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ABSTRACT

Objective: New techniques have emerged to aid in preventing inadequate margins in oral squamous cell carcinoma (OSCC) surgery, but studies comparing different techniques are lacking. Here, we compared narrow band imaging (NBI) with fluorescence molecular imaging (FMI), to study which intraoperative technique best assesses the mucosal tumour margins.

Materials and Methods: NBI was performed in vivo and borders were marked with three sutures. For FMI, patients received 75 mg of unlabelled cetuximab followed by 15 mg cetuximab-800CW intravenously-two days prior to surgery. The FMI borders were defined on the excised specimen. The NBI borders were correlated with the FMI outline and histopathology.

Results: Sixteen patients were included, resulting in 31 NBI and 30 FMI measurements. The mucosal border was delineated within 1 mm of the tumour border in 4/31 (13 %) of NBI and in 16/30 (53 %) FMI cases ($p = 0.0008$), and within 5 mm in 23/31 (74 %) of NBI and in 29/30 (97 %) of FMI cases ($p = 0.0048$). The median distance between the tumour border and the imaging border was significantly greater for NBI (3.2 mm, range −6.1 to 12.8 mm) than for FMI (0.9 mm, range −3.0 to 7.4 mm; $p = 0.028$). Submucosal extension and previous irradiation reduced NBI accuracy.

Conclusion: Ex vivo FMI performed more accurately than in vivo NBI in mucosal margin assessment, mainly because NBI cannot detect submucosal extension. NBI adequately identified the mucosal margin especially in early-stage and not previously irradiated tumours, and may therefore be preferable in these tumours for practical and cost-related reasons.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy in the head and neck region, with a yearly global incidence of over 300 000 [1]. The preferred treatment consists of surgery, possibly followed by postoperative (chemo)radiotherapy, depending on histopathological features of the resected specimen [2]. The goal of surgical treatment is complete tumour removal, with preservation of healthy tissue in this delicate anatomical and functional area. Unfortunately, inadequate margins (<5 mm) occur in up to 85 % of primary OSCC resections, especially in locally advanced tumours [3–5], which are associated with increased risk of local recurrence, more aggressive adjuvant therapy and worsened disease-specific survival rates [6–8]. Currently, surgeons rely mainly on visual and tactile information to assess the surgical resection margin, often supported by fresh frozen sections, of which the utility is controversial [9]. Thus, efforts are made to improve margin assessment, for example through optical techniques such as fluorescence molecular imaging (FMI) and narrow band imaging

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(NBI) [10–12].

NBI is a non-invasive technique that uses blue and green light to visualize (sub)mucosal vascular patterns [13–15]. Filtering techniques like NBI are available on commercially available cameras, which allow easy switching between conventional white light imaging and NBI [16,17]. NBI uses wavelengths of 415 nm (blue) and 540 nm (green), which are maximally absorbed by haemoglobin and thus aid in the visualization of blood vessels [18], thereby allowing the detection of aberrant blood vessel patterns associated with tumour angiogenesis. NBI reaches a tissue penetration of 240 μm and is suitable for in vivo mucosal margin assessment of tumours arising from thin mucosa, such as the floor of mouth, buccal mucosa or lateral tongue, which comprise over 50 % of OSCCs [19,20]. NBI cannot visualize blood vessels ex vivo and cannot be used for deep margin assessment due to the absence of intraepithelial papillary capillary loops in the wound bed, typical for mucosal malignancies.

FMI uses exogenous fluorescent tracers specifically targeting cancer cells to visualize tumour up to several millimetres depth [10,12]. Previous clinical trials have shown the potential of FMI targeting epidermal growth factor receptor, which is overexpressed in 90 % of all OSCCs, for margin assessment in OSCC surgery [10–12]. Assessment of the EGFR expression prior to FMI is not required. This technique can be used *in*- and ex vivo, since tracers specifically bind to tumour tissue, and remain bound even after excision of the tumour [10,21,22]. Ex vivo imaging allows for controlled imaging parameters, and both mucosal and deep margins can be analysed within minutes, which makes it suitable for intraoperative margin correction [23,24].

In this study, we aimed to compare how FMI and NBI perform in mucosal OSCC margin assessment. Secondly, we assessed in which tumour or patient types the techniques can be applied best.

Material and methods

Clinical trial design

This feasibility study was performed at the Department of Oral & Maxillofacial Surgery and the Department of Otorhinolaryngology/ Head and Neck Surgery of the University Medical Centre Groningen, the Netherlands. The data were obtained from two independent clinical trials investigating cetuximab-800CW for margin assessment in OSCC surgery and NBI for the detection of (pre)malignant mucosal HNSCC lesions. Both studies were approved by the Institutional Review Board of the UMCG (METc 2016/395 and 2015/152) and conducted according to the Dutch Act on Medical Research involving Human Subjects (WMO) and the principles of the Declaration of Helsinki (adapted version Fortaleza, Brazil, 2013). The trials were registered at www.clinicaltrials.gov (NCT03134846) and www.trialregister.nl (NL6052). Informed consent was obtained prior to any study-related procedure. Patients with biopsy-confirmed OSCC in an anatomic location suitable for NBI (i.e., floor of mouth, ventral tongue, or buccal mucosa) scheduled for tumour resection were included in this study. Exclusion criteria are provided in the supplementals.

Production and administration of cetuximbab-800CW

Clinical grade cetuximab-800CW (peak excitation and emission wavelength of 778 and 795 nm, respectively) was produced in the Good Manufacturing Practice (GMP) facility of the UMCG and released by a certified Qualified Person (QP). A detailed description of the production process has been described previously [25]. Briefly, commercially available cetuximab (Erbitux®) 5 mg/mL was conjugated to the near-infrared fluorescence dye IRDye800CW (LI-COR Biosciences, Lincoln, NE, USA) and purified using PD-10 buffer exchange columns (GE Healthcare, Chicago, IL, USA). Cetuximab-800CW was formulated in a sodium-phosphate buffer at a 1 mg/mL concentration.

The study drugs were administered two days before surgery, as deemed optimal in our phase-I trial [10]. First, patients received 2 mg of clemastine intravenously-one hour prior to any study drug administration for safety reasons, according to standard of care cetuximab administration. Then, 75 mg cetuximab was administered followed by 15 mg cetuximab-800CW one hour later. Preloading patients with unlabelled cetuximab was found to lead to higher tumour-to-background ratios in our previous trial [10]. Patients were monitored for at least one hour following the last tracer administration, and a 12-lead ECG was obtained to evaluate QTc time. If no complications occurred, patients were discharged.

Intraoperative imaging

Tumour borders were consecutively assessed by NBI and FMI (Fig. 1). NBI-endoscopic procedures were performed under general anaesthesia, using an Olympus HD camera head with a 0°, 5.4 mm telescope and Evis Exera CLV-180 light source (Olympus BV, Zoeterwoude, the Netherlands) [26]. Three sutures were placed to delineate the tumour based on in vivo NBI signal (i.e., Takano's classification types III and IV)[2]. These were placed on selected areas of the tumour where sutures could be placed relatively easily. Although the resection margins could not be analysed in their entirety, this method allows for clinical point-to-point comparison with subsequent ex vivo FMI. All NBI examinations were performed by the same head and neck surgical oncologist specialized in NBI [BP].

After NBI assessment, a wide-field fluorescence imaging system (Explorer Air, SurgVision, Groningen, the Netherlands) was used for in vivo imaging. Tumours were excised with a standard of care clinical margin of 1 cm. Subsequently, back-table FMI was performed on the freshly excised specimen using a closed-field imaging system with an 800 nm channel for cetuximab-800CW detection (Pearl-Trilogy®, LI-COR BioSciences Inc., Lincoln, NE, USA). The mucosal border of the fluorescence signal was determined by the average of in triplicate manual delineation. The location of the NBI based suture was scored as within, on or outside the FMI border. After completion of all imaging procedures, the specimen was submitted to the Department of Pathology for formalin fixation.

Specimen imaging and correlation with histopathology

Incisions were made on the ex vivo formalin-fixed specimen on the location of the NBI based sutures to be able to relocate them during microscopic examination (Fig. 1B). Subsequently, the specimen was sliced in 3 mm thick tissue slices which were embedded in paraffin. Only incisions placed perpendicular to the direction of specimen slicing could be included for further analysis since only these demarcations can be visualized with tumour simultaneously (Fig. 1B). The formalin-fixed, paraffin embedded tissue blocks were scanned in a fluorescence flatbed scanner (Odyssey-CLx®, LI-COR BioSciences Inc., Lincoln, NE, USA). Tissue sections of 4 μm thick were cut and haematoxylin & eosin (H&E) stained for histopathology.

FMI signal was delineated on the tissue block images using different thresholds of 25 %, 50 %, and 75 % of the maximum value within each tissue block. This was done on the first two patients, to determine the optimal correlation between the tumour and FMI border. The optimal value was used for all patients to allow for standardized analysis of results. The NBI incisions and ex vivo FMI borders in the tissue blocks were cross-correlated with histopathology. To evaluate the clinical impact, the distance between the NBI border and tumour border was measured, irrespective of tumour depth. To ensure head-to-head comparison, the

[2] Takano JH, Yakushiji T, Kamiyama I, Nomura T, Katakura A, Takano N, et al. Detecting early oral cancer: narrowband imaging system observation of the oral mucosa microvasculature. Int J Oral Maxillofac Surg. 2010;39:208–13. https://doi.org/10.1016/j.ijom.2010.01.007.

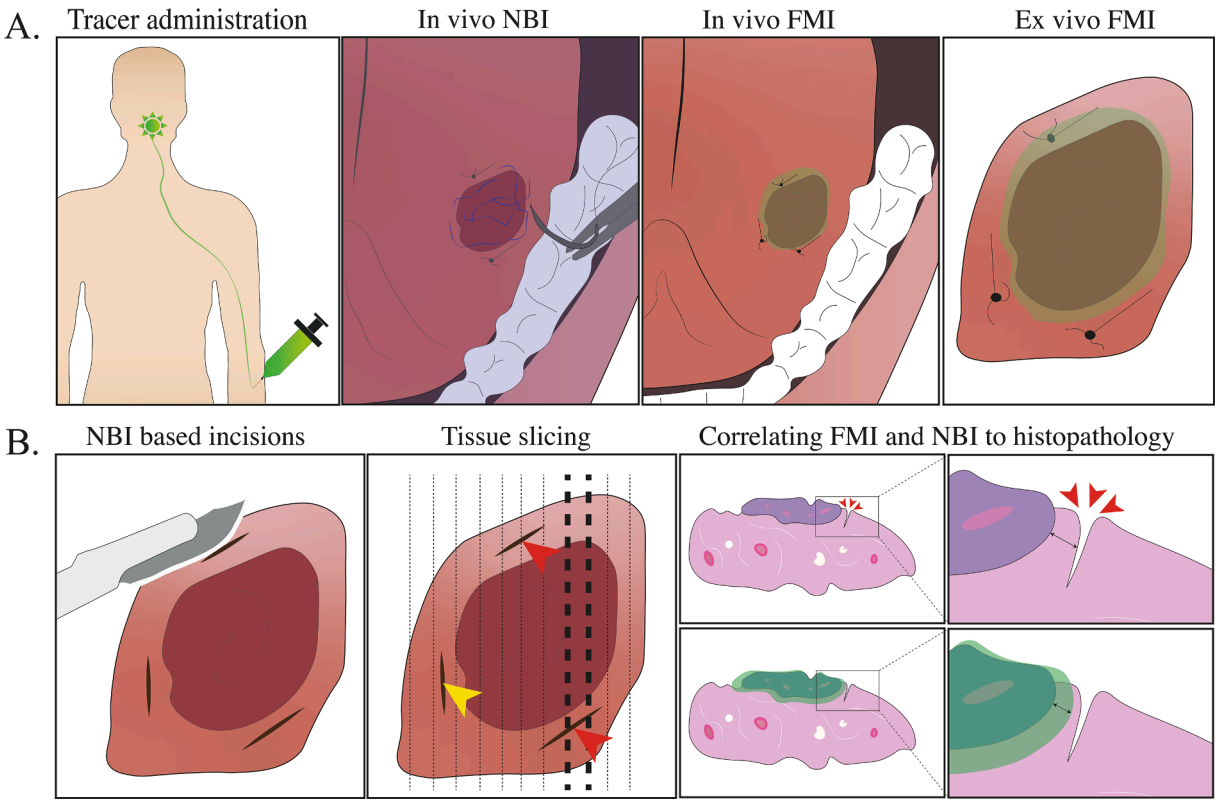

Fig. 1. Overview of study workflow. A) Overview of intraoperative imaging workflow. Two days prior to surgery, patients were intravenously administered with 75 mg of cetuximab and 15 mg of cetuximab-800CW. Then, in vivo NBI and FMI was performed, in these pictures a left sided floor of mouth cancer was depicted. During NBI, the tumour border was identified and marked with surgical sutures. These were clearly visible on the excised specimen. B) On the location of the sutures, incisions were made on the excised specimen. The specimen was cut into tissue slices. Here, the incisions placed parallel to the slicing (yellow arrowhead) could not be identified on the H&E, but the incisions perpendicular to slicing (red arrowheads) could. After paraffin embedding, these tissue slices were cut in 4 μm tissue sections (bold dotted line is the corresponding tissue slice) and NBI incisions (red arrowheads) and FMI results were correlated with histopathology. Abbreviations: NBI, Narrow Band Imaging; FMI, Fluorescence Molecular Imaging. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

FMI distance to the tumour border was measured at the same site of the NBI based incision.

The mucosal resection margins were measured on all tumour slices according to standard of care. The clinical border was defined as the surgical resection margin minus 5 mm as measured on the H&E tissue section. Final histopathological margin status was classified as tumour-positive (<1 mm), close (1–5 mm) or tumour-negative (>5 mm), according to the Royal College of Pathologists [27].

Statistical analysis

FMI analysis was performed using ImageJ Fiji (version 2.3.0/1.53f). Data were tested using the Mann-Whitney test for non-parametric data, and presented as median values with ranges, in millimetres. For statistical analysis and graph design, GraphPad Prism (version 9.2.0, GraphPad Software Inc, San Diego, California, USA) was used.

Results

Study population

Between May 2019 and April 2021, 16 patients were included. Six patients were female (38 %), and the median age was 68 (29 to 82) (Table 1). The cohort consisted of T1 (n = 8), T2 (n = 4), T3 (n = 1), and T4 (n = 3) tumours. These were located on the lateral tongue (n = 12), floor of mouth (n = 3) and buccal mucosa (n = 1). Seventeen out of 48 NBI measurements were lost due to standard of care surgery and pathology workup. In these cases, the suture was either placed outside the clinical excision margin of 1 cm (n = 4) or the suture was placed parallel to the direction of the tissue slicing of the specimen during pathology processing, and could therefore not be included in the tissue section (n = 13) (see Fig. 1B). Of the 31 remaining measurements, one FMI measurement was lost due to photobleaching of the tissue slice, resulting in 30 FMI measurements. We found three grade 1 adverse events (one related to the study drug) and three serious adverse events, all unrelated

Table 1
Patient demographics and tumour characteristics of all patients.

Characteristic	n
Median age, y (range)	68 (29–82)
Female, n (%)	6 (38)
History of radiotherapy in head and neck area, n (%)	4 (25)
Tumour location, n (%)	
Lateral tongue	12 (75)
Floor of mouth	3 (19)
Buccal mucosa	1 (6)
T-classification, n (%)	
T1	8 (50)
T2	4 (25)
T3	1 (6)
T4	3 (19)
Median invasion depth, mm (range)	5.8 (0.9–20)
Diameter, mm (range)	22.5 (4–50)

to any study procedure (Supplementary Table 1). In histopathological examination, in 1/16 patients a positive mucosal surgical margin was found (<1 mm), in 8/16 patients a close margin (1–5 mm), and in 7/16 a clear surgical margin (>5 mm).

Distance between tumour border and the NBI and FMI defined mucosal borders

In 26 out of 31 (84 %) cases the NBI border was delineated outside the tumour. In these 26 cases, the median distance between the NBI border and tumour border was 3.1 (0.3–12.8) mm. In five cases the suture was placed within the tumour, at a median distance of −3.5 (−0.6 to −6.1) mm. A representative example of our imaging results is shown in Fig. 2.

In the first two patients (five measurements), a threshold of 50 % of the maximum value led to the best results for the FMI border, with a median distance of 6.0 (0.7–13.3) mm between FMI and tumour border (Supplementary Figure 1).

To estimate the clinical accuracy of both techniques, the number of measurements set within 1 mm and 5 mm (i.e., corresponding to a positive and close surgical resection margin) of the tumour border were scored. Compared to FMI borders, NBI borders were set less frequently both within 1 mm of the tumour (4/31 and 16/30, respectively; $p = 0.0008$), and 5 mm of the tumour border (23/31 and 29/30, respectively; $p = 0.0048$) (Fig. 3). The FMI border was defined outside the tumour border in 23 out of 30 (77 %) cases, and within the tumour in seven cases. This was not different from the performance of NBI ($p = 0.72$). In these 23 cases, the median distance between FMI border and tumour border was 1.1 (0.0–7.4) mm. In the seven cases the median distance was −0.4 (−0.1 to −3) mm. The median distance from the tumour border to all 30 FMI borders was significantly shorter than the median distance to all 31 NBI borders (0.9 vs 3.0 mm, $p = 0.028$). The clinical border to the tumour was set within the tumour in 3/30 cases. The average distance of the clinical border to the tumour was 6.4 mm, significantly larger than both NBI and FMI ($p = 0.046$ and $p < 0.0001$, respectively).

Ex vivo whole specimen correlation of NBI and FMI

In order to also compare NBI to FMI on the whole specimen rather than only in histopathology, all NBI sutures were evaluated in relation to the FMI borders on the whole specimen. Out of the 30 FMI measurements that could be correlated to histopathology, 19 NBI sutures were placed outside the FMI border on the whole specimen, and of these 16 were also set outside the FMI border on the tissue section. In two cases, the NBI suture was set within the FMI border, and these were also set within the FMI border on the tissue section. For the nine sutures placed on the FMI border on the whole specimen, the median distance to the tumour was 0.2 (−3.1 to 6.6) mm on the tissue slice.

Influence of radiotherapy on imaging results

In our cohort, 4/16 patients had been treated with radiotherapy (RT) of the oral cavity prior to participation in the study. All NBI and FMI borders were set outside the tumour border in the four post-RT patients. The median distance between tumour and NBI border was significantly larger in the post-RT group (4.4 (1.9–10.9) mm) compared to the non-RT group (2.7 (−6.1 to 11.6) mm) ($p = 0.04$). In the post-RT group, 44 % of NBI borders were placed at > 5 mm from the tumour border compared to 9 % in the non-RT group. For FMI, the median distance from the FMI to the tumour border was also significantly smaller in the post-RT group with 0 % of borders placed at > 5 mm of the tumour border (2.0 (0.6–4.2) mm) compared to the non-RT group (11% >5 mm, 0.6 (−3.0 to 7.4) mm) ($p = 0.003$) (Fig. 3).

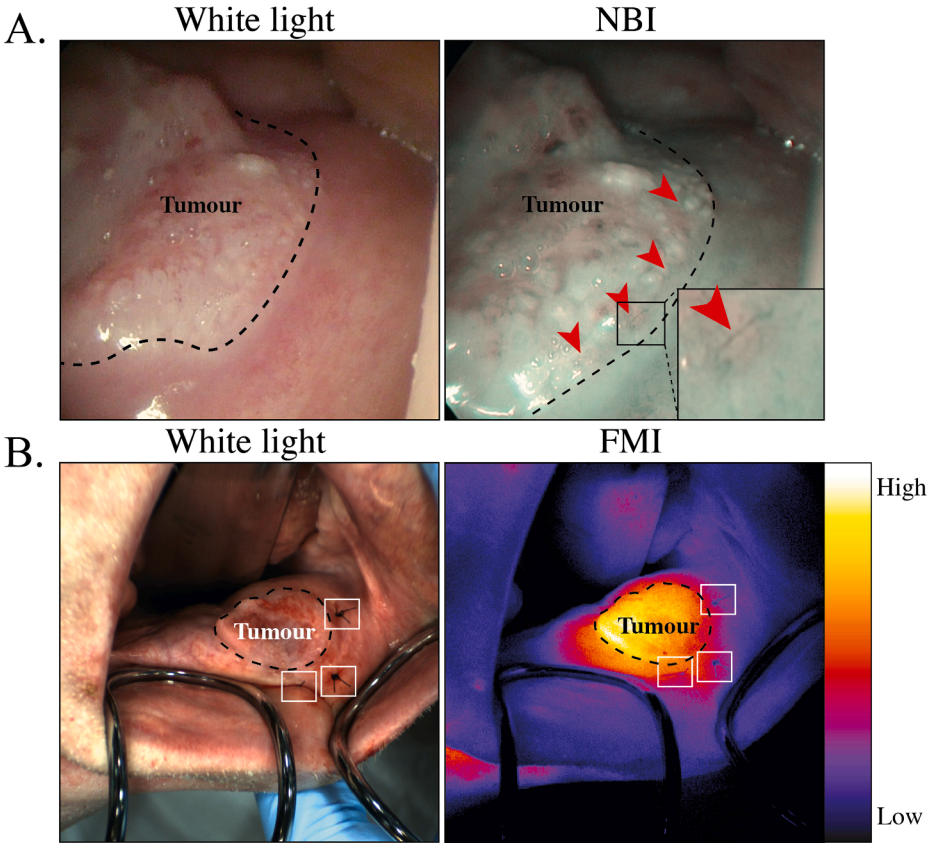

Fig. 2. In vivo narrow band imaging and fluorescence molecular imaging. A) White light and narrow band images of a patient with a tumour in the buccal mucosa. Imaging was performed at 3 cm, which is the optimal distance for NBI. The dotted line indicates the tumour border defined by white light imaging (left) and NBI (right). The red arrows indicate aberrant blood vessel patterns suspicious for tumour. B) White light and fluorescence images of the same patient. Imaging was performed at 20 cm from the tissue of interest, optimal for FMI. The white rectangles indicate the sutures placed based on NBI. Increased fluorescence signal is observed in the tumour. Abbreviations: NBI, Narrow Band Imaging; FMI, Fluorescence Molecular Imaging. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

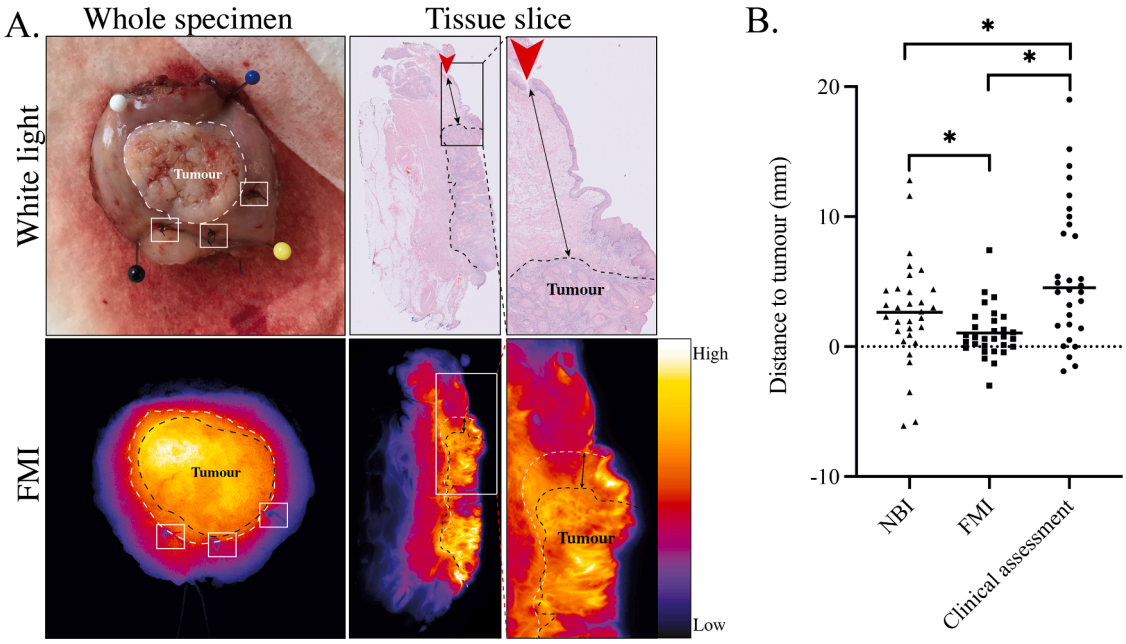

Fig. 3. Correlating imaging results to histopathology. A) Excised specimen of a tumour in the buccal mucosa (see also Fig. 2). The top row shows the white light images with the NBI based sutures (white rectangles). On the tissue section the incision is indicated with a red arrow, the distance from the incision to the tumour border was measured. The bottom pictures show an FMI image of the whole specimen, and the correlation of FMI results to histopathology. The black dotted line indicates the tumour as indicated by white light (whole specimen) and H&E (tissue section). The white dotted line indicates the fluorescence border. B) Scatterplot of the distance of all NBI and FMI measurements to the tumour border, compared to the tumour border determined by clinical assessment using white light imaging (resection margin minus 5 mm). Abbreviations: NBI, Narrow Band Imaging; FMI, Fluorescence Molecular Imaging.

Imaging defined borders in relation to adjacent dysplasia

The mucosa between the imaging defined borders and the tumour border was evaluated in all tissue sections. The tissue was classified as healthy mucosa, inflamed, grade of dysplasia (high and low), or unidentifiable. In patients with high grade dysplasia, the border was defined outside both tumour and dysplasia in 4/5 cases for NBI and 5/5 cases for FMI. The histopathological grade of dysplasia for did not affect imaging performances.

Influence of tumour characteristics on imaging results

The distances between the tumour border and the NBI and FMI borders were compared between mucosally and submucosally extending tumours. For NBI, the distances between the tumour border and the NBI border was non-significantly larger in the mucosal group (3.1 (−3.5 to 12.8) mm) compared to the submucosal group (2.0 (−6.1 to 4.5) mm) ($p = 0.09$). In the two cases where the suture was placed in malignant mucosa, the distances between the NBI and tumour border were −3.5 and −1.2 mm (i.e., <5 mm). The other three sutures were placed within submucosal extension, in two cases at > 5 mm from the tumour border (−0.6, −5.8, and −6.1 mm). In FMI, we observed no difference in distances between tumours extending into the mucosa (0.7 (−0.9 to 4.2) mm) and into the submucosa (0.3 (−3.0 to 7.4) mm) ($p = 0.37$). The influence of *T*-classification on distance to the tumour border was compared in early-stage tumours (T1-T2) and locally advanced tumours (T3-T4). For NBI, we observed no difference in distance between early-stage tumours (2.5 (−3.5 to 12.8) mm) compared to locally advanced tumours (3.4 (−6.1 to 11.6) mm) ($p = 0.82$). In FMI, we observed a non-significant tendency for early-stage tumours (1.2 (−0.9 to 7.4) mm) to have a larger distance compared to locally advanced tumours (0.6 (−3.0 to 3.8) mm) ($p = 0.059$). A comparison of imaging characteristics is provided in Table 2.

Table 2
Comparison of narrow band imaging and fluorescence molecular imaging.

	Narrow band imaging	Fluorescence molecular imaging
Penetration depth	240 μm	Up to several mm
Accuracy	+	++
Margin assessment	Mucosal	Mucosal and deep
Wide field imaging	Yes	Yes
Ex vivo imaging	No	Yes
Acquisition time	Real-time	Real-time
Availability	On standard endoscopes	Requires fluorescence cameras
Invasive	No	Yes
Risks	None	Possible adverse reaction to tracer administration
Preparation	None	Administration of contrast agent
Tumour location	Selected locations with thin mucosa	All OSCCs
Tumour size	Most suitable for early-stage tumours	Suitable for early-stage and locally advanced tumours
Extra costs	None	Cameras and fluorescent tracers

Discussion

This study shows a head-to-head comparison of NBI and FMI for intraoperative margin assessment in OSCC patients. We demonstrate that FMI is more accurate compared to NBI in assessing mucosal tumour borders, since FMI borders are determined significantly more often within 1 and 5 mm of the actual tumour border compared to NBI. Maintaining a wider surgical resection (i.e., 5 mm after resection, which is standard of care) margin around the imaging-based delineations would have overcome tumour-positive mucosal resection margins in nearly all cases. This image-guided tumour delineation would have resulted in only two (6 %) tumour-positive resection margins for NBI and none for FMI, which demonstrates that these techniques have

potential to reduce current tumour-positive margin rates of 12–43 % reported in standard of care surgery [3–5].

A few studies report on NBI for surgical margin assessment in OSCC [13–15]. Tirelli et al. showed in OSCC and oropharyngeal SCC that NBI led to a more widely set margin (11 ± 3 mm) compared to white light imaging alone, and resulted in only one tumour-positive margin (6.3 %) for NBI, while cancer was found in 62.5 % of patients in the area between the white light and NBI margin [13]. Evaluating our NBI results, the median distance to the tumour was 3.2 (−6.1 to 12.8) mm. The slightly wider NBI margins could be a result of field cancerization and related changes that may have occurred on molecular level, but may not appear in histopathology yet. On molecular level, a study of Farah et al. showed that mRNA and miRNA expression profiles have fewer abnormalities and had a greater biological distinction to tumour in surgical margins determined by NBI compared to more conservative margins determined by white light in OSCC [28]. Moreover, clustering of differentially expressed genes by principal component analysis revealed that none of the NBI margin samples were clustered with tumour samples, while 22 % of the white light margin samples were [14]. The two missed cases, i.e. where the NBI border was set within the tumour at a distance greater than 5 mm from the tumour border, were both submucosally extending tumours. The inability of NBI to detect these tumours can be explained by the limited penetration depth of NBI (240 μm). Standard of care preoperative imaging or palpation can, however, detect submucosal extension and may be used for patient selection. Previous studies suggest that NBI is a safe technique resulting in considerably lower tumour-positive margins than conventional white light examination [13–15]. We find that this is the case, especially in early-stage primary tumours with no submucosal extension. In tumours without submucosal extension, surgical resection based on NBI margin assessment would have resulted in no tumour-positive margins. NBI (or other filtering systems) are already available, non-invasive and is easy to use by pushing a button on an endoscope and is already available and approved for clinical use.

We have shown excellent sensitivity and accuracy for FMI, with most measurements set within 1 mm of the tumour, and all within 5 mm, resulting in no missed tumour-positive margins. This was also observed in previous FMI studies for margin assessment during OSCC surgery. Fakurnejad et al. reported on intraoperative, back-table, EGFR-targeted FMI for mucosal margin assessment [29]. In their approach, the areas showing the highest fluorescence signals on the mucosal margin, which they defined as the sentinel margin, correlated with significantly shorter tumour margin distances than areas showing low fluorescence signal. Furthermore, they demonstrated that FMI showed improved margin assessment compared to the surgeon [30]. Also, in a study of our group [10], all cases with a tumour-positive resection margin (<1 mm) were identified using back-table FMI, which contained both mucosal and deep margins.

In previously irradiated patients, none of the imaging defined borders were set within the tumour. However, all NBI borders of > 5 mm occurred in this post-RT group, and distances between tumour and NBI borders were significantly larger. External beam radiation potentially alters the normal arrangement of the blood vessels, and hampers the identification of intraepithelial papillary capillary loops, which are NBI characteristics for malignancies [31]. For FMI, the influence of (chemo) radiotherapy has been studied during fluorescence endoscopy. It has been shown that residual tumour could still be adequately identified using fluorescence in patients with incomplete response, when compared to patients with a complete response [32].

In our relatively small study population, only 5/31 measurements contained high grade dysplasia. Comparing both techniques for detecting high dysplasia would be especially interesting, since we expect it to be included in the NBI defined border (high grade dysplasia is hardly distinguishable from malignant tissue), but also in FMI, where higher fluorescent signal is seen with increasing grade of dysplasia [33]. In our cohort, high grade dysplasia was identified in all cases of FMI and in 4/5 of NBI, which makes a comparison on a larger scale promising.

For the clinical application of NBI and FMI in OSCC, we found that NBI performs well in high grade dysplasia and smaller, early-stage tumours, since in these cases no tumour-positive margins were missed. NBI fails to detect submucosal extension, and deep margin assessment is not possible. We therefore think that NBI can be especially useful in early-stage tumours for mucosal surgical margin assessment. Moreover, since NBI endoscopes are available in more institutes than FMI cameras, NBI can be used more readily for mucosal margin assessment than FMI. NBI is cheaper and does not require the administration of exogenous tracers. Both NBI and FMI show improved performance compared to clinical assessment, of which the border was defined as the surgical resection margin minus 5 mm as measured on the H&E tissue section, since the average distances to the tumour border were smaller using either technique.

FMI is more accurate in delineating the tumour border. It performs well in both locally advanced and early-stage tumours, and it can be used for both mucosal and deep margin assessment. Currently, it does come with more logistic challenges than NBI. Therefore, it is most suitable for clinically challenging, locally advanced tumours where tumour-positive margins are anticipated.

Conclusion

In summary, FMI seems more accurate in defining the mucosal tumour borders than NBI. However, NBI is easy to apply and still adequately identified the mucosal tumour borders in early-stage primary tumours. Ultimately, both techniques show adequate margin detection compared to standard of care, which reduces the risk of local recurrence, and may improve patient prognosis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2022.106099.

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65(2):87–108. https://doi.org/10.3322/caac.21262.
[2] Shanti RM, O'Malley BW. Surgical management of oral cancer. Dent Clin North Am 2018;62(1):77–86. https://doi.org/10.1016/j.cden.2017.08.005.
[3] Woolgar JA, Triantafyllou A. A histopathological appraisal of surgical margins in oral and oropharyngeal cancer resection specimens. Oral Oncol 2005;41(10):1034–43. https://doi.org/10.1016/j.oraloncology.2005.06.008.
[4] Smits RWH, Koljenović S, Hardillo JA, ten Hove I, Meeuwis CA, Sewnaik A, et al. Resection margins in oral cancer surgery: Room for improvement. Head Neck 2016;38(S1):E2197–203. https://doi.org/10.1002/hed.24075.
[5] Wong LS, McMahon J, Devine J, McLellan D, Thompson E, Farrow A, et al. Influence of close resection margins on local recurrence and disease-specific survival in oral and oropharyngeal carcinoma. Br J Oral Maxillofac Surg 2012;50(2):102–8. https://doi.org/10.1016/j.bjoms.2011.05.008.
[6] Buchakjian MR, Tasche KK, Robinson RA, Pagedar NA, Sperry SM. Association of main specimen and tumor bed margin status with local recurrence and survival in

oral cancer surgery. JAMAOtolaryngol Head Neck Surg 2016;142:1191–8. https://doi.org/10.1001/jamaoto.2016.2329.
[7] Buchakjian MR, Ginader T, Tasche KK, Pagedar NA, Smith BJ, Sperry SM. Independent predictors of prognosis based on oral cavity squamous cell carcinoma surgical margins. Otolaryngol Head Neck Surg 2018;159(4):675–82. https://doi.org/10.1177/0194599818773070.
[8] Binahmed A, Nason RW, Abdoh AA. The clinical significance of the positive surgical margin in oral cancer. Oral Oncol 2007;43(8):780–4. https://doi.org/10.1016/j.oraloncology.2006.10.001.
[9] Shapiro M, Salama A. Margin analysis: squamous cell carcinoma of the oral cavity. Oral Maxillofac Surg Clin North Am 2017;29(3):259–67. https://doi.org/10.1016/j.coms.2017.03.003.
[10] Voskuil FJ, de Jongh SJ, Hooghiemstra WTR, Linssen MD, Steinkamp PJ, de Visscher SAHJ, et al. Fluorescence-guided imaging for resection margin evaluation in head and neck cancer patients using cetuximab-800CW: A quantitative dose-escalation study. Theranostics 2020;10(9):3994–4005. https://doi.org/10.7150/thno.43227.
[11] Rosenthal EL, Warram JM, de Boer E, Chung TK, Korb ML, Brandwein-Gensler M, et al. Safety and tumor specificity of Cetuximab-IRDye800 for surgical navigation in head and neck cancer. Clin Cancer Res 2015; 21: 3658–3666. https://doi.org/10.1158/1078-0432.CCR-14-3284.
[12] Gao RW, Teraphongphom NT, van den Berg NS, Martin BA, Oberhelman NJ, Divi V, et al. Determination of Tumor Margins with Surgical Specimen Mapping Using Near-Infrared Fluorescence. Cancer Res 2018; 78: 5144–5154. https://doi.org/10.1158/0008-5472.CAN-18-0878.
[13] Tirelli G, Piovesana M, Gatto A, Tofanelli M, Biasotto M, Boscolo Nata F. Narrow band imaging in the intra-operative definition of resection margins in oral cavity and oropharyngeal cancer. Oral Oncol 2015;51(10):908–13. https://doi.org/10.1016/j.oraloncology.2015.07.005.
[14] Farah CS, Dalley AJ, Nguyen P, Batstone M, Kordbacheh F, Perry-Keene J, et al. Improved surgical margin definition by narrow band imaging for resection of oral squamous cell carcinoma: A prospective gene expression profiling study. Head Neck 2016;38(6):832–9. https://doi.org/10.1002/hed.23989.
[15] Farah CS. Narrow Band Imaging-guided resection of oral cavity cancer decreases local recurrence and increases survival. Oral Dis 2018;24(1-2):89–97. https://doi.org/10.1111/odi.12745.
[16] Staníková L, Walderová R, Jančatová D, Formánek M, Zeleník K, Komínek P. Comparison of narrow band imaging and the Storz Professional Image Enhancement System for detection of laryngeal and hypopharyngeal pathologies. Eur Arch Otorhinolaryngol 2018;275(7):1819–25. https://doi.org/10.1007/s00405-018-4987-3.
[17] Hawkshaw MJ, Sataloff JB, Sataloff RT. New concepts in vocal fold imaging: A review. J Voice 2013;27(6):738–43.
[18] Nonaka S, Saito Y. Endoscopic diagnosis of pharyngeal carcinoma by NBI. Endoscopy 2008;40(04):347–51. https://doi.org/10.1055/s-2007-995433.
[19] Bagan J, Sarrion G, Jimenez Y. Oral cancer: Clinical features. Oral Oncol 2010;46(6):414–7. https://doi.org/10.1016/j.oraloncology.2010.03.009.
[20] Piazza C, Del Bon F, Paderno A, Grazioli P, Perotti P, Barbieri D, et al. The diagnostic value of narrow band imaging in different oral and oropharyngeal subsites. Eur Arch Otorhinolaryngol 2016;273(10):3347–53. https://doi.org/10.1007/s00405-016-3925-5.
[21] van Keulen S, van den Berg NS, Nishio N, Birkeland A, Zhou Q, Lu G, et al. Rapid, non-invasive fluorescence margin assessment: Optical specimen mapping in oral squamous cell carcinoma. Oral Oncol 2019;88:58–65. https://doi.org/10.1016/j.oraloncology.2018.11.012.
[22] van Keulen S, Nishio N, Birkeland A, Fakurnejad S, Martin B, Forouzanfar T, et al. The sentinel margin: Intraoperative ex vivo specimen mapping using relative fluorescence intensity. Clin Cancer Res 2019; 25: 4656–4662. https://doi.org/10.1158/1078-0432.CCR-19-0319.
[23] Koller M, Qiu S-Q, Linssen MD, Jansen L, Kelder W, de Vries J, et al. Implementation and benchmarking of a novel analytical framework to clinically evaluate tumor-specific fluorescent tracers. Nat Commun 2018;9(1). https://doi.org/10.1038/s41467-018-05727-y.
[24] Voskuil FJ, Vonk J, van der Vegt B, Kruijff S, Ntziachristos V, van der Zaag PJ, et al. Intraoperative imaging in pathology-assisted surgery. Nat Biomed Eng 2022;6(5):503–14. https://doi.org/10.1038/s41551-021-00808-8.
[25] Linssen MD, ter Weele EJ, Allersma DP, Lub-de Hooge MN, van Dam GM, Jorritsma-Smit A, et al. Roadmap for the Development and Clinical Translation of Optical Tracers Cetuximab-800CW and Trastuzumab-800CW. J Nucl Med 2019;60(3):418–23. https://doi.org/10.2967/jnumed.118.216556.
[26] Zwakenberg MA, Dikkers FG, Wedman J, Halmos GB, van der Laan BFAM, Plaat BEC. Narrow band imaging improves observer reliability in evaluation of upper aerodigestive tract lesions. Laryngoscope 2016;126(10):2276–81. https://doi.org/10.1002/lary.26008.
[27] Helliwell T, Woolgar JA. Standards and datasets for reporting cancers. Dataset for histopathology reporting of nodal excisions and neck dissection specimens associated with head and neck carcinomas. London, UK: The Royal College of Pathologists; 2013.
[28] Farah CS, Fox SA, Dalley AJ. Integrated miRNA-mRNA spatial signature for oral squamous cell carcinoma: a prospective profiling study of Narrow Band Imaging guided resection. Sci Rep 2018;8:823–913. https://doi.org/10.1038/s41598-018-19341-x.
[29] Fakurnejad S, Krishnan G, van Keulen S, Nishio N, Birkeland AC, Baik FM, et al. Intraoperative Molecular Imaging for ex vivo Assessment of Peripheral Margins in Oral Squamous Cell Carcinoma. Front Oncol 2020;9. https://doi.org/10.3389/fonc.2019.01476.
[30] Krishnan G, van den Berg NS, Nishio N, Kapoor S, Pei J, Freeman L, et al. Fluorescent Molecular Imaging Can Improve Intraoperative Sentinel Margin Detection in Oral Squamous Cell Carcinoma. J Nucl Med 2022;63(8):1162–8.
[31] Zabrodsky M, Lukes P, Lukesova E, Boucek J, Plzak J. The Role of Narrow Band Imaging in the Detection of Recurrent Laryngeal and Hypopharyngeal Cancer after Curative Radiotherapy. Biomed Res Int 2014;2014:175398–9. https://doi.org/10.1155/2014/175398.
[32] Tjalma JJJ, Koller M, Linssen MD, Hartmans E, de Jongh S, Jorritsma-Smit A, et al. Quantitative fluorescence endoscopy: an innovative endoscopy approach to evaluate neoadjuvant treatment response in locally advanced rectal cancer. Gut 2020;69(3):406–10. https://doi.org/10.1136/gutjnl-2019-319755.
[33] Fakurnejad S, van Keulen S, Nishio N, Engelen M, van den Berg NS, Lu G, et al. Fluorescence molecular imaging for identification of high-grade dysplasia in patients with head and neck cancer. Oral Oncol 2019;97:50–5. https://doi.org/10.1016/j.oraloncology.2019.08.008.