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CURRENT OPINION

Image-guided surgery in oral cancer: toward improved margin control

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Purpose of review
The aim of this review is to discuss recent studies on the assessment of tumor extension and resection margins by different intraoperative techniques allowing for image-guided surgery of oral cancer.

Recent findings
There are different in-vivo and ex-vivo intraoperative techniques to improve margin control of which intraoperative ultrasound and targeted fluorescence-guided resections have high potential clinical value and are closest to clinical implementation.

Summary
In oral cancer surgery, resection margins, particularly deep margins, are often inadequate. Intraoperative frozen section does not improve resection margin control sufficiently. Specimen-driven intraoperative assessment for gross analysis of suspected margins reduces the amount of positive resection margins substantially but leaves still room for improvement. Mucosal staining methods, optical coherence tomography and narrow band imaging can only be used for superficial (mucosal) resection margin control. Spectroscopy is under investigation, but clinical data are scarce. Intraoperative ex-vivo imaging of the resection specimen by magnetic resonance and PET/computed tomography may be used to assess resection margins but needs more research. Intraoperative in-vivo ad ex-vivo ultrasound and targeted fluorescence imaging have high potential clinical value to guide oral cancer resections and are closest to clinical implementation for improved margin control.

Keywords
image-guided surgery, oral cancer, resection margin

INTRODUCTION

In oral cancer, surgery is the primary choice of treatment. Complete resection of the tumor and the removal of positive lymph nodes is pivotal for local and regional control and thereby survival. Resection margins with tumors cells less than 1 mm of the surgical margins are defined as 'positive', less than 5 mm as 'close' and with at least 5-mm healthy tissue as 'negative' in oral cancer [1]. On the contrary, up to 85% of the resection margins in oral cancer are inadequate (close or positive), with up to 40% positive resection margins. Most of these inadequate margins are found at the deep (submucosal) margins. The majority of patients with positive margins require adjuvant treatment, that is reresection during a second surgical procedure or (chemo)irradiation, to prevent local recurrence [2]. Moreover, these adjuvant treatments are associated with increased morbidity, use of scarce resources and costs. This high rate of inadequate margins and its consequences urges the need for surgical guidance in the operating theatre.

Today, the only commonly available method for surgical guidance in oral cancer surgery is frozen section of tissue biopsies. Although this technique has been used for decades since it is the only intraoperative technique available, it has some major drawbacks. First of all, since only a selected amount of the resection specimen is investigated, this lead to sampling error. As a result, sensitivity of frozen

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KEY POINTS

- Intraoperative *in-vivo* and *ex-vivo* imaging techniques have the potential to improve resection margin control.
- Optical coherence tomography and narrow band imaging can be used to visualize *in vivo* the mucosal extension of the tumor.
- Spectroscopy may be used for real-time intraoperative assessment of the resection surface.
- Intraoperative in-vivo ultrasound-guided resections followed by ex-vivo ultrasound of the resection specimen improves margin control.
- Intraoperative in-vivo and ex-vivo fluorescent imaging using targeted tracers allows for a sharp tumor delineation potentially improving resection margins.

section is low (approximately 50%) which could lead up to 12.9% conversion from 'negative' margins by frozen section to 'positive' final histopathology margins [3,4]. Second, even if positive margins are found by frozen section, revising of initial positive margins to final negative histopathology margins using immediate intraoperative reresection was only achieved in 57.9% in a recent study [4]. Third, a recent review revealed that margin revision of initially positive margins to 'negative' based on frozen section guidance does not equate to an initially negative margin and does not significantly improve local control [5]. Together, this leads to a high cost–benefit ratio with minor improvement for the patient [6,7].

To improve resection margin control in oral cancer, multiple imaging techniques have been studied last decade. Here, we provide an overview of different techniques with clinical trials in the last 2 years that have the potential to improve resection margin control in oral cancer and discus what progress should be made in the following years to implement these techniques in the clinic.

IMAGE-GUIDED SURGERY

The most ideal technique for surgical guidance in oral cancer is a technique that provides the surgeon with direct information of the complete mucosal and deep resection margins with microscopic accuracy during the resection (*in vivo*). On the contrary this method is not (yet) available. Image-guided techniques are used *in vivo* (during surgery in the oral cavity) or *ex vivo* (on the resection specimen), perform guidance for the mucosal or deep margin and provide information using gross analysis of the whole surface or selected (microscopic) analysis. In-vivo guidance has the advantage to guide the surgeon in a proactive way instead of reactive way, in case of positive or close resection margins at the resection specimen. Ex-vivo control also introduces the problem of relocating inadequate margins in the wound bed. A problem which can be dealt with using parallel tagging; in which the surgeon places numbered tags in a pair-wise manner on both sides of the resection line, both superficially and deep in the wound bed [8]. Most positive resection margins are found in the deep (submucosal) surgical margins (84%) and only 16% in the mucosal surgical margin [9]. Although direct feedback of the status of the whole surface is ideal, microscopic involvement of margins cannot always ruled out. This limited accuracy could require subsequent analysis on a microscopic level.

Gross examination of resection specimen

Specimen-driven intraoperative assessment is an ex-vivo method for gross analysis of suspected margins. After the resection is completed, the surgeon consults a pathologist and after visual and palpate inspection of the resection specimen, the pathologist makes one or more parallel incisions of suspicious areas perpendicular to the tissue surface. This enables the visualization and measurement of gross resection margin of healthy tissue on the cross-sectional side with a ruler. Using this method, Smits *et al*. reduced the amount of positive resection margins from 43% in the period 2010–2012 to 16% in the period 2013–2017. Although this method screens larger areas than frozen section, it still depends on tactile information from the surgeon, which could lead to sampling errors comparable with frozen section. Furthermore, this method is unable to detect microscopic or perineural growth of the tumor front [10▪,11▪].

Visualization of superficial extension

For superficial tumor delineation there are several mucosal staining methods, for example Lugol's iodine solution and Toluidine blue, but these techniques are not often used in clinical practice due to their limited sensitivity and specificity. Other techniques to visualize the mucosal extension of the tumor include optical coherence tomography (OCT) and narrow band imaging (NBI).

OCT is a technique that uses the interference of light to generate two-dimensional cross-sectional images. It can be considered to be the optical analog of ultrasound; it detects back-reflected light, instead of sound. Its resolution is 10–50 times better than ultrasound, but its penetration depth is limited to less than 1 mm. A systematic review (search date

May 2018) found that OCT was able to distinguish between benign and (pre)malignant oral lesions with high accuracy [12]. The status of the basal membrane was the most important parameter for differentiating squamous cell carcinoma from oral dysplasia or benign disorders. The interobserver and intraobserver agreements of OCT measurements are very good [13]. However, no intraoperative studies were performed for real-time evaluation of the surgical resection margins.

NBI is an endoscopic technique that uses blue and green light to enhance mucosal and submucosal blood vessels, leading to better detection and delineation of (pre)malignant lesions by visualization of aberrant blood vessel patterns [14]. A systematic review (search date December 2018) on the use of NBI in transoral microscopic laser surgery of laryngeal carcinoma reported a significant reduction of positive superficial margins in 577 patients treated with and 526 patients without intraoperative use of NBI (52 and 29%, respectively $P < 0.05$) [15]. Another systematic review (search date September 2020) reported that also in oral and oropharyngeal cancer surgery the use of NBI was associated with significantly less positive superficial margins than in a historical control group. However, the resection extent was sometimes mistakenly increased due to nonoptimal specificity and further studies are needed to determine the subsequent functional impact [16▪].

Spectroscopy imaging

Spectroscopy imaging uses the reflection of tissue to discriminate between tissues. Since tissue composition affect the absorption and scattering properties, each tissue has its own ‘optical fingerprint’. Hyperspectral imaging (HIS) uses a broadband white light source to generate tissue specific spectra to discriminate between healthy and tumor tissue. A recent ex-vivo trial in tongue cancer showed that HIS of diffuse reflection (400–1700 nm) using both a near infrared wavelength camera (950–1700 nm for superficial spectra) and a visible wavelength range (VIS) camera (400–1000 nm for deeper spectra) was able to distinguish relatively large tumor areas from tongue muscle with a combined accuracy of 82% in a perpendicular slice of the resection specimen [17]. Although HIS has the potential to screen the tumor bed in-vivo real-time, no trials for detection of small tumor deposits intraoperatively have been published yet.

Raman spectroscopy uses spectral differences between normal and malignant tissue, is an objective tool that can provide real-time information about the molecular composition of tissue and has the potential to provide an objective intraoperative assessment of the entire resection surface. Raman spectrometry can discriminate oral squamous cell carcinoma from healthy surrounding tissue based on the higher water concentration in tumor. The water concentration changes across the tumor border can be used for objective intraoperative assessment of the resection margins [18,19]. Despite many promising results, there are still hurdles to be overcome for clinical adoption and application in routine oncologic diagnostic and surgical procedures [20]. On the contrary, no clinical trials on the use of Raman spectroscopy in oral cancer surgery are reported yet.

Conventional imaging

Several conventional imaging modalities have been used to improve margins in oral cancer; that is ultrasound, MRI and PET-computed tomography (CT). Ultrasound is the most extensive studied technique, particularly in tongue cancer, which has the advantage that it could be used both *in vivo* (sites in reach of ultrasound probe) and *ex vivo* on the resection specimen. Baek *et al.* [21] published in 2008 the first case-controlled study which compared margin control between conventional resection and ultrasound-guided resection. They inserted a 25-ga spinal needle 1.5 cm off the deep margin of the tumor (the deep resection margin) under sonography which guided the surgeon during the resection. Although this resulted in improved margin control, the needles tended to move during the resection [21]. Bulbul *et al.* [22▪] improved the ultrasound-guided resection technique using a hockey stick shaped probe without the need of needles and recently published their cohort of 44 tongue cancer patients (23 ultrasound-guided and 21 conventional), with improvement of margin control: no cases of microscopic cutthrough and a significant reduction of need for adjuvant therapy were observed in the ultrasound-guided group [22▪]. A study using ultrasound for ex-vivo control of the resection specimen of tongue cancer suspended in ultrasound gel in 31 patients showed high accuracy of the deep resection margin measurement in 31 patients: the mean (SD) deep resection margins measured on the ultrasound differed by 1.1 (0.9) mm from those reported by the pathologist [23▪]. In a feasibility study de Koning *et al.* [24▪] also found significant improvement of margin control using in-vivo ultrasound-guided resections followed by ex-vivo ultrasound of the resection specimen in ten tongue cancer patients compared with a large cohort of conventional treated tongue cancer patients: 70 vs. 17% free (negative or close) margins. See Fig. 1 for an example of a case.

Two ex-vivo MRI studies of the same team investigated the accuracy of both a preclinical 7T scanner (10 patients) and clinical 3T whole body (10

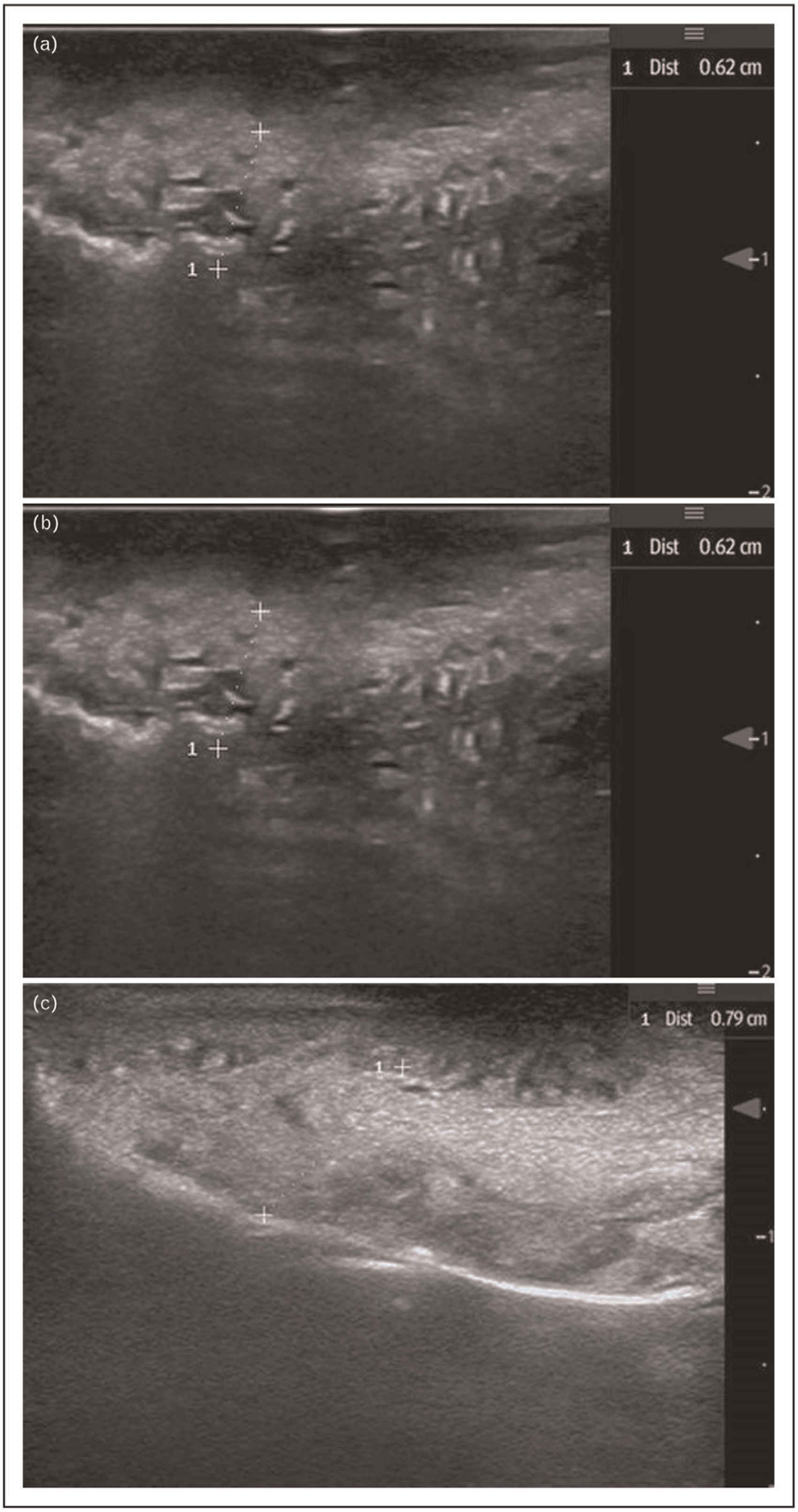

FIGURE 1. (a) Intraoral ultrasound for in-vivo determination of tumor-thickness. (b) The deep resection plane has reached the middle of the tumor. The resection plane, which here reached the middle of the tumor, is visible as a white border during in-vivo examination. (c) Ex-vivo determination of resection margin.

patients) for assessment of resection margins of the fresh tongue specimens. Using a small-bore 7T magnetic resonance (MR) machine in six of seven specimens with a pathologically determined invasion depth of the tumor of at least 3 mm, the tumor could be recognized with a resection margin within a 2 mm range as compared with histopathology. In three specimens with an invasion depth of less than 1 mm, the tumor was not visible on MR [25]. Using a 3T clinical whole-body MRI in ten tongue specimens the minimal tumor-free margins were assessed by two radiologist. Although the radiological evaluation was within a clinically realistic time frame of under 30 min, the positive predictive values for identification of margins less than 5 mm was low (5–38%), which makes MRI not yet feasible to accurately assess the surgical margin status [26].

In a proof-of-concept study, eleven resection specimens of eight head and neck malignancies were scanned with a micro-PET-CT imaging device after administration of 4MBq/kg 18F-FDG approximately one hour prior to surgery. This allowed the three-dimensional delineation of 18F-FDG using submillimetric PET/CT imaging, but differentiation between inflamed and dysplastic tissue versus malignant tissue was complicated due to increased peritumoral inflammation and thereby accurate prediction of the resection margin. The use of more tumor-specific PET-tracers is needed to resolve this problem [27].

Fluorescence imaging

For several decades continuous attempts have been made to light up the tumor with fluorescent agents for the purpose of finding or delineation of cancers, or identifying inadequate margins after removal [28]. In the past years, the field has shifted from using untargeted fluorescent tracers such as 5-ALA or indocyanine green (ICG) to the use of tracers directed to tumor-specific characteristics to overcome nonspecific tracer uptake [29]. In the first clinical trial with a targeted tracer in head and neck cancer, cetuximab, which is directed against the epidermal growth factor receptor (EGFR), was coupled to the fluorescent dye 800CW. The 800CW dye is excited and detected in the infrared spectrum which holds a favorable range wavelength for depth of interrogation of the tissue and less autofluorescence signals than excitation within the VIS. Cetuximab-800CW was selectively taken up in head and neck carcinomas which showed the potential of targeted fluorescence imaging [30]. Several other tracers directed against EGFR were developed. Panitunumab [31] and an EGFR-affibody [32] are currently explored in clinical trials both using the 800CW dye as the fluorescent probe. Affibodies are smaller fragments of regular antibodies with the advantage of rapid uptake and less side effects than regular antibodies. No data on the diagnostic accuracy are yet available. Recently a first in human trial was completed using the tumor microenvironment as the target. A pH sensitive nanobody ONM-100 consisting of micelles that break up below a pH-threshold and releases ICG in the tissue was first explored in head and neck tumors [33,34,35▪]. In 13 patients with histologically proven HNSCC fluorescence images of the excised surgical specimen and of the surgical cavity after intravenous administration of ONM-100 24 ± 8 h before surgery were analyzed. All six tumor-positive surgical margins were detected immediately after excision using fluorescence-guided intra-operative imaging. Postoperative analysis showed a median tumor-to-background ratio of the fluorescent lesions on the resection margin of 3.36. The authors concluded that the binary mechanism of ONM-100 allows for a sharp tumor delineation in all patients, giving the surgeon a clinical tool for real-time margin assessment, with a high sensitivity [35▪].

Several targeted tracers show potential for intraoperative fluorescent margin assessment although larger trials showing positive predictive values and better local control are currently lacking. Which tracer ultimately will be selected for general use by clinicians remains to be decided. Selection most likely will be based on clinical performance, side effects and general availability. Intraoperative in-vivo fluorescent imaging of head and neck cancers is complex since most tumors cannot be evenly illuminated due to the localization in the upper aero digestive tract. Therefore, tissue margin analysis is *ex vivo* performed on the excised specimen in camera systems that can be used in the operating room. See Fig. 2 for an example of a case. The strategy for targeted fluorescence imaging therefore is expected to be combined with intraoperative frozen section analysis of fluorescent spots identified on the excised specimen.

CONCLUSION

Specimen-driven intraoperative assessment for gross analysis of suspected margins needs a close collaboration between surgeon and pathologist and reduces the amount of positive resection margins but leaves still room for improvement of margin control. Mucosal staining methods, OCT and NBI have an intrinsic inability to image structures other than the (sub-)mucosa and can only be used for superficial margin detection. For detection of deep margins and clinical real-time margin assessment several techniques are currently under investigation. Spectroscopy is under investigation for

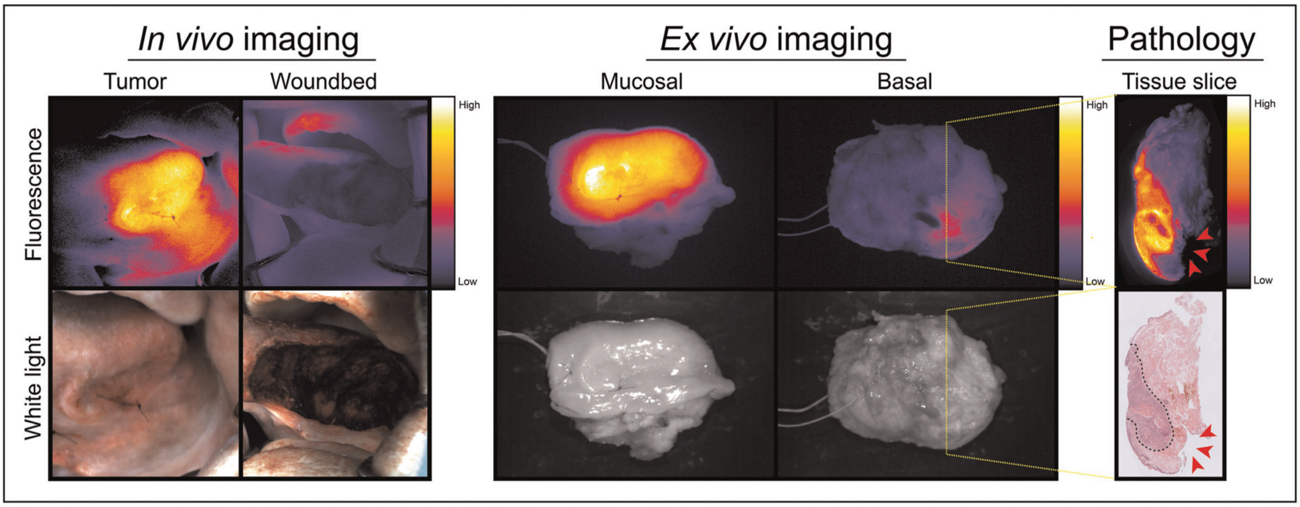

FIGURE 2. Example of a case showing the added value of fluorescence guided surgery after the intravenous injection of cetuximab-800CW two days prior to surgery. Images on the left show intraoperative in vivo imaging of fluorescence of a squamous cell carcinoma of the tongue and the wound bed negative of fluorescence after removal of the tumor. The images in the middle show intraoperative ex-vivo specimen analysis of the tumor/mucosal side and the basal margin in which a fluorescent spot reveals a close margin. On the right the confirmation of the status of the margin with histopathology, including H&E staining with demarcation of the extension of the carcinoma and clearly a close margin.

objective real-team intraoperative assessment of the resection surface. Imaging techniques with high potential clinical value to guide head and neck tumor resections closest to clinical implementation are in-vivo and ex-vivo intraoperative ultrasound and targeted fluorescence-guided resections. It is likely that some of these techniques can be used in combination or complementary. For coming to a strategy, clinical trials are required showing the value of these (combinations of) techniques.

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Conflicts of interest

There are no conflicts of interest.

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