## Different Perspective

# Early Detection of Oral Neoplasia: Watching with New Eyes

*Perspective on Roblyer et al., p. 423*

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**Editor's Note:** The editors invited two perspectives—one more detailed, one more open-ended—on the important article in this issue of the journal by Roblyer et al. on new imaging technology for neoplasia screening in the oral cavity. Whereas the perspective by the Rosin group (Poh et al.) puts autofluorescence imaging and the Roblyer et al. report under a figurative microscope, this different perspective both examines the Roblyer et al. study and the broader implications of evolving imaging technology for detecting cancer and intraepithelial neoplasia.

Yogi Berra said, "You can observe a lot just by watching." Since an estimated 47,560 new cases and 11,260 deaths from cancers of the oral cavity, oropharynx, and larynx (3% of all new cancers and 2% of all cancer deaths) occurred in the United States in 2008 (1), it is time to start "watching" these sites with new tools and insights. Despite advances in diagnostic tools and treatment modalities, overall survival rates for these cancers have improved little over the last three decades (2). The main reasons for treatment failure are second primary tumors in patients with early-stage disease (stages I and II) and local recurrence and metastases in patients with locally advanced disease (2). These cancers result from multistep carcinogenesis, which involves increasing degrees of mucosal atypia and dysplasia and molecular (epigenetic and genetic) alterations (3) and "field cancerization," in which the multistep changes occur over large areas of the carcinogen-exposed upper aerodigestive tract epithelium [the seminal field hypothesis was proposed over 50 years ago by Slaughter et al. (4) based on work in the oral cavity]. Therefore, there are three potential approaches to reduce the incidence of head and neck cancer: first, early detection and local control of high-risk focal precursor lesions; second, decrease an individual's exposure to carcinogens; and third, systemic chemopreventive agents to halt or reverse carcinogenesis in individuals exposed to a risk factor(s) and/or diagnosed with a precursor lesion (5, 6). The effect of local control by removing head and neck precursor lesions has not been established as a method of reducing cancer risk (7). Although numerous changes contribute to epithelial carcinogenesis, histologically defined intraepithelial neoplasia (IEN), or premalignant lesions, is still considered to be better than any individual molecular marker for predicting cancer risk (8, 9).

Among head-and-neck cancers, oral neoplasia is particularly amenable to imaging because of the accessibility of its epithelial surface and the frequency of its routine screening by dentists. Once established for oral cancer screening, successful imaging tools could also be used in other cancers. Oral IEN initially appears as white or red patches (oral leukoplakia or erythroplakia, respectively) and carries a 17.5% risk of malignant transformation, or 36.4% in cases of dysplastic oral IEN, at 8 years (10). More precise definitions of risk are necessary; however, because carcinogenesis is multifocal and multiclonal, even within the same lesion, not all IEN progresses to cancer or can be readily detected and measured, and, at present, no specific drug can target all the causative genetic changes preceding or within IEN. To proceed with screen-detected lesions and determine if excision or chemopreventive intervention is warranted, more definitive screening and risk-assessment measures are required.

Loss of heterozygosity profiling is one of the better current methodologies for refining the risk of progression of histologic IEN. Loss of heterozygosities at specific loci in the genome encoding tumor suppressor genes are powerful predictors of oral cancer development. Several allelic losses have been shown to be early events of head and neck tumorigenesis (7, 11-13). Loss of heterozygosity at 3p and 9p is not only frequent but also predicts progression to invasive cancer (7, 11-13) and is a very powerful predictor of a second oral malignancy at previously treated oral cancer sites (13). Moreover, loss of heterozygosity findings have genetically confirmed the concept of field cancerization by showing the clonal relationship of transformed cells in large areas of mucosa (11, 14). Microsatellite analysis at the 9p, 3p, 17p, 8p, 13q, and 18q chromosomal regions and mutation analysis for p53 have shown that genetically altered mucosa remains after treatment in a majority of patients, a finding with potentially significant clinical implications (15). These findings confirmed earlier studies of the feasibility of p53 alterations as a tool for molecular staging and fingerprinting of head and neck tumors and underscore the need for a molecular basis for risk characterization (11). Despite the great potential of this type of approach, reagents and devices that can detect these molecular markers are not suitable at present as rapid screening tools for cancers of epithelial surfaces (i.e., they require tissue sampling and are too costly, time consuming, and labor intensive), and development of alternatives that complement loss of heterozygosity are necessary.

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Although some progress has been made in reducing carcinogen exposure (e.g., through tobacco use cessation) and in chemopreventive interventions, clearly more work is needed. There also remain several limitations to current oral cancer screening methods that could be addressed by improved cytologic methods and new imaging approaches. Current screening approaches rely too heavily on clinical experience for visual recognition because early neoplastic lesions have the appearance of more common benign lesions and many patients are reluctant to undergo biopsy (16). Staining with vital dyes has been used to increase screening sensitivity, but it has low specificity (i.e., also detects nonneoplastic lesions) and also requires expertise for interpretation. The alternative of using brush biopsy for cytology delays diagnosis relative to visual inspection with or without vital dyes and has an unknown false-negative rate. Better cytologic methods, including measurements of allelic imbalance in tissue from visible lesions as well as from normal-appearing mucosa, have improved screening accuracy in detecting oral neoplasia (17) but are still limited by delayed diagnosis. Optical technologies offer promising new ways to detect the changes associated with carcinogenesis. For example, the recently Food and Drug Administration–approved VELscope (LED Dental, Inc.; refs. 18, 19) offers a marked improvement over previous devices for measuring autofluorescence; the interpretation of its findings, however, still relies heavily on subjective evaluations from the image reader. Optical fibers and microscopes also have limitations, including the inherent limitation of small fields of view, which requires measurements on small areas that are deemed suspect by visual inspection. Interrogation of small regions, or point measurements, offered by microscopic or spectroscopic methods are only useful for visually obvious lesions because the user needs to determine the position of the optical device before making the measurements. Therefore, a wide-field optical method for efficiently detecting intrinsic changes in the appearance of neoplastic tissue, relative to normal tissue, which does not allow user bias or require user interpretation, is required for effectively screening and interrogating large epithelial surfaces areas.

The work from the laboratory of Richards-Kortum published by Roblyer et al. (20) in this issue of the journal describes the use of a computer algorithm that has been trained with clinical data to interpret data from a wide-field autofluorescence optical system. This algorithm provides a quantitative and objective assessment of differences in the appearance of normal and neoplastic tissue. This system is a significant improvement over previous approaches because much of the guesswork is removed through the use of a trained algorithm. This study evaluated the optical system in 56 patients with oral lesions and 11 normal subjects, establishing a classification algorithm using learning and test sets. It was used to quantify the decrease in the ratio of red-to-green autofluorescence in neoplasia relative to normal tissue, assessing it on a pixel-by-pixel basis within the regions of interest for each patient. Use of autofluorescence is an excellent and logical approach to early detection because it has long been established that neoplasia "looks different" than normal tissue, and the strategy described here aims to quantify this difference in appearance using specific colors (i.e., wavelengths of light) and accumulated experience from known data sets for oral cancer cases.

The optical approaches that are based on the inherently different "look" between neoplasia and normal tissue can be powerful strategies because the use of intrinsic optical properties obviates the need for exogenous dyes and probes (21). Moreover, the physiologic and molecular changes that cause these differences in appearance have been studied intensively, enabling molecular assessments from the optical properties. Intrinsic differences in the optical properties of tissue can be due to changes in absorbance (22), scatter (23), or fluorescence (24–28), and these changes can result from inflammation, increased cellularity, altered cellular anatomy (e.g., cell shape and nuclear volume), appearance of tumor stroma, and biochemical differences associated with cellular proliferation and tissue reorganization. The study by Roblyer et al. shows us a way of looking at neoplasia with a highly sensitive and unbiased eye. This study focuses on autofluorescence, or changes in fluorescence without the addition of exogenous fluorescent dyes, which is largely a function of tissue composition attributable to changes in collagen and elastin, and in concentrations of biomolecules such as hemoglobin and other porphyrins. Detection strategies based on intrinsic changes have the advantage of not requiring use of extrinsic dyes or contrast agents; however, the interpretation of subtle changes in optical properties of tissues has caused ambiguity in the past. The contribution of this study to the field is the development and testing of a well-trained algorithm for interpreting the data and removing user error. Optical differences can be very specific for a given change in the tissue, but the differences are often slight and subject to variation. The algorithm described here accounts for nuances and variability in the signal, resulting in a screening tool that is both user-friendly and low-cost. These advances will enable reliable, wide-spread use of autofluorescence screening that potentially will reach the patients in greatest need.

Alternative approaches would largely require the use of exogenous contrast agents. Developing exogenous contrast agents is a focus for many research groups looking at a variety of cancer types (29-33). Because many epithelial surfaces are readily available for optical inspection and exogenous probes may be applied topically, a large number of these probes use markers that can be detected with visible and near-IR light. The molecular etiologies of many cancers are shared; therefore, the large number of emerging molecular probes will likely have utility in the early detection of oral and other cancers.

The autofluorescence study of Roblyer et al. highlights the importance of standardization in imaging and the use of automated analyses for cancer screening. Imaging tools that target cellular processes, such as proliferation and apoptosis, or physiologic changes such as hypoxia and angiogenesis have been developed. Alternatively, key cell-surface markers of neoplasia or dysregulated cellular physiology associated with rapid cellular proliferation can be targeted with specific molecular probes. Examples of imaging both physiologic and molecular changes are seen in the study of angiogenesis, where changes in blood flow can be assessed or localization of molecular probes that target a mediator of angiogenesis (e.g., vascular endothelial growth factor) are determined (34). Optical imaging approaches are being developed in other IEN settings, for example, to enhance early detection in the cervix and colon-rectum (16). The use of changes in autofluorescence and the use of probes for epidermal growth factor

receptor have been evaluated in cervical IEN. These tools will enhance standard screening and may complement established screening approaches for these tissues (35). Colonoscopy with IEN removal (polypectomy) is the standard of care for colorectal cancer risk reduction and has been proven to be significantly effective in this regard (8, 9). Despite the anatomic changes associated with colon carcinogenesis that are readily detected (e.g., raised polyps), a number of flat lesions can be missed and cancers can be hidden behind folds in the colon (36). Dye-spray chromoendoscopy has been used to reduce the number of missed lesions, and further studies will determine if it will further reduce cancer risk (36). Chromoendoscopy is a method of anatomic imaging that reveals changes in tissue structure, but it does not detect molecular changes. The development and use of molecular-imaging probes and the microscopes and cameras that detect binding will complement these methods, further decreasing miss rates and identifying more-aggressive adenomas of any size or surface contour (30). This strategy is being actively investigated, and such molecular probes will help in detecting aggressive IEN at other tissue sites including the oral cavity.

Molecular probes can be designed in various ways so that they are activated, or "light up," when they interact with their molecular target (34). This is an effective means of reducing noise and improving detection. A variety of labels can be used to create optical probes that include fluorescent dyes (33) and dots (37, 38), chromogens (39) and scattering particles (40). These probes enhance the optical differences between normal and malignant tissues and can be used to molecularly phenotype the cancer. The molecular specificity of the probes gives them the potential for image-guidance and to predict and monitor responses to specific therapies. As such, these new tools will have tremendous clinical potential; however, there will be significant barriers to translation given the regulatory requirements for using molecular diagnostic tools in patients and high-risk subjects. Although already useful for screening, imaging tools based on tissue autofluorescence cannot reveal the molecular basis of neoplasia with the specificity needed for guiding molecularly targeted drug interventions. However, the practical ease of translation gives these tools a significant advantage over approaches that require the use of exogenous reagents for contrast. The development of multiparameter optical methods will enable the complementary use of methods such as those described by Roblyer et al. and any number of molecularly targeted probes. These probes could help in selecting patients most likely to benefit from a specific molecular-targeted intervention. Therefore, this complementary use will present the best of both worlds and offer the greatest opportunity for early detection and personalized intervention by building on the strengths of each approach.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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