HHS Public Access

Author manuscript

Ann Surg Oncol. Author manuscript; available in PMC 2020 November 01.

Published in final edited form as:

Ann Surg Oncol. 2019 November ; 26(12): 4070–4080. doi:10.1245/s10434-019-07645-9.

Margin Analysis in Head and Neck Cancer: State of the Art and Future Directions

Michael M. Li, MD, **Sidharth V. Puram, MD, PhD**, **Dustin A. Silverman, MD**, **Matthew O. Old, MD**, **James W. Rocco, MD, PhD**, **Stephen Y. Kang, MD**

Division of Head and Neck Oncology, Department of Otolaryngology - Head and Neck Surgery, The James Cancer Hospital and Solove Research Institute, The Ohio State University, Columbus, OH

Abstract

Background.—The status of surgical margins is the most important prognosticator for patients undergoing surgical resection of head and neck squamous cell carcinoma (HNSCC). Despite this, analysis of surgical margins is fraught with inconsistencies, including the ways in which margins are sampled and interpreted. Fundamentally, even the definition what constitutes a “clear” (or negative) margin may vary between institutions, surgeons, and pathologists.

Methods.—The PubMed database was queried for articles relevant to the topic, and experts in the field were consulted regarding key articles for inclusion. Abstracts were reviewed and the full text was accessed for articles of particular interest.

Results.—Data regarding various approaches to traditional margin analysis have been published without consensus. Several next-generation technologies have emerged in recent years that hold promise.

Conclusion.—An overview and appraisal of traditional margin analysis techniques are provided. Additionally, we explore novel technologies that may assist in more accurate margin assessment, guide the extent of surgical resections intraoperatively, and inform decisions regarding adjuvant treatment postoperatively.

The status of the surgical margin is the most important prognosticator in the surgical treatment of head and neck cancer.[1,2] Traditionally, margin status is determined intraoperatively through the use of frozen sections, with the identification of a positive margin on frozen histopatho-logic analysis driving additional resection; the ultimate goal is a negative, intraoperative, frozen margin.[3] Postoperatively, the final histopathologic analysis guides adjuvant treatment and bears substantial prognostic significance; the association between positive margins and decreased survival as well as increased rates of locoregional recurrence have been well-established for all head and neck sub-sites.[4,5] A positive surgical margin may increase local recurrence by 90% and has been shown to increase the risk of all-cause mortality at 5 years by 90% in oral cavity cancer.[5,6] Furthermore, unlike other

S. Y. Kang, MD Stephen.kang@osumc.edu.

DISCLOSURE The authors have nothing to disclose.

prognostic factors, such as T or N stage, the status of the surgical margin is under surgical control.

Despite the importance of margin status, many questions remain unanswered, including the definition of a negative versus “close” margin and whether final margin status should be obtained from the main surgical specimen or the surgically resected wound bed. In addition, there is a contrast between the amount of tissue required to obtain a negative surgical margin between head and neck subsites, such as the oral cavity, oropharynx, and larynx. Additional challenges exist when assessing surgical margins in the setting of field cancerization, submucosal spread, and bone invasion.[7,8] To address these issues, various technologies with the potential to optimize margin analysis intraoperatively and postoperatively have arisen in recent years. Among these, optical imaging—often enhanced with an antibody conjugated fluorescent molecule—holds significant promise in guiding intraoperative resection, but large-scale clinical studies have yet to take place. Likewise, few clinical studies have occurred in the field of molecular margins, wherein interrogation for specific mutations, translocations, or other genetic alterations are performed. While this method is further limited by cost, such genetic analyses may guide recommendations for postoperative adjuvant treatment, especially in the setting of dysplastic or “close” margins. In this review, we provide an overview of current surgical margin analysis techniques and the next-generation technologies in surgical margin analysis.

DEFINING THE SURGICAL MARGIN

It is a widely accepted principle that surgical resection with clear margins remains the most critical objective of a successful extirpative head and neck surgery.[9] Despite this, consensus regarding the definition of a “clear” or negative surgical margin is lacking, with a high degree of variability in how margins are evaluated in both the intra- and postoperative settings. In a survey of American Head and Neck Society members, a plurality (46%) of surgeons defined a clear margin as >5 mm on microscopic examination. Yet, strikingly, the remaining 54% of surgeons—more than half of those surveyed—utilized differing interpretations when defining a clear margin, with responses ranging from >1 high-power field to >1 cm, to the absence of ink present on the tumor specimen.[3] These heterogeneous responses represent the complexity and challenging reality of margin sampling, with varying interpretations between both surgeons and pathologists. While algorithms for surgical tissue sampling and histopathologic assessment are dependent on tumor type and anatomic location, the methods by which those margins are analyzed greatly influences the need for re-resection. In contrast to so-called “breadloaf” sectioning, in which serial transverse cross-sections of tissue are evaluated, recent advances in complete circumferential peripheral and deep margin assessment (CCPDMA) have significantly decreased recurrence rates, particularly for nonmelanoma skin cancers.[10–12] In contrast to cross-sectional analysis, CCPDMA allows for comprehensive examination of the entirety of the surgical margin. Mohs micrographic surgery, a form of CCPDMA, has been demonstrated to be superior to standard histopathologic sectioning, particularly when attention to tissue-sparing techniques and cosmesis is paramount, with decreased recurrence rates for cutaneous basal and squamous cell carcinoma.[11,13]

Another major source of variability is related to the source of tissue, namely, whether tissue is sampled from the surgical wound bed or the tumor itself. Seventy-six percent of surgeons reported sampling from the tumor bed (defect-driven sampling) as opposed to the resected specimen itself (specimen-driven sampling).[3] In early-stage oral cancer, Amit et al. reported a significantly increased rate of negative final margins utilizing a specimen-driven approach compared with a defect-driven approach (84% vs. 55%, respectively).[14] Similarly, Varvares and colleagues concluded that a specimen-driven approach may be a more accurate reflection of resection adequacy and thus a better predictor of local control.[15] Others have advocated for specimen-driven sampling of *en bloc* resections with face-to-face handoffs between surgeon and pathologist to ensure adequate and precise histopathologic sampling.[16] One potential advantage of specimen-driven sampling may be improved accuracy during re-resection; when surgeons were asked to identify and subsequently reidentify sampling sites in a defect-driven approach after 5 minutes, the mean error for mucosal margins was 9 mm and 12 mm for deep margins.[17] Thus, even while a majority of surgeons report a preference for defect-driven sampling, evidence suggests that specimen-driven margin sampling may yield clear margins at a higher rate.

DEEP SURGICAL MARGINS

Analysis of the deep surgical margin represents further complexity in margin analysis. During *en bloc* resection, the peripheral mucosal margins are easier to evaluate through visualization and palpation compared with deep margins. Additionally, resection at the deep margin may be limited by anatomic constraints at select subsites, including the oropharynx and retromolar trigone. Theoretically, features, such as lymphovascular invasion (LVI) and perineural invasion (PNI), allow for deep infiltration of tumor. These features are unrecognizable intraoperatively via visual inspection and palpation and may thereby up-stage tumors during permanent histopathologic analysis.[18] Sampling at the deep margin may not be feasible at certain subsites (e.g., oropharynx, parapharyngeal space) due to the presence of immediately adjacent neurovascular structures. These practical constraints may contribute to higher rates of margin positivity when sampling from the deep surgical margin. In a study of 301 oral cavity and oropharyngeal squamous cell carcinoma (SCC) specimens, 70 (23.3%) resections had positive margins; of these specimens, 61 (87.1%) were discovered to have a positive deep margin.[19] The study also demonstrated that the deep margin alone was responsible for a positive margin in 50 of the 70 (71.4%) resections. Given the association between a positive surgical margin and worse oncologic outcomes, particular attention must be paid to the deep margin.

Some authors have suggested that so-called piecemeal resection may yield higher rates of negative margins at the deep margin. Piecemeal resection generally encompasses a stepwise resection with serial histopathologic analysis.[20,21] In a series of 42 patients, a combination of narrow band imaging (NBI) and carbon dioxide laser was used to perform piecemeal resection of oral cavity and oropharyngeal tumors, revealing increased rates of clear margins in the piecemeal resection group.[21] In this study, the authors analyzed intraoperative margins from each segment resected until a negative margin was achieved and argue that compared with traditional *en bloc* resection, piecemeal resection allows for more comprehensive margin analysis, especially at the deep margin.[21] Similar findings were reported by Choi et

al. who demonstrated the utility of transoral bisected resection (TBR) among 75 patients with cT1-T2N0 oral tongue SCC as a form of piecemeal resection. In this study, the midportion of the tumor was incised deeply until healthy, native tissue was encountered, with subsequent delineation of the peripheral margins needed for *en bloc* transoral extirpation.[20] The authors contend that similar techniques may provide a more adequate deep resection margin, particularly for early-stage tongue SCC. While piecemeal resection techniques allow for margin mapping in three-dimensional (3D) orientation, validated prospective studies are required to assess fully the long-term efficacy of these methods, including data on additional surgical time for approaches, which rely on extensive, intraoperative, frozen-section analyses.

BONE MARGINS

Analysis of bone margins poses orthogonal challenges for intraoperative, frozen-section analysis. In advanced oral cavity tumors, which often present with osseous mandibular cortical erosion, such an analysis represents the crux of successful oncologic surgery. However, as opposed to mucosal margins, frozen analysis of cortical bone cannot be reliably performed as specimens require decalcification and dedicated analysis on permanent pathologic examination. This process requires 7 days—after oncologic surgery and complex reconstruction have already been completed.[22,23] As a result, surgeons may sample cancellous bone from the osteotomized edge of mandible. Although a common technique, a series by Bilodeau et al. comparing curette specimen of marrow and sampling of the inferior alveolar nerve to final histopathologic analysis reported specificity of 50% with a sensitivity of 100%.[24] In contrast, Forrest and colleagues reported a 98.3% concordance between frozen-section analysis of the cancellous bone and final pathologic analysis of the cortical bone.[25] In efforts to expedite analysis of cortical bone, Weisberger et al. experimented with microwaving tissue immersed in formalin and sectioning with a diamond-bladed band saw, thereby reducing processing time to two hours. This group reported 100% correlation between this technique and conventional decalcification in a sample of 10 specimens.[26] Nieberler et al. further expedited analysis of cortical bone by utilizing cytopathologic analysis of cortical bone scrapings obtained with either a scalpel or cytobrush. Overall, cytopathologic analysis resulted in 95% sensitivity and 96% specificity compared with histopathologic analysis.[27] Perhaps due to the small sample sizes in these studies and varied sensitivity in using frozen analysis of bony margins, only 21% of surgeons report utilizing frozen-section analysis to evaluate osseous margins.[3] In an attempt to simplify the issue, Singh et al. proposed performing mandibular osteotomies at least 15-mm away from clearly visualized bony or mucosal disease to ensure clear bone margins.[28] Regardless of method, intraoperative sampling of the bone margin should be performed when osseous involvement is encountered.

ACCURACY OF FROZEN SECTIONS

During frozen-section analysis, the tissue is frozen, stained by hematoxylin and eosin (H&E), and analyzed by a surgical pathologist, with results reported in real time to guide intraoperative decision-making and need for re-re-section. Challenges inherent with frozen-section histopathologic analysis include tissue distortion during sampling, transport,

sectioning, tissue staining, and the inability to use additional tumor-specific stains. Other sources of error include sampling error by the surgeon and interpretive error by a pathologist, in which a section is misread under the microscope. Despite these challenges, multiple studies have reported a high rate of concordance between frozen sections and final analysis of the main specimen with values routinely exceeding 96% in academic centers.[29–31] While surgery at academic institutions and increasing facility volume have been associated with lower rates of positive margins, there have not been studies examining the relationship between these factors and the concordance of frozen and final margins.[32] Instead, it appears the majority of positive margins occur at sites that are not sampled intraoperatively.[29–31] Furthermore, as many as 22.1% of all cases with intraoperative frozen sections interpreted as negative may have a close or positive margin on analysis of the main specimen, implying sampling error.[30] These findings suggest that while frozen-section sampling is reliable, interpretive, and especially sampling, error remain persistent challenges undermining its accuracy.

When a positive frozen margin is identified, standard of care is to re-resect with the ultimate goal of achieving a negative frozen margin. Remarkably, Du and colleagues reported that 22.9% of cases demonstrated a positive margin, which was subsequently re-resected to negative; however, patients with an initial positive margin, even when re-resected to negative, have been shown to perform poorly compared with those in which negative margins were obtained during initial resection.[30] Ettl et al. showed that a positive frozen margin on the initial resection— again, even when revised to negative—was the strongest predictor of locoregional recurrence in a study of 156 patients, despite a lack of association between an initial positive margin and disease-specific survival (DSS).[33] Patel et al. demonstrated that microscopic tumor “cut-through” on initial frozen section, when revised to negative margins, had an independently adverse effect on locoregional control and DSS in the presence of regional disease.[34] A potential factor affecting the results in each of the aforementioned studies is the presence or absence of adjuvant treatment, which may influence locoregional and DSS, and cannot be controlled for in retrospective studies. Importantly, tumors with positive margins on initial resection are rarely a reflection of surgeon skill or technical errors; instead, it appears that when a negative final frozen margin is obtained, the presence of an initially positive frozen margin may represent a tumor with more aggressive biology, especially in the presence of other adverse features, such as LVI or PNI.

DEFINING A “CLOSE” MARGIN

To date, no consensus on what constitutes a close margin has been established or what steps should be taken when this scenario is encountered. The significance of a close margin may change based on the specific anatomic subsite, surgical approach, and disease pathology. In early-stage (Tis or T1) glottic SCC, laser cordectomy is commonly utilized given the goals of functional conservation, minimizing penumbral tissue insults, and relative ease of re-resection. In this situation, studies have demonstrated that utilizing a 1- or 2-mm cutoff to define a negative surgical margin has no effect on locoregional recurrence or DFS.[35,36]

In several series focusing on oral cavity cancer using 1–5 mm as the cutoff to define a close margin, analyses have failed to identify an association between close margins and worse locoregional recurrence (LRR) or DFS.[37–42] Ch'ng et al. did not identify a difference in LRR or DFS when comparing clear margins to a close surgical margin (≤ 5 mm from tumor edge).[40] Others have replicated this finding with regards LRR but interestingly have suggested that a cutoff of 2.2 mm is predictive of worse DFS, because patients with a margin of 2.3–5.0 mm had similar survival to patients with ≥ 5-mm margins.[41] Based on their own data and a comprehensive review, Ch'ng et al. reasoned that while a close margin alone may not warrant adjuvant treatment, in the setting of other adverse features, such as PNI or LVI, adjuvant therapy may be warranted.[40]

Clearly, a complex interplay between biological, logistical, and technical factors exists when considering accurate margin analysis—a summary of which is provided in Table 1. To address these challenges, several "next-generation" technologies have arisen with the goal of addressing shortcomings. The remainder of this review will aim to provide an overview of these technologies and their significance in clinical practice—a summary of which is provided in Table 2.

ADVANCED IMAGING

In this section, we describe techniques that utilize advanced imaging and fluorescent agents to assess margin status. Of these, 5-aminolevulinic acid (5-ALA) is among the most studied fluorescent agents, especially in malignant glioma.[43,44] 5-ALA is a metabolite of the heme synthesis pathway, which preferentially concentrates in malignant cells, and is metabolized to protoporphyrin-IX (PP-IX). PPIX fluoresces when exposed to 405 nm light, creating differential fluorescence between tumor and normal tissue. Although promising in other contexts, studies utilizing 5-ALA for head and neck tumor identification have not borne out this excitement. When using 5-ALA as an oral rinse before surgery, fluorescence was identified in both normal and dysplastic epithelium. In three of four cases within one case series, invasive carcinoma did not fluoresce, whereas the overlying normal epithelium did.[45] Enthusiasm for this approach has been further limited by its poor soft-tissue penetration (1 mm).

High-resolution microendoscopy (HRME) utilizes a fluorescence microscope to allow for real-time, intraoperative microscopic evaluation of tissue following the application of a fluorescent agent. Acquired images are evaluated for histologic changes indicative of malignancy by a pathologist. In a study of 33 patients with oral cavity and oropharyngeal tumors, Miles et al. demonstrated 95.1% accuracy with HRME and topical application of a nonspecific fluorophore in differentiating between neo-plastic and benign mucosa with 96% specificity and 95% sensitivity.[46] Limitations of HRME include its poor depth of penetration, which is limited to 50–100 microns, precluding evaluation of submucosal spread and deep margins. Moreover, images may be distorted by tissue auto-fluorescence and prior radiation. Although this technology remains promising, many of the challenges experienced with standard frozen-section H&E analyses persist with HRME, including the presence of sampling bias, because the surgeon determines which tissue to sample. Thus, questions regarding whether to apply a specimen or defect-driven approach reemerge, along

with the possibility of obtaining false-negative results secondary to sampling error. As a notable logistical constraint, a pathologist is required to interpret the image, limiting the ease of implementation of HRME technology for intraoperative margin assessment.

In recent years, near-infrared (NIR) fluorescence imaging has emerged as an alternative modality for augmenting residual tumor detection in vivo. NIR light exists in the 700–900 nm range and is invisible to normal human vision. Compared with white light, NIR has a greater depth of tissue penetration (up to 10 mm) and avoids auto-fluorescence, creating a greater signal-to-noise ratio.[47,48] NIR imaging is performed with a fluorescent agent and charge coupled diode (CCD) camera, which converts fluorescence signal into high-resolution, grayscale images. Several fluorophore options exist with the most common being indocyanine green (ICG), Cy5.5, Cy 7, and Irdye800-CW.[48] Various imaging systems are available and range from handheld intraoperative devices to closed systems for visualizing resected specimens. The specificity of NIR has recently been enhanced through conjugating NIR-based agents to cancer-specific antibodies (e.g., cetuximab or panitimumab).[48] Using IRDye800 conjugated to epidermal growth factor, Keereweer and colleagues demonstrated a high tumor to background signal in a murine model of oral cavity SCC.[49] Van driel et al. validated this work in follow-up studies, while Atallah et al. demonstrated 50% improvement in DFS in a murine oral cavity SCC model using a fluorescently conjugated integrin antibody to guide surgical resection of the tumor.[50,51] In a phase I study, a cetuximab-IRDye800CW conjugate was utilized in nine patients, with the authors demonstrating a strong correlation between EGFR immunohistochemistry and NIR fluorescence intensity (i.e., fluorescent signal faithfully recapitulated EGFR protein levels).[47] More recently, a phase I clinical trial utilizing panitumumab-IRDye800CW and NIR was completed by Gao et al., demonstrating the feasibility of this imaging system in identifying tumor presence at resected margins with a sensitivity of 100% and specificity of 90% when using an open-field imaging system to perform in situ imaging.[52] The authors also utilized a closed-field imaging system to image resected tumor specimens and create a fluorescence map of the specimen, allowing for comprehensive analysis of margin status in a resected specimen. In a follow-up study, the authors used a closed system imaging device to create an optical specimen map of resected specimens, demonstrating 95% sensitivity for identifying tumor within 5 mm of the specimen surface and 100% sensitivity within 2 mm; of note, tumors were able to be imaged intraoperatively in approximately 7 min.[53] Furthermore, white-light overlays of the fluores-cent map were created, offering the promise of more accurate re-resection.

Antibody-guided fluorescent imaging techniques are not without limitation. While EGFR is expressed in many head and neck tumors, tumor heterogeneity may affect sensitivity and specificity of imaging techniques. As EGFR is expressed in normal tissue, auto-fluorescence may confound results and lead to unnecessary resection. Additionally, De Boer and colleagues noted low mean fluorescence intensity in regions of well-differentiated keratinizing cells and necrotic regions.[47] While NIR does offer an improved depth of imaging over techniques, such as HRME, the penetration depth is limited to 5–10 mm, which may limit the analysis of large, anatomically complex tumors. Although van Keulen et al. demonstrated the ability to comprehensively image a tumor along with its deep margin,

few patient studies have been performed with most limited to small cohorts of patients with oral cavity and oropharyngeal tumors.[53]

SPECTROSCOPY

In addition to antibody-directed fluorescent imaging, fluorescence spectroscopy (FS) has emerged as another option for margin analysis. FS is based on the physical property that molecules emit a specific wavelength of light when excited by an exogenous electromagnetic source. The emission of biological molecules is dependent on the concentration and ability of a given molecule to absorb, and subsequently emit, energy that is appreciated as fluorescence.[54] When adapted for use in tissue analysis, FS devices consist of a light source and, most commonly, a charge-coupled device. Emission spectra of cancerous and benign tissues differ—a characteristic that may be augmented by applying an exogenous fluorophore that enhances resolution between differing spectra. Closely related to FS are Raman spectroscopy (RS) and infrared spectroscopy (IS). RS and IS are complementary techniques that interrogate the vibrational and rotational properties of molecules by examining the scattering of incident light.[55] Compared with antibody-guided fluorescence, there is a paucity of literature on FS and RS in determining margin status. In a study of 28 patients with oral cavity carcinoma, Francisco et al. found that in 2 patients with recurrent disease, the spectra at a given surgical margin obtained intraoperatively and interpreted as negative on histopathologic analysis were similar to the spectra of the tumor itself.[56] This observation suggests that RS may capture underlying molecular changes at tumor margins that are unable to be assessed by traditional techniques.

More recently, multiple investigators have combined spectroscopic techniques in the same device. Jermyn et al. devised a handheld intraoperative device combining IS, RS, and diffuse reflectance spectroscopy (DRS).[57] Using this device, the authors analyzed 15 brain tumors (primary tumors, metastatic melanoma, or metastatic colon cancer) and found intraoperative cancer detection accuracy, sensitivity, and specificity rates of 97%, 100%, and 93%, respectively. Furthermore, the combination of the three techniques was shown to improve accuracy and sensitivity over each technique alone. Notably, each spectral tracing was obtained in 8 s, suggesting the feasibility of interrogating numerous sites intraoperatively. Compared with antibody-guided fluorescent imaging where a camera must be transported into the field, smaller handheld devices may increase operative efficiency and represent a viable alternative to more cumbersome and labor intensive methods.

Other handheld devices have been developed, including a mass spectrometer that analyzes aerosolized tissue from monopolar cautery (i.e., “bovie smoke”). In a study of electrosurgical aerosol produced from ex vivo and in vivo breast samples, St. John et al. reported a 90.9% sensitivity and 98.8% specificity with mean acquisition times of 1.8 s to differentiate between normal and cancerous tissue.[58] While these technologies hold promise, human studies are limited by small sample sizes with an absence of late-phase clinical trials. Similar to the aforementioned techniques, spectroscopic techniques cannot provide an objective measure of margin distance and instead provide a binary assessment of margins as either positive or negative and thus fail to address the clinical challenge of accurately predicting patients at risk for local recurrence in the setting of clear margins. Such

technology is further limited by the need to cauterize the margin being assessed, leading to tissue shrinkage and interfering with immunohistochemistry based assessments that may be required to validate this technology.

MOLECULAR MARGIN ANALYSIS

Molecular margin analysis is a promising alternative with the potential to predict more accurately the presence of tumor at the surgical margin. In their seminal article, Brennan et al. investigated the utility of protein 53 (*p*53) mutation as a molecular marker for surgical margins.[59] Through analysis of *p*53 mutations from 25 histologically negative margins, the authors found that 13 patients harbored a *p*53 mutation within the histologically negative surgical margin. Furthermore, 5 (38.5%) of these patients with *p*53 mutations recurred locally within 7 months, whereas none of the 12 patients with both negative histo-logic and mutational margins recurred ($p = 0.02$). In addition to staging implications, these findings suggest that molecular margins have the potential to more accurately identify a subset of patients who may require adjuvant treatment and would not have otherwise been candidates for such therapy.

Studies expanding on this prior work have demonstrated mixed results. Pierssens et al. did not find *p*53 overexpression to be associated with local recurrence rates but instead identified chromosomal instability (measured by copy number variation via fluorescence in situ hybridization [FISH] at chromosomes 1 and 7) as a risk factor for local recurrence and worse progression-free survival.[60] In contrast, van Houten et al. found that *p*53 mutations, rather than overexpression, were associated with local recurrence in a prospective study of 76 patients with HNSCC deemed to have histologically negative margins.[61] Although a genetic mutation-based strategy to determine the true status of a surgical margin appears attractive, it is associated with several limitations.[62] A diverse spectrum of *p*53 mutations exists, thus rendering simple assays capable of capturing all possibilities of mutations difficult to design. Moreover, tumors without specific *p*53 genetic mutations may not be detected in assays. Additionally, the phenomenon of field cancerization further complicates its utility in clinical practice as several series have found a close association with *p*53 expression in dysplastic tissues.[63–65] Thus, the presence of *p*53 alterations at surgical boundaries may actually be a predictor of premalignancy rather than an overtly positive margin in some settings. Finally, sampling bias remains an issue as testing the entirety of the resection surface is necessary in order to reduce the overall false negative rate.

To overcome the specificity and complexity of a *p*53-based analysis, the utility of more general molecular biomarkers has been explored including eukaryotic translocation initiation factor 4E (eIF4E), which is over-expressed in the majority of HNSCC tumors and other premalignant oral cavity lesions.[66] In a study of 65 patients with HNSCC, Nathan et al. found elevated levels of eIF4E in all tumors, with positive eIF4E expression in 55.4% of patients with histologically negative surgical margins. Interestingly, eIF4E expression appears to have prognostic significance: more than half of the eIF4E-positive patients developed locoregional recurrence, whereas only 7% of patients with an eIF4E-negative margin recurred.

Similar to a mutation-based approach, recent developments have focused on the impact of molecular biomarkers including epigenetic alterations, microsatellite instability (MSI), and loss of heterozygosity on margin status in head and neck tumors.[67–73] Epigenetic alterations, namely the presence of gene methylation, was demonstrated by Hayashi et al. as a predictor of poor recurrence-free survival (hazard ratio [HR] 3.31; 95% confidence interval [CI] 1.30–8.46; $p = 0.012$), independent of standard histologic factors in deep surgical margin samples.[67] Roh et al. demonstrated that hypermethylation present on tissue imprints may be feasible for the molecular detection of residual SCC at the deep surgical margin and may correlate with locoregional recurrence.[73] Epigenetic changes have been found to be associated with carcinogenesis and genomic instability in other disease processes, including the development of sporadic colorectal cancer.[72,74,75] Microsatellite alterations including loss of heterozygosity and MSI also have been demonstrated to correlate with surgical margin positivity and locoregional recurrence rates.[69,70] A prospective analysis by Liu et al. investigated the association between microsatellite variations in tumor-free surgical margins and recurrence in 145 patients with oral cavity SCC and identified microsatellite alterations in 69.0% of patients, 85 specimens with loss of heterozygosity and 55 with MSI.[69] In patients with MSI at the surgical margin, an increased risk of local recurrence was appreciated on multivariable analysis (odds ratio [OR] 7.17; 95% CI 3.49–14.73). Thus, more universal approaches to assessing tumor biology may hold greater promise when considering the development of therapeutic interventions.[76]

As such, broader genetic assessment of individual tumors holds great promise with translational studies at the forefront of identifying high-risk patients. In an analysis of The Cancer Genome Atlas (TCGA), Mroz et al. demonstrated that mutant allele tumor heterogeneity (MATH) values calculated from whole-exome sequencing (WES) of bulk tumor DNA provides improved prognostication over traditional clinical and molecular characteristics.[76] The authors demonstrated correlation between higher MATH (higher heterogeneity) scores and decreased overall survival (OS). Furthermore, compared with low MATH patients, patients with a high degree of tumor heterogeneity were more than twice as likely to expire (HR 2.18; 95% CI 1.44–3.30; $p < 0.001$). Puram et al. performed single cell sequencing of matched head and neck primary tumors and lymph node metastases in human subjects.[77] The authors profiled transcriptomes from > 6000 single cells from 18 head and neck cancer patients and identified a unique subset of malignant cells within and between tumors, which express a partial epithelial-to-mesenchymal transition (p-EMT), which spatially localized to the leading edge of malignant tumors. The presence of the p-EMT program was uncovered as an independent predictor of nodal metastasis, tumor grade, and adverse pathologic features, including extranodal extension and LVI and effectively provided a deeper insight into the broader HNSCC ecosystem. While similar studies remain largely in their infancy, future research should include dedicated single-cell analyses of tumor margins to address more definitively the heterogeneity that may be present within tumor specimens. Despite the promise of molecular margin techniques, the application of these analyses may be barred by several constraints, including high financial costs and the amount of time required for complete and accurate margin assessment. As complicated extirpations and complex reconstruction would be completed before analysis of a given patient’s unique

molecular data, significant improvements are required before such technologies may be introduced into the intraoperative setting.

CONCLUSIONS

While the surgical margin is the most important predictor of outcomes in head and neck cancer, significant variation and challenges exist in obtaining a negative margin and evaluating the surgical margin. Improvements may be made within the framework of traditional techniques, including standardizing definitions and how margins are sampled. While, frozen-section analysis is reliable, it is subject to sampling error. Specimen-based sampling may reduce the rates of false negatives. Tumor within 5 mm of the cut edge if often defined as a “close” margin; however, a more stringent cutoff of 3 mm may portend truly worse outcomes.

Next-generation techniques offering rapid intraoperative assessment of margin status are promising. Optical imaging techniques, specifically those capable of generating fluorescent tumor specimen maps, hold great potential in guiding intraoperative resection. Other techniques, such as electrocautery aerosol analysis, still in their infancy, also may mature into viable intraoperative margin surveillance methods. Postoperatively, the field of molecular margins holds particular promise in allowing improved prognostication of tumor behavior but remains limited by the high cost and limited generalizability of such approaches. Given the complex nature of margin analysis, it is likely that a combination of techniques will enhance the ability to assess accurately the surgical margin and to improve surgical outcomes in head and neck cancer.

REFERENCES

1. Jesse RH, Sugarbaker EV. Squamous cell carcinoma of the oropharynx: why we fail. Am J Surg. 1976;132(4):435–8. [PubMed: 1015532]
2. Loree TR, Strong EW. Significance of positive margins in oral cavity squamous carcinoma. Am J Surg. 1990;160(4):410–4. [PubMed: 2221245]
3. Meier JD, Oliver DA, Varvares MA. Surgical margin determination in head and neck oncology: Current clinical practice. The results of an International American Head and Neck Society Member Survey. 2005;27(11):952–8.
4. McMahon J, O’Brien CJ, Pathak I, et al. Influence of condition of surgical margins on local recurrence and disease-specific survival in oral and oropharyngeal cancer. Br J Oral Maxillofac Surg. 2003;41(4):224–31. [PubMed: 12946663]
5. Eldeeb H, Macmillan C, Elwell C, Hammod A. The effect of the surgical margins on the outcome of patients with head and neck squamous cell carcinoma: single institution experience. Cancer Biol Med. 2012;9(1):29–33. [PubMed: 23691451]
6. Binahmed A, Nason RW, Abdoh AA. The clinical significance of the positive surgical margin in oral cancer. Oral Oncol. 2007;43(8):780–4. [PubMed: 17174145]
7. Slaughter DP. Surgical management of intraoral cancer. Am J Roentgenol Rad Ther Nucl Med. 1955;73(4):605–10; discussion, 635–8.
8. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. Cancer. 1953;6(5):963–8. [PubMed: 13094644]
9. Looser KG, Shah JP, Strong EW. The significance of “positive” margins in surgically resected epidermoid carcinomas. 1978;1(2):107–11.

10. Lane JE, Kent DE. Surgical margins in the treatment of nonmelanoma skin cancer and mohs micrographic surgery. Curr Surg. 2005;62(5):518–26. [PubMed: 16125611]
11. Minton TJ. Contemporary Mohs surgery applications. Curr Opin Otolaryngol Head Neck Surg. 2008;16(4):376–80. [PubMed: 18626258]
12. Cohen DK, Goldberg DJ. Mohs micrographic surgery: past, present, and future. Dermatologic Surg. 2019;45(3):329–39.
13. Weinstein MC, Brodell RT, Bordeaux J, Honda K. The art and science of surgical margins for the dermatopathologist. Am J Dermatopathol. 2012;34(7):737–45. [PubMed: 23000878]
14. Amit M, Na'ara S, Leider-Trejo L, et al. Improving the rate of negative margins after surgery for oral cavity squamous cell carcinoma: a prospective randomized controlled study. Head Neck. 2016;38 Suppl 1:E1803–9. [PubMed: 26685937]
15. Varvares MA, Walker RJ, Chiosea S. Does a specimen-based margin analysis of early tongue cancer better predict local control? Laryngoscope. 2016;126(11):2426–7. [PubMed: 27313090]
16. Hinni ML, Ferlito A, Brandwein-Gensler MS, et al. Surgical margins in head and neck cancer: a contemporary review. Head Neck. 2013;35(9):1362–70. [PubMed: 22941934]
17. Kerawala CJ, Ong TK. Relocating the site of frozen sections: is there room for improvement? Head Neck. 2001;23(3):230–2. [PubMed: 11428454]
18. Berdugo J, Thompson LDR, Purgina B, et al. Measuring depth of invasion in early squamous cell carcinoma of the oral tongue: positive deep margin, extratumoral perineural invasion, and other challenges. Head Neck Pathol. 4 26 2018.
19. Woolgar JA, Triantafyllou A. A histopathological appraisal of surgical margins in oral and oropharyngeal cancer resection specimens. Oral Oncol. 2005;41(10):1034–3. [PubMed: 16129652]
20. Choi N, Cho JK, Lee EK, Won SJ, Kim BY, Baek CH. Transoral bisected resection for T1–2 oral tongue squamous cell carcinoma to secure adequate deep margin. Oral Oncol. 2017;73:70–6. [PubMed: 28939079]
21. Tirelli G, Boscolo Nata F, Gatto A, et al. Intraoperative margin control in transoral approach for oral and oropharyngeal cancer.
22. Mayer A, Royer MC, Summerlin DJ, et al. Rapid mandible margins for intraoperative assessment. Am J Otolaryngol. 2015;36(3):324–9. [PubMed: 25630848]
23. Garcia-Donas JG, Dalton A, Chaplin I, Kranioti EF. A revised method for the preparation of dry bone samples used in histo-logical examination: five simple steps. Homo Int Zeitschrift vergleichende Forschung Menschen. 2017;68(4):283–8.
24. Bilodeau EA, Chiosea S. Oral squamous cell carcinoma with mandibular bone invasion: intraoperative evaluation of bone margins by routine frozen section. Head Neck Pathol. 2011;5(3):216–20. [PubMed: 21512783]
25. Forrest LA, Schuller DE, Karanfilov B, Lucas JG. Update on intraoperative analysis of mandibular margins. Am J Otolaryngol. 1997;18(6):396–9. [PubMed: 9395016]
26. Weisberger EC, Hilburn M, Johnson B, Nguyen C. Intraoperative microwave processing of bone margins during resection of head and neck cancer. Arch Otolaryngol Head Neck Surg. 2001;127(7):790–3. [PubMed: 11448351]
27. Nieberler M, Hausler P, Drecoll E, et al. Evaluation of intraoperative cytological assessment of bone resection margins in patients with oral squamous cell carcinoma. Cancer Cytopathol. 2014;122(9):646–56. [PubMed: 24753505]
28. Singh A, Mair M, Singhvi H, et al. Incidence, predictors and impact of positive bony margins in surgically treated T4 stage cancers of the oral cavity. Oral Oncol. 2019;90:8–12. [PubMed: 30846181]
29. Ord RA, Aisner S. Accuracy of frozen sections in assessing margins in oral cancer resection. J Oral Maxillofacial Surg. 1997;55(7):663–9.
30. Du E, Ow TJ, Lo YT, et al. Refining the utility and role of Frozen section in head and neck squamous cell carcinoma resection. Laryngoscope. 2016;126(8):1768–1775. [PubMed: 27113207]
31. DiNardo LJ, Lin J, Karageorge LS, Powers CN. Accuracy, utility, and cost of frozen section margins in head and neck cancer surgery. Laryngoscope. 2000;110(10 Pt 1):1773–1776. [PubMed: 11037842]

32. Nocon CC, Ajmani GS, Bhayani MK. Association of facility volume with positive margin rate in the surgical treatment of head and neck cancerassociation of facility volume with positive margin rate in head and neck cancer surgical treatment. Association of facility volume with positive margin rate in head and neck cancer surgical treatment. JAMA Otolaryngol Head Neck Surg. 2018;144(12):1090–7. [PubMed: 30347018]
33. Ettl T, El-Gindi A, Hautmann M, et al. Positive frozen section margins predict local recurrence in R0-resected squamous cell carcinoma of the head and neck. Oral Oncol. 2016;55:17–23. [PubMed: 27016013]
34. Patel RS, Goldstein DP, Guillemaud J, et al. Impact of positive frozen section microscopic tumor cut-through revised to negative on oral carcinoma control and survival rates. Head Neck 2010;32(11):1444–51. [PubMed: 20091833]
35. Bertino G, Degiorgi G, Tinelli C, Cacciola S, Occhini A, Benazzo M. CO2 laser cordectomy for T1–T2 glottic cancer: oncological and functional long-term results. Eur Arch Oto-Rhino-Laryngol. 2015;272(9):2389–95.
36. Hendriksma M, Montagne MW, Langeveld TPM, Veselic M, van Benthem PPG, Sjögren EV. Evaluation of surgical margin status in patients with early glottic cancer (Tis-T2) treated with transoral CO(2) laser microsurgery, on local control. Eur Arch Oto-rhino-laryngol. 2018;275(9):2333–40.
37. Wong LS, McMahon J, Devine J, et al. Influence of close resection margins on local recurrence and disease-specific survival in oral and oropharyngeal carcinoma. Br J Oral Maxillofacial Surg. 2012;50(2):102–8.
38. Dillon JK, Brown CB, McDonald TM, et al. How does the close surgical margin impact recurrence and survival when treating oral squamous cell carcinoma? J Oral Maxillofacial Surg. 2015;73(6):1182–8.
39. Barry CP, Ahmed F, Rogers SN, et al. Influence of surgical margins on local recurrence in T1/T2 oral squamous cell carcinoma. 2015;37(8):1176–80.
40. Ch'ng S, Corbett-Burns S, Stanton N, et al. Close margin alone does not warrant postoperative adjuvant radiotherapy in oral squamous cell carcinoma. 2013;119(13):2427–37. [PubMed: 23576156]
41. Zanoni DK, Migliacci JC, Xu B, et al. A proposal to redefine close surgical margins in squamous cell carcinoma of the oral tongue. JAMA Otolaryngol Head Neck Surg. 2017;143(6):555–60. [PubMed: 28278337]
42. Tasche KK, Buchakjian MR, Pagedar NA, Sperry SM. Definition of "close margin" in oral cancer surgery and association of margin distance with local recurrence rate. JAMA Otolaryngol Head Neck Surg. 2017;143(12):1166–72. [PubMed: 28445581]
43. Hadjipanayis CG, Widhalm G, Stummer W. What is the surgical benefit of utilizing 5-aminolevulinic acid for fluorescence-guided surgery of malignant gliomas? Neurosurgery. 2015;77(5):663–73. [PubMed: 26308630]
44. Motekallemi A, Jeltema H-R, Metzemaekers JDM, van Dam GM, Crane LMA, Groen RJM. The current status of 5-ALA fluorescence-guided resection of intracranial meningiomas-a critical review. Neurosurg Rev. 2015;38(4):619–28. [PubMed: 25736455]
45. Leunig A, Mehlmann M, Betz C, et al. Fluorescence staining of oral cancer using a topical application of 5-aminolevulinic acid: fluorescence microscopic studies. J Photochem Photobiol B. 2001;60(1):44–9. [PubMed: 11386680]
46. Miles BA, Patsias A, Quang T, Polydorides AD, Richards-Kortum R, Sikora AG. Operative margin control with high-resolution optical microendoscopy for head and neck squamous cell carcinoma. Laryngoscope. 2015;125(10):2308–16. [PubMed: 26059758]
47. de Boer E, Warram JM, Tucker MD, et al. In Vivo Fluorescence Immunohistochemistry: Localization of Fluorescently Labeled Cetuximab in Squamous Cell Carcinomas. Sci Rep. 2015;5:10169. [PubMed: 26120042]
48. Iqbal H, Pan Q. Image guided surgery in the management of head and neck cancer. Oral Oncol. 2016;57:32–9. [PubMed: 27208842]
49. Keereweer S, Kerrebijn JD, Mol IM, et al. Optical imaging of oral squamous cell carcinoma and cervical lymph node metastasis. Head Neck. 2012;34(7):1002–8. [PubMed: 21987435]

50. van Driel PB, van de Giessen M, Boonstra MC, et al. Characterization and evaluation of the artemis camera for fluorescence-guided cancer surgery. Mol Imaging Biol. 2015;17(3):413–23. [PubMed: 25344146]
51. Atallah I, Milet C, Coll JL, Reyt E, Righini CA, Hurbin A. Role of near-infrared fluorescence imaging in head and neck cancer surgery: from animal models to humans. Eur Arch Otorhinolaryngol. 2015;272(10):2593–600. [PubMed: 25115313]
52. Gao RW, Teraphongphom NT, van den Berg NS, et al. Determination of Tumor Margins with Surgical Specimen Mapping Using Near-Infrared Fluorescence. Cancer Res. 2018;78(17):5144–54. [PubMed: 29967260]
53. van Keulen S, van den Berg NS, Nishio N, et al. Rapid, noninvasive fluorescence margin assessment: Optical specimen mapping in oral squamous cell carcinoma. Oral Oncol. 2019;88:58–65. [PubMed: 30616798]
54. Ramanujam N Fluorescence spectroscopy of neoplastic and nonneoplastic tissues. Neoplasia. 2000;2(1–2):89–117. [PubMed: 10933071]
55. Auner GW, Koya SK, Huang C, et al. Applications of Raman spectroscopy in cancer diagnosis. Cancer Metastasis Rev. 12 19 2018.
56. Francisco AL, Correr WR, Pinto CA, et al. Analysis of surgical margins in oral cancer using in situ fluorescence spectroscopy. Oral Oncol. 2014;50(6):593–9. [PubMed: 24630901]
57. Jermyn M, Mercier J, Aubertin K, et al. Highly accurate detection of cancer with intraoperative, label-free, multimodal optical spectroscopy. Cancer Res. 2017;77(14):3942. [PubMed: 28659435]
58. St John ER, Balog J, McKenzie JS, et al. Rapid evaporative ionisation mass spectrometry of electrosurgical vapours for the identification of breast pathology: towards an intelligent knife for breast cancer surgery. Breast Cancer Res. 2017;19(1):59. [PubMed: 28535818]
59. Brennan JA, Mao L, Hruban RH, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. N Engl J Med. 1995;332(7):429–35. [PubMed: 7619114]
60. Pierssens D, Borgemeester MC, van der Heijden SJH, et al. Chromosome instability in tumor resection margins of primary OSCC is a predictor of local recurrence. Oral Oncol. 2017;66:14–21. [PubMed: 28249643]
61. van Houten VM, Leemans CR, Kummer JA, et al. Molecular diagnosis of surgical margins and local recurrence in head and neck cancer patients: a prospective study. Clin Cancer Res. 2004;10(11):3614–20. [PubMed: 15173067]
62. Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med. 2007;357(25):2552–61. [PubMed: 18094376]
63. Yang XH, Ding L, Fu Y, et al. p53-positive expression in dys-plastic surgical margins is a predictor of tumor recurrence in patients with early oral squamous cell carcinoma. Cancer Manage Res. 2019;11:1465–72.
64. Cruz IB, Snijders PJ, Meijer CJ, et al. p53 expression above the basal cell layer in oral mucosa is an early event of malignant transformation and has predictive value for developing oral squamous cell carcinoma. J Pathol. 1998;184(4):360–8. [PubMed: 9664901]
65. Singh J, Jayaraj R, Baxi S, et al. Immunohistochemical expression levels of p53 and eIF4E markers in histologically negative surgical margins, and their association with the clinical outcome of patients with head and neck squamous cell carcinoma. Molec Clin Oncol. 2016;4(2):166–72. [PubMed: 26893854]
66. Nathan CO, Franklin S, Abreo FW, Nassar R, De Benedetti A, Glass J. Analysis of surgical margins with the molecular marker eIF4E: a prognostic factor in patients with head and neck cancer. J Clin Oncol. 1999;17(9):2909–14. [PubMed: 10561370]
67. Hayashi M, Wu G, Roh JL, et al. Correlation of gene methylation in surgical margin imprints with locoregional recurrence in head and neck squamous cell carcinoma. Cancer. 2015;121(12):1957–65. [PubMed: 25773145]
68. Mao L, Clark D. Molecular margin of surgical resections–where do we go from here? Cancer. 2015;121(12):1914–6. [PubMed: 25773010]
69. Liu SA, Wang CC, Jiang RS, Wang WY, Lin JC. Genetic analysis of surgical margins in oral cavity cancer. Br J Surg. 2018;105(2):e142–9. [PubMed: 29341160]

70. Lin JC, Wang CC, Jiang RS, Wang WY, Liu SA. Impact of microsatellite alteration in surgical margins on local recurrence in oral cavity cancer patients. Eur Arch Otorhinolaryngol. 2017;274(1):431–9. [PubMed: 27430224]

71. Szukala K, Brieger J, Bruch K, et al. Loss of heterozygosity on chromosome arm 13q in larynx cancer patients: analysis of tumor, margin and clinically unchanged mucosa. Med Sci Monit. 2004;10(6):Cr233–40. [PubMed: 15173666]

72. Matsuzaki K, Deng G, Tanaka H, Kakar S, Miura S, Kim YS. The relationship between global methylation level, loss of heterozygosity, and microsatellite instability in sporadic colorectal cancer. 2005;11(24):8564–9.

73. Roh JL, Westra WH, Califano JA, Sidransky D, Koch WM. Tissue imprint for molecular mapping of deep surgical margins in patients with head and neck squamous cell carcinoma. Head Neck. 2012;34(11):1529–36. [PubMed: 22223471]

74. Laytragoon-Lewin N, Rutqvist LE, Lewin F. DNA methylation in tumour and normal mucosal tissue of head and neck squamous cell carcinoma (HNSCC) patients: new diagnostic approaches and treatment. Med Oncol. 2013;30(3):654. [PubMed: 23824644]

75. Thomas GR, Nadiminti H, Regalado J. Molecular predictors of clinical outcome in patients with head and neck squamous cell carcinoma. Int J Exp Pathol. 2005;86(6):347–63. [PubMed: 16309541]

76. Mroz EA, Tward AD, Hammon RJ, Ren Y, Rocco JW. Intra-tumor genetic heterogeneity and mortality in head and neck cancer: analysis of data from the Cancer Genome Atlas. PLoS Med. 2015;12(2):e1001786. [PubMed: 25668320]

77. Puram SV, Tirosh I, Parikh AS, et al. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. Cell. 2017;171(7):1611–24.e1624. [PubMed: 29198524]

TABLE 1

Overview of margin status and summary of key points

Topic	Summary of key points	Conclusion
What defines a “clear” (negative) margin?	> 5 mm, > 1 high-power field, > 1 cm, and no ink on tumor are all accepted definitions of a “clear” margin	A plurality of surgeons accept > 5 mm from the tumor edge as “clear”
	What defines a clear margin may vary by subsite and disease stage	
What defines a “close” margin?	Varying definitions of a “close” margin exist; < 5 mm is the most common	There is no clear definition on what defines a close margin and thus, the oncologic significance of a close margin is not well-defined. Using a more stringent cutoff of 3 mm may portend worse outcomes in patients with close margins
What clinical significance might a close margin hold?	Comparing close margins (< 5 mm) vs. clear margins has not demonstrated any difference in local recurrence rates or OS	Close margins in the setting of other histopathologically adverse features may portend worse outcomes
	A close margin in itself may not have an effect on survival or local recurrence; however, in the setting of adverse features (e.g., LVI, PNI), close margins have been associated with higher rates of local recurrence and worse DFS	
	Studies comparing clear or negative margins vs. incrementally close margins in 1-mm increments have identified 2—3 mm as a cutoff where rates of local recurrence increase	
What is defect-driven vs. specimen-driven sampling?	Margins may be sampled from either the defect bed (e.g., defect-driven) or the tumor itself (e.g., specimen-driven)	Specimen-driven margin sampling may improve rates of clear margins. Regardless, a face-to-face handoff between surgeon and pathologist may improve the
	Studies have demonstrated improved clear margin rates when margins are sampled via a specimen-driven approach	accuracy of margin sampling
Where are positive margins most likely to occur?	Deep margins, rather than mucosal margins, are most likely to be positive	Special attention should be paid to the deep margin when performing resection
	Deep margins are more difficult to examine and assess via palpation and visualization	
	Resection of the deep margin may be limited anatomically (e.g., oropharynx)	
Are frozen margins reliable?	At skilled academic centers, there is a 96% concordance between frozen margins and final pathology results	Sampling error occurs more frequently than interpretive error. Overall, there is good concordance between frozen and final pathology
	Up to 22% of frozen margins may be read as negative, but reveal close or positive margins on final pathology	
	Errors may arise in either sampling (e.g., a positive margin is not sampled) or on interpretation (e.g., a positive margin is misinterpreted under the microscope)	
What should be done with a frozen margin that returns as	The standard of care is to re-resect to a negative margin, as feasible	Re-resection, with the goal of a negative margin should be the goal. While an initial positive frozen
positive intraoperatively?	A positive margin on initial frozen section, even when revised to a negative margin, has been associated with increased rates of local recurrence and decreased OS	margin itself is not an indication for adjuvant therapy, surgeons should be aware that it may belie underlying aggressive disease

LVI lymphovascular invasion, *PNI* perineural invasion, *DFS* disease-free survival, *OS* overall survival

TABLE 2

Comparison of next-generation margin techniques

Technique	Underlying principle	Advantages	Disadvantages	Other
5-aminolevulinic acid (5-ALA)	5-ALA is a natural metabolite of the heme synthesis pathway that preferentially accumulates in tumor cells	Easy to use	Low sensitivity and specificity in some oral cavity studies	May be utilized as a sensitizer in photodynamic therapy
		May use differential fluorescence to guide resection intraoperatively	Limited soft tissue penetration May be limited by auto-fluorescence	
High-resolution microendoscopy (HRME)	Fluorescence microscope which captures images with contact of tissue	Rapid, easily adaptable to intraoperative use	Requires histopathologic training to review images	
			Poor soft tissue penetration	
			Limited by auto-fluorescence	
Near-infrared (NIR) fluorescence	Infrared light induces fluorescence from tissue which is captured by a camera	Less noise from autofluorescence	EGFR is the most common target of antibody-guided imaging and may not be expressed in all tumoral tissue	
		May be used in conjunction with antibody-targeted dyes	Depth of penetration is 5–10 mm	
		Closed field imaging of a resection specimen may create a ‘‘margin map’’		
Spectroscopy (Raman and Fluorescence)	Molecules are excited by incident light and the resulting emission spectra differs between normal tissue and tumor	Limited studies demonstrate high sensitivity and specificity	Existing studies in humans and the head and neck are limited and thus it is unknown how well RS and FS may handle dysplasia	Multiple spectroscopic techniques may be combined in one device
		Intraoperative assessment of a given margin may be resulted in under 10 s		
Molecular margins	Margins may harbor underlying cancerous changes despite benign histopathologic appearance	May be used to identify genetic markers of aggressive disease that are otherwise missed	Results may take weeks to obtain	Potential to be used to guide adjuvant treatment

RS Raman spectroscopy, *FS* Fluorescence spectroscopy, *EGFR* epidermal growth factor receptor