
**eAppendix.** Association of Main Specimen and Tumor Bed Margin Status With Local Recurrence and Survival in Oral Cancer Surgery

**eReferences.**

**eTable 1.** Frequency of Margin Results Under Various Definitions of Involved

**eTable 2.** Margin outcomes for various grouping categories, represented in 2x2 tables

**eTable 3.** Correlating Frozen Margins to Specimen Margins, including Local Recurrence Rate

**eFigure 1.** Observed Survival Based on Margin Groups

This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix
Association of Main Specimen and Tumor Bed Margin Status With Local Recurrence and Survival in Oral Cancer Surgery

We conducted a review of previously published articles related to margin assessment in head and neck cancer surgery, with particular attention to oral cavity squamous cell carcinoma, and summarized the results by grouping findings related to particular questions.

Whether a microscopically positive margin is a negative prognostic factor
In the Looser et al.\(^1\) review of 1775 head and neck epidermoid carcinomas treated with surgery during the 1960’s, they found 62 patients had a microscopically positive margin; their definition of positive was broad, including close (within 5mm), dysplasia or CIS at the margin, or invasive cancer. Local recurrence developed in 71% with a positive margin versus 32% with negative margins. The 5-year survival was 30% with a positive margin and 36% with negative margins. Several points from this study were clear: a microscopically positive margin does increase the risk of local recurrence, however a negative margin does not completely remove the risk; and margin status did not predict survival.

Other studies support this conclusion and go further to suggest that a positive margin does affect survival. Byers et al.\(^2\) reported a local recurrence rate of 16% with negative final margins and 80% with positive final margins; the 2-year survival rate was 66% if negative and 5% if final margins were positive. Zieske et al. in 349 patients with HNSCC report a 7% local recurrence rate for negative and 30% for positive final margins; the 3-year recurrence free survival rate was 60% for negative and 6% for positive final margins. Loree et al.\(^3\) looked at 398 patients undergoing surgery for OSCC and reported a local recurrence rate of 18% if the final margins were negative and 36% if positive, and the 5-year survival rate was 60% versus 52%, respectively (statistically significant).

Sutton et al.\(^4\) studied 200 patients with OSCC in the 1990s and reported a local recurrence rate of 12% for negative margins, 33% for close (<5mm), and 55% for positive margins, and the 5-year disease-free survival was 78%, 47%, and 11%, respectively. On multivariate analysis the margin status was the strongest predictor of survival.

In more recent series, however, the prognostic significance of microscopic positive margins has again been challenged. McMahon et al.\(^5\) studied 332 patients with oral or oropharyngeal SCC and found that margin status and other factors were associated with local recurrence and disease-specific survival on univariate analysis; however, on multivariate regression, only perineural invasion was associated with local recurrence, and only nodal involvement predicted survival. They suggested that positive margin status is one of several factors that suggests aggressive tumor biology, but is not independently predictive of prognosis, and intervention directed at changing the margin status (ie more aggressive resection) will theoretically not change outcomes. Brandwein-Gensler et al.\(^6\) reported in a series of 168 patients with OSCC that the local recurrence rate was 20% for negative margins, 13% for close (<5mm), and 26% for positive, without a statistically significant difference in local recurrence or survival based on margin status. However, they did find that histopathologic criteria other than margin status did predict local recurrence and survival, including perineural invasion, lymphocytic response, and worst pattern of invasion. Barry et al.\(^7\) found in a series of 295 OSCC a trend for a relationship between clear, close, and involved margins on local recurrence, but on univariate and multivariate analysis the margin status was not a significant predictor of local recurrence.

These studies are similar in that they assess margins based on the oriented main resection specimen (whether they used intraoperative frozen sections as part of the analysis or relied solely on the final permanent sections). However, the definitions of a positive margin varied, including the presence of invasive cancer at the inked edge,\(^4,6,8\) or within 1mm,\(^7\) including CIS,\(^2\) or also including dysplasia and <5mm.\(^1,3\)

Whether frozen section assessment accurately identifies a positive margin
Multiple studies report on the accuracy of frozen section diagnoses in head and neck surgery. Of 1947 frozen sections sampled for margin assessment from 1990-1991, Gandour-Edwards et al.\(^9\) reported an accuracy of 98.1%. DiNardo et al.\(^10\) examined 420 frozen samples from 1997-1999 and reported an accuracy of 98.3%, with a sensitivity of 88.8% and specificity of 98.9%.
However, in accurately identifying positive final margins (based on the main specimen), frozen sections do not perform as well. Ord et al. evaluated 49 patients with OSCC who had frozen section margins sampled from the excised specimen, demonstrating a sensitivity of 30%, specificity 97%, and prevalence of positive margin of 20% (definition of positive included dysplasia, CIS, and close <5mm). Spiro et al. reported data for 150 patients with OSCC from 1987-1993, with half of the margins sampled from the patient and half from the tumor specimen. This study found a sensitivity of 29%, specificity 100%, and accuracy 89%; the prevalence of positive margins was 16% (definition of positive included close <5mm). DiNardo et al. published 80 patients with HNSCC from 1997-1999 with a sensitivity of 34%, specificity of 100%, and accuracy of 71% for the evaluation of close and positive final margins by frozen section. Pathak et al. reviewed 229 patients with OSCC from 1973-2003 undergoing intraoperative frozen section margin assessment from the tumor specimen and found a sensitivity of 70% (definition of positive including close <5mm). Maxwell et al. analyzed a multi-institution cohort of OCSCC from 1986-2012, with 100 patients who had frozen sections sampled from the tumor bed; these data demonstrated a sensitivity of 30%, specificity 100%, and accuracy 79%; the prevalence of positive margins was 24%. In contrast to these studies, Patel et al. found in a series of 547 patients with OCSCC from 1994-2008, where they obtained frozen section samples from the tumor bed, a prevalence of positive margins on the main specimen of 10%, but impressively they had 100% sensitivity of frozen section for identifying positive main specimen margins, allowing for revision to negative margins (definition of positive included only invasive cancer at the inked margin).

Whether frozen margins should be assessed based on the resection specimen, or as samples from the patient in the tumor bed

Margin sampling from the tumor bed is the most prevalent practice amongst head and neck surgeons. In the published studies we reviewed, intraoperative frozen section margins, if obtained, have either been sampled from the specimen, the patient (tumor bed), or both. Given how common tumor bed margin sampling is amongst head and neck surgeons, it is surprising that there has been only one report of outcomes related to margin sampling and focusing on just this technique. The several papers which compared the two intraoperative margin assessment techniques suggested that false negatives may occur equally with either approach, while margin sampling from the main specimen showed the best correlation with local control and survival.

Whether re-excision after a positive margin can ‘clear’ the margin and re-establish a new negative margin, and whether this benefits the patient

In the Byers et al. review of 216 patients with surgically treated head and neck cancer who had intraoperative margins evaluated on the main specimen, they reported only a small difference in local recurrence (14% versus 20%) or 2-year survival (67% versus 62%) whether the margin was negative or initially positive but cleared to negative through additional surgery. For patients who continued to have a positive margin at the conclusion of the surgery, 80% had local recurrence with 2-year survival of 5%. The exclusion criteria from this study are important to keep in my mind, as nearly 60% of patients treated during this time period were excluded from analysis for reasons including radiation treatment and death from distant disease or other causes without evidence of local recurrence. The study concluded that intraoperative frozen analysis identifying a microscopically positive margin may benefit the patient if the margin can subsequently be cleared to negative.

In the past decade, a number of retrospective studies have re-examined this conclusion, providing conflicting findings.

Brandwein-Gensler et al. reported local recurrence rates for four different margin groups: initially negative (20%), initially positive but subsequently rendered negative (26%), final margin close <5mm (13%), and final margin remains positive (26%); they found no significant difference in survival between these groups.

Pathak et al. reported a series of 416 OCSCC patients with 55% undergoing intraoperative frozen section evaluation of margins and 45% without frozen assessment, based on the surgeon’s preference. They found no difference in survival, local recurrence (25% versus 20%), or probability of achieving clear final margins (70% versus 67%) between the groups. However, the probability of local recurrence was associated with the final margin status of the main specimen, with patients with positive margins recurring twice as often as negative margins, whether frozen was performed or not. They attributed this in part to sampling error in frozen assessment, as 30% of the involved or close margins were not identified by frozen section.
Patel et al.\textsuperscript{10,14} examined 547 OCSCC patients and found that 10% had positive initial frozen section margins subsequently revised to a negative, and these patients had a higher local recurrence rate but not worse survival. These patients with microscopic tumor cut-through were more likely to have extracapsular extension and more likely to be treated with adjuvant radiation, however the positive resection specimen margin remained a significant predictor of local recurrence on multivariate analysis. There was a strong trend for microscopic tumor cut-through to also be associated with poor disease-specific survival on multivariate analysis.

Kwok et al.\textsuperscript{11,22} found for 321 HNSCC patients with intraoperative frozen section margin evaluation that patients with negative margins or initially positive revised to negative had similar 5-year survival (72\% versus 76\%), and that survival was better than patients with persistently positive final margins (58\%).

Varvares et al.\textsuperscript{20} reported 108 patients with oropharyngeal or OCSCC and found a significant difference in local control and mortality, respectively, for a) negative (not close) margins (3.4\%, 0\%), b) initially positive resected to negative margins (29\%, 29\%), and c) persistently positive margins (8.3\%, 58\%).

Maxwell et al.\textsuperscript{21} concurred in their study of 280 early stage OCSCC patients, reporting that the main glossectomy specimen margin status (showing microscopic tumor cut-through) was significantly related to local recurrence-free survival, while the final tumor bed margin was not predictive.

Frozen section analysis is performed on many more patients than could ultimately benefit from it, if you consider that only patients with a positive frozen margin ultimately cleared to negative would benefit from the intraoperative assessment of their margin status. Byers et al.\textsuperscript{2} calculated that 30\% of their patients derived benefit from frozen assessment. Using the same criteria, Ord et al.\textsuperscript{11} concluded that of the 49 consecutive patients in their study, only 1 patient (2\%) benefited from frozen section assessment, and this was partly attributed to the low sensitivity (30\%) for positive margin identification in this study, as well as the inability to obtain final negative margins. Kwok et al.\textsuperscript{22} calculated that 15\% of their patients benefited from frozen section assessment of the margins.

That frozen section-guided re-excision of surgical margins may not directly benefit patients is also supported by the fact that there is often no additional invasive cancer identified in the re-resection specimen. The prevalence of cancer in the additional resection specimens after initial positive margins varies in reports but is overall low, including 27\%.\textsuperscript{16} and 22\%.\textsuperscript{19}


**eTable 1.** Frequency of Margin Results Under Various Definitions of Involved

<table>
<thead>
<tr>
<th>N=406</th>
<th>Initial Frozen</th>
<th>Final Operative</th>
<th>Tumor Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninvolved</td>
<td>Involved</td>
<td>Uninvolved</td>
</tr>
<tr>
<td>Positive</td>
<td>332</td>
<td>74</td>
<td>395</td>
</tr>
<tr>
<td>Positive and CIS</td>
<td>257</td>
<td>149</td>
<td>363</td>
</tr>
<tr>
<td>Positive and &lt;1mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, CIS, &lt;1mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, CIS, &lt;5mm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**eTable 2.** Margin outcomes for various grouping categories, represented in 2x2 tables

A) Test performance of intraoperative frozen section (IFS) compared to the final permanent section (PS) diagnosis of the same tissue sample

<table>
<thead>
<tr>
<th></th>
<th>PS +</th>
<th>PS -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS +</td>
<td>387</td>
<td>8</td>
<td>395</td>
</tr>
<tr>
<td>FS -</td>
<td>14</td>
<td>2899</td>
<td>2913</td>
</tr>
<tr>
<td>Total</td>
<td>401</td>
<td>2907</td>
<td>3308</td>
</tr>
</tbody>
</table>

B) Comparison of tumor bed (TB) frozen section diagnosis to tumor specimen (TS) diagnosis (considering an involved margin to be positive, and CIS)

<table>
<thead>
<tr>
<th></th>
<th>TS +</th>
<th>TS -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB +</td>
<td>63</td>
<td>86</td>
<td>149</td>
</tr>
<tr>
<td>TB -</td>
<td>52</td>
<td>205</td>
<td>257</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>291</td>
<td>406</td>
</tr>
</tbody>
</table>

C) Comparison of tumor bed frozen section diagnosis to tumor specimen diagnosis (considering an involved margin to be just positive)

<table>
<thead>
<tr>
<th></th>
<th>TS +</th>
<th>TS -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB +</td>
<td>33</td>
<td>41</td>
<td>74</td>
</tr>
<tr>
<td>TB -</td>
<td>61</td>
<td>271</td>
<td>332</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>312</td>
<td>406</td>
</tr>
</tbody>
</table>

A) Test performance of intraoperative frozen section (IFS) compared to the final permanent section (PS) diagnosis of the same tissue sample
B) Comparison of tumor bed (TB) frozen section diagnosis to tumor specimen (TS) diagnosis (considering an involved margin to be positive, and CIS)
C) Comparison of tumor bed frozen section diagnosis to tumor specimen diagnosis (considering an involved margin to be just positive)
eTable 3. Correlating Frozen Margins to Specimen Margins, including Local Recurrence Rate

<table>
<thead>
<tr>
<th>N=344</th>
<th>Tumor Specimen Margin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Initial Frozen Margin</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>7% (57)</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>25% (4)</td>
</tr>
<tr>
<td>CIS</td>
<td>29% (7)</td>
</tr>
<tr>
<td>Positive</td>
<td>27% (11)</td>
</tr>
<tr>
<td>All</td>
<td>13% (79)</td>
</tr>
</tbody>
</table>
eFigure 1. Observed Survival Based on Margin Groups
A) Frozen margin involved/uninvolved, B) Final margin involved/uninvolved, C) Specimen margin involved/uninvolved. Involved includes invasive cancer or CIS at the margin, as well as very close (<1mm) to the specimen margin.

A)

Frozen Margin
Chi−sq 4.1, p = 0.044

B)

Final Margin
Chi−sq 5.1, p = 0.024

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Follow-up Time, Months

Survival Probability

Uninvolved includes positive, very close, and CIS

Chi-sq 22.6, p = 0