise approach, from lateral to deep margins, is needed for eventual local control of this disease.

Screening is believed to be the key to prevent this disease. However, lack of effective treatment can lead to futile screening efforts. Furthermore, screening may create more harm in causing stress and anxiety, especially among patients with precancerous conditions. Moreover, repetitive ineffective treatment not only is costly to patients and society but also can result in physical and psychological morbidity to patients. We now provide a very promising solution to this situation with effective treatment for early-stage cancer.

In this single-center retrospective study, selection bias cannot be avoided. The sample size, length of follow-up, and novelty of the optical approach for this long-standing problem are unique. In addition, a small subgroup, constituting approximately 5% of patients with SCC, had poor pathologic findings (ie, perineural invasion or positive margins) and received post-surgical adjuvant radiotherapy or chemoradiotherapy; these treatment modalities might affect the outcome data. A randomized clinical study is required to obtain level 1 evidence and change usual practice. This study has also set the stage for the pan-Canadian, multicenter, phase 3 Canadian Optically Guided Approach for Oral Lesions Surgical (COOLS) Trial funded by the Terry Fox Research Institute.⁴³ The COOLS Trial

first started in October 2010^{43,44} and has completed the accrual of 400 patients across Canada. This trial will not only assess local disease-free survival (primary end point) but also examine the field shift using molecular tools (nuclear phenotype score⁴⁵ and loss of heterozygosity^{41,46}); the trial will also examine the economic health impact using the incremental cost-effectiveness ratio for cost per avoided local recurrence and cost per quality-adjusted life-year for the cost-effectiveness. An integrative knowledge translation planning is one of the major goals. At the end of the trial, we hope that the development of a user-friendly knowledge translation package can facilitate the rollout of the study results into communities and ultimately into global cancer care centers.

Conclusions

This study's results support the use of FV as the strongest single independent factor in the control of local recurrence and provide a possible effective modality to control early-stage oral cancer and high-grade preinvasive oral lesions. If validated in the multicenter prospective COOLS Trial, level I evidence will support this novel approach to be implemented in the clinical setting to change practice and, subsequently, improve patients' outcomes.

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Study concept and design: Poh, Anderson, Durham, MacAulay, Rosin.
Acquisition, analysis, or interpretation of data: Poh, Durham, Chen, Berean, MacAulay.
Drafting of the manuscript: Poh.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Poh, Chen, MacAulay.
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