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COMPUTER ASSISTED ANALYSIS OF THE MICROVASCULATURE IN METASTASIZED AND NONMETASTASIZED SQUAMOUS CELL CARCINOMAS OF THE TONGUE

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Abstract: Background. Quantification of microvessels in solid malignancies is regarded as a potential test to predict their clinicobiologic behavior. However, discordant results have been reported for head and neck cancer that may be explained by varying methods.

Methods. In this retrospective study, we therefore quantified the microvasculature in 20 nonmetastasized and 20 metastasized squamous cell carcinomas of the tongue, using recently developed methods. For immunohistochecmical visualization of the vessels, we used anti-CD34 with a signal amplification step based on the catalyzed deposition of biotinsylated tyramine. This protocol results in enhanced staining quality compared with standard protocols. For each tumor, a representative tissue section was systematically sampled with 40 to 60 standardized test fields. True color image analysis system was used to measure microvessel density (MVD) and to obtain additional information with regard to size categories of vessels.

Results. Remarkably, in the group of nonmetastasized tumors, the MVD was greater than in the metastasized tumors (p = .007). However, the microvessels with a diameter in the range of 10 to 15 μm predominated in the group of metastasized tongue carcinomas (p = .03). A logistic regression model based on the percentage of vessels smaller than 5 μm, classified 85% of patients with a metastasized tumor correctly and 75% of patients with a nonmetastasized tumor, independently of the clinical stage of the tumor.

Conclusions. These results suggest that only vessels with a diameter larger than 10 μm, consistent with functional vessels, play a role in the process of metastasis. Further research more specifically into structural and functional characterization of blood and lymphatic vessels might help provide more insight into the relationship between microvasculature and the pathogenesis of metastasis in tongue carcinomas. © 2002 Wiley Periodicals, Inc.


Angiogenesis in Tongue Carcinomas

HEAD & NECK July 2002  643
Angiogenesis is essential for neoplastic progression and metastasis. The microvessel density (MVD) was found to correlate with tumor progression and occurrence of metastasis in a variety of tumors. Most often vessels are manually counted in so-called angiogenic hot spots, defined as the area with the highest density of microvessels in a tissue section, identified under low-power magnification. Many studies on angiogenesis in head and neck squamous cell carcinomas (HNSCCs) have been performed. Discordant results were found for the measured MVDs in different studies but also for the relation between MVDs and the metastatic behavior. These discrepancies may be caused by differences in tumor location, the choice of antibodies, and differences in immunohistochemical protocols to visualize the vessels. Furthermore, manual selection of hot spots is liable to interobserver variation. In tongue carcinoma studies, both higher and lower counts in metastasized tumors, as well as no differences between metastasized and nonmetastasized tumor, were reported.

To the best of our knowledge, no angiogenesis studies on HNSCCs addressed the possibility that subclasses of vessels are preferentially used for metastasis, as was shown for xenografts of melanoma cell lines. Image analysis, which is needed to identify such subclasses of vessels in immunohistochemically stained tissue sections, requires high standards of staining quality, such as low background staining, high signal-to-noise ratio, and small intraspecimen and interspecimen variations in staining intensity.

In a recent study on HNSCCs, it was shown that anti-CD34 monoclonal antibodies with amplification at sites of immunoreactivity facilitated the use of a modified true color model for recognition of stained objects by an image analysis system. In the latter study, it was shown that averaging the 10 most vascularized fields of view found by a systematic tissue sampling procedure resulted in an overall acceptable reproducibility.

The aims of this study were first to use these recently developed protocols and procedures to assess the characteristics of the microvasculature in relation to metastatic behavior in tongue carcinomas and second to explore the possibilities of microvascular parameters to predict the metastatic behavior of tongue carcinomas.

**MATERIALS AND METHODS**

**Patients.** In a retrospective study, we retrieved from our archives 40 resection specimens of primary SCCs of the mobile tongue (anterior two thirds), of which a follow-up of at least 24 months was available. The following inclusion criteria were used to select the patients. None of the patients had been treated otherwise earlier or in the follow-up. There was no evidence of a second primary tumor at the time of presentation or during follow-up. The distribution between metastasized and nonmetastasized tumors was predetermined on 20 to 20. In 16 of 20 patients with a metastasized tumor, a neck dissection was simultaneously performed with treatment of the primary tumor, and in all these cases lymph node metastasis was microscopically confirmed. The remaining four patients had microscopically confirmed metastases developed during follow-up. In the group of patients with a nonmetastasized tumor, there was no evidence of lymph node or distant metastases at the time of treatment or during follow-up. In 19 of the 20 patients with nonmetastasized tongue carcinomas, a neck-dissection was performed at the time of surgical treatment of the primary tumor, and no evidence of metastasis was found by the pathologist during routine examination of the lymph nodes. After initial treatment, no adjuvant treatment to the neck was given for this group. During follow-up, the patients were seen every 2 months for physical examination, and chest x-rays were taken every 6 months. The distribution between the clinical T stages according to the UICC is given in Table 1.

**Immunohistochemistry.** Four-micrometer thick, formalin-fixed, paraffin-embedded tissue sections were mounted on Superfrost slides (Menzel Gläser, Braunschweig, Germany) and dried overnight at 56°C. The sections were deparaffinized, treated with 1% H2O2 in methanol (20 min), and rinsed in phosphate-buffered saline (PBS, pH 7.4) three times for 5 minutes. After preincubation with normal horse serum 100% (Vector Laboratories, Burlingame, CA) for 20 minutes at room temperature (RT), an overnight incubation with anti-CD34 (QB-END, Biogenex, San Ramon, CA) 1:2 in PBS/1% bovine serum albumin (PBS/1% BSA, Sigma, St. Louis, MO) was performed at 4°C, followed by three steps of 10-minute rinsing in PBS. Next, incubation with biotinylated horse anti-mouse antibodies (Vectastain ABC-kit, Vector Laboratories) 1:200 in PBS/
Table 1. Clinical tumor size distribution.

<table>
<thead>
<tr>
<th>T</th>
<th>Observed</th>
<th>Predicted</th>
<th>T</th>
<th>Observed</th>
<th>Predicted</th>
<th>N</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>4</td>
<td>6</td>
<td>T1</td>
<td>9</td>
<td>7</td>
<td>13</td>
<td>85</td>
</tr>
<tr>
<td>T2</td>
<td>12</td>
<td>10</td>
<td>T2</td>
<td>9</td>
<td>11</td>
<td>21</td>
<td>81</td>
</tr>
<tr>
<td>T3</td>
<td>4</td>
<td>5</td>
<td>T3</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
<td>1</td>
<td>T4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>22</td>
<td>Total</td>
<td>20</td>
<td>18</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>

Observed frequencies per clinically determined T stage; predicted frequencies by the logistic regression model, after cross-validation. *n*, number of patients per T stage; percentage, correct classification per T stage; NA, not applicable.

1% BSA (Sigma) for 30 minutes at RT and three rinsing steps of 5 minutes in PBS. Subsequently, the tissue sections were incubated during 45 minutes with peroxidase (PO)-labeled avidin-biotin complex (ABC<sup>®</sup>, ABC Elite kit, Vector Laboratories) 1:100, with three rinsing steps in PBS of 5 minutes. Signal amplification was performed by catalyzed amplification of reporter deposition (CARD) as described previously. Biotinylated tyramine (BT) solution was prepared dissolving 100 mg sulfo-succinimidyl-6-(biotinimido)hexanoate (NHS-LC-biotin) (Pierce, Rockford, IL) in 40 mL 50 mM borate buffer, pH 8.0. Next, 30 mg tyramine-HCl (Sigma) was added. The mixture was agitated overnight at RT and filtered. Before application, BT was diluted 1:50 in PBS with 0.01% H<sub>2</sub>O<sub>2</sub>. Incubation with BT solution was performed during 5 minutes at RT, with precipitation of BT at sites of PO reactivity. Deposited BT was detected with an extra incubation with ABC<sup>®</sup> for 15 minutes. Finally, peroxidase was visualized with 0.05% diamonobenzidine tetrahydrochloride (DAB, Sigma) for 5 minutes (Figure 1A). The sections were rinsed in tap water, slightly counterstained with Mayer’s hematoxylin, rinsed in tap water, dehydrated, and mounted with Permount (Fisher Scientific, Fair Lawn, NJ).

Tissue Sampling. The systematic tissue sampling procedure that was used in this study has been described elsewhere in more detail. In short, the slides were examined under low-power magnification (×4 objective) to identify areas containing tumor tissue. Next, neighboring fields in a horizontal and vertical direction were stored on disk in true color format, with a higher magnification (×10 objective), thus reciprocating from left to right, starting at the top, going to the bottom through the slide. In cases of a large tumor area, two or more neighboring fields were skipped in a horizontal direction, so as not to exceed 60 fields. Only fields containing enough vital tumor tissue were included. Fields containing more than 50% vessel-rich structures such as striated muscle, lamina propria, or peripheral nerves were skipped because of the prevailing presence of preexisting (ie, non-tumor-induced) vessels in these structures. In this manner, 40 to 60 fields were sampled for each tumor section. After recognition of the vessel profiles by CIAS (Figure 1B), the images with the highlighted vessel contours were shown on an image monitor for interactive removal of small areas in the fields containing nerves, muscle cells, lamina propria, necrotic tissue, artifacts, prominent keratin pearls, and incorrectly recognized vessel profiles. From the corrected images, the vascular parameters were computed by CIAS.

Computerized Image Analysis. Details of the image analysis used in this study are described elsewhere. In short, image analysis was performed using a Vidas<sup>®</sup>plus system (Kontron GmbH, Eching, Germany). Images were recorded with a three-chip CCD camera (DXC-325P, Sony, Tokyo, Japan) mounted on a conventional light microscope (Axioskop, Carl Zeiss, Jena, Germany), using a ×10 objective (numerical aperture 0.3). Microscopic fields were digitized and stored on magneto-optical disks (Borsu Systema, Lelystad, The Netherlands) as true color (24-bit RGB) images. The size of each field was 0.17 mm<sup>2</sup>, measuring 0.400 × 0.425 mm. The pixel size was 0.8 × 0.8 μm. Segmentation was done with an algorithm based on an adaptation of the HSI model, in which the RGB to HSI transform is applied to optical densities for the individual RGB channels, instead of intensities. MVD, defined as the number of CD34 positive objects in a measurement field, was determined.
after interactive correction. In addition, the area (AREA), perimeter (PERIM), and diameter (DIAM) of the individual vessels were measured. On the basis of DIAM, the vessels were subdivided in the following four categories. CAT1 vessels with DIAM < 5 μm, CAT2 vessels with 5 ≤ DIAM < 10 μm, CAT3 vessels with 10 ≤ DIAM < 15 μm, and CAT4 vessels with DIAM ≥ 15 μm. Then the percentage of each diameter category per patient was computed and added as parameter %CAT1, %CAT2, %CAT3, and %CAT4, respectively.

Statistics. Data analyses were performed using SPSS statistical software package (SPSS 9.0 for Windows, SPSS Inc., Chicago, IL). On the basis of the results of a previous study, we chose to include only the 10 most vessel-rich fields per patient, because this gave the best combination of reproducibility and discriminating power between the groups. An ANOVA was used to assess differences between the groups of patients with metastasized and nonmetastasized tumors. A forward likelihood-ratio stepwise logistic regression analysis was performed on the aforementioned parameters to select the best discriminating parameters. The selected parameters were used to construct a linear discriminant function DF to predict the occurrence of metastasis. Cross-validation was performed using the “leave-one-out” procedure to reduce possible bias, because the same data sets are used both to construct the model and to assess the classification performance of the predictor.

RESULTS
A total of 19,427 vessels was assessed in 2181 fields, distributed over 40 patients in two groups. The results are summarized in Table 2. In the group of nonmetastasized tongue carcinomas, the mean MVD was significantly greater than in the group of metastasized tumors (p = .007). In the latter group a greater value of the diameter was found than in the group of nonmetastasized tumors, but the difference was not significant (p = .06).

Comparison of the group of nonmetastasized tumors revealed that in the former group the microvessels with DIAM < 5 μm (p < .001) predominated, whereas vessels with 10 ≤ DIAM < 15 μm were more numerous in metastasized tumors (p = .03) (Table 2, Figure 2). Differences between metastasized and nonmetastasized tumors for the other vascular parameters and the clinically determined T stage were not significant at significance level of .05. The stepwise logistic regression, applied on all vascular parameters and T stage, selected only one parameter, namely %CAT1 vessels. The discriminant function (DF) obtained by logistic regression analysis is given by the formula: DF = 4.6743 – 0.2262 × %CAT1. Application of this DF resulted in 80% overall correct classifications. In the groups of meta-

FIGURE 1. Typical immunohistochemically stained tissue section of a tongue carcinoma used in this study. Consecutive sections, 10 × objective, actual size: 0.400 × 0.425 mm. (A) Microvessels visualized with CD34/DAB, resulting in brown profiles against blue Mayer’s hematoxylin background staining. (B) Microvessel recognition by Computerized Image Analysis System (CIAS) added in green.
sized and nonmetastasized tumors, the percentages of correct classifications were 85% and 75%, respectively. The “leave-one-out” cross-validation procedure resulted in the same classifications (Table 3). For the T1, T2, and T3 tumors, the percentages of correct classifications were 85%, 81%, and 80%, respectively (Table 1). In our series there was only one patient with a T4 tumor, and this tumor was erroneously classified as metastasized by the logistic regression model. The estimated relative risk to have a metastasized carcinoma is 17 times higher in patients with a DF value > 0, corresponding with %CAT1 < 20.7%, than patients with DF value < 0.

A second observer independently measured the same tissue sections of 10 patients to assess the interobserver reproducibility of the classification results of the discriminant function. In one case there was an inconsistent classification of the metastatic behavior between both observers (Cohen’s kappa = 0.78, where kappa > 0.75 indicates a strong interobserver agreement).

**DISCUSSION**

The most remarkable result of this study was that significantly higher MVDs were found in the group of nonmetastasized tumors than in the group of metastasized tumors. Other studies on HNSCC, which disclosed significant difference between metastasized and nonmetastasized tumors, reported higher values of MVDs in the former group (Table 4). Other investigators found lower MVDs in metastasized HNSCC than in the nonmetastasized tumors, but the differences between both groups were not significant. Our observation in tongue carcinomas is not unique, because in a recent study on renal cell carcinomas, it was also found that tumors without metastasis had a higher number of microvessels than tumors with metastases, and the difference was highly significant. They found that MVD was the most important factor to predict the occurrence of metastatic disease and concluded that the inverse correlation between MVD and metastatic disease implies that the relation between MVD and metastasis formation is more complex than assumed hitherto.

![Percentage of microvessels counts in relation to vessel diameter](image)

**FIGURE 2.** Proportional microvessel counts in relation to vessel diameter. Percentages of microvessels within the groups metastasized (striped columns) versus nonmetastasized tumors (open columns) per diameter category 0–5, 5–10, 10–15, and >15 μm. Bars represent upper limit of 95% confidence interval.

**Table 2.** Vessel parameters used in this study with their means and standard deviation (SD) for the groups metastasized and nonmetastasized tumors and the significance level as obtained from an ANOVA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonmetastasized</th>
<th>Metastasized</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVD</td>
<td>32.9</td>
<td>25.5</td>
<td>.007</td>
</tr>
<tr>
<td>AREA</td>
<td>161</td>
<td>174</td>
<td>.40</td>
</tr>
<tr>
<td>PERIM</td>
<td>57.4</td>
<td>60.6</td>
<td>.30</td>
</tr>
<tr>
<td>DIAM</td>
<td>7.8</td>
<td>8.4</td>
<td>.06</td>
</tr>
<tr>
<td>%CAT1</td>
<td>25.1</td>
<td>17.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%CAT2</td>
<td>53.6</td>
<td>55.7</td>
<td>.40</td>
</tr>
<tr>
<td>%CAT3</td>
<td>17.1</td>
<td>22.2</td>
<td>.03</td>
</tr>
<tr>
<td>%CAT4</td>
<td>4.2</td>
<td>4.6</td>
<td>.70</td>
</tr>
</tbody>
</table>

**Table 3.** Prediction results.

<table>
<thead>
<tr>
<th></th>
<th>Nonmetastasized</th>
<th>Metastasized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>15 (75%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>Metastasized</td>
<td>3 (15%)</td>
<td>17 (85%)</td>
</tr>
</tbody>
</table>

Observed frequencies of metastasized and nonmetastasized tumors and predicted frequencies by the logistic regression model, given by DF = 4.6743 + 0.2262 × %CAT1, after cross-validation.
Table 4. MVDs reported in literature.

<table>
<thead>
<tr>
<th>Author</th>
<th>Tissue</th>
<th>Antibody</th>
<th>Mean value ± SD for vessels per mm² in hot spot</th>
<th>Mean value ± SD for vessels per mm² overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymphnode –</td>
<td>Lymphnode +</td>
</tr>
<tr>
<td>Gasparini</td>
<td>HNSCC</td>
<td>CD31</td>
<td>97.4 ± 46.6</td>
<td>75.0/107.0*</td>
</tr>
<tr>
<td>Leedy</td>
<td>T1,2 longue scc</td>
<td>F VIII</td>
<td>65.6/137.8*</td>
<td>59.4/118.9*</td>
</tr>
<tr>
<td>Shpitzer</td>
<td>T1 tongue scc</td>
<td>F VIII</td>
<td>50.0 ± 13.7*</td>
<td>103.4 ± 40.7*</td>
</tr>
<tr>
<td>Penfold</td>
<td>Oral scc</td>
<td>CD31</td>
<td>116 ± 24</td>
<td>168 ± 56</td>
</tr>
<tr>
<td>Gleich</td>
<td>T1 tongue</td>
<td>CD31</td>
<td>193.0</td>
<td>192.0</td>
</tr>
<tr>
<td></td>
<td>T2,4 longue</td>
<td>F VIII</td>
<td>168.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ FOM² scc</td>
<td>CD31</td>
<td>157.7 ± 78</td>
<td></td>
</tr>
<tr>
<td>Pazouki</td>
<td>Oral scc</td>
<td>F VIII</td>
<td>148 ± 53</td>
<td>18.3*</td>
</tr>
<tr>
<td>Moriyama</td>
<td>Tongue scc</td>
<td>CD31</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>Hegde</td>
<td>HNSCC</td>
<td>F VIII or CD31</td>
<td>36.6</td>
<td></td>
</tr>
<tr>
<td>Hegde</td>
<td>Tongue² scc</td>
<td>F VIII or CD31</td>
<td>29.9*</td>
<td>71*</td>
</tr>
<tr>
<td>Galto</td>
<td>HNSCC</td>
<td>CD31</td>
<td>107.8 ± 62.2*</td>
<td>82.3 ± 49.5*</td>
</tr>
<tr>
<td>Present study</td>
<td>T1,4 longue scc</td>
<td>CD34</td>
<td>264.7 ± 72.1*</td>
<td></td>
</tr>
</tbody>
</table>

Results reported in HNSCC microvessel density studies, recalculated into counts per mm² ± standard deviation (SD, where available). Values for the selected hot spot and mean overall values depend on available data. Bold figures indicate a significant difference (p < .05) between metastasized and nonmetastasized tumors as reported by the respective authors.

*Depending on magnification (200/400 x respectively).
1Per 250 x magnification field.
2Floor of the mouth.
3Per 200 x magnification field.
4Two cases from HN group.
5Median.
6Value for single hottest field, as opposed to top 10 fields from Table 2.
7Value for all fields in tissue section, as opposed to top 10 from Table 2.

Indeed, an inverse correlation between MVD and metastatic behavior seems to be in conflict with Folkman’s concept concerning the development of metastasis, in which the complex process of angiogenesis plays an important role.¹ The process of angiogenesis is characterized by a series of events, namely migration of endothelial cells, sprout formation, endothelial cell proliferation, fusion of sprouts, and eventually maturation of newly formed vessels by formation of basement membrane and the appearance of pericytes.³¹,³² It is plausible to assume that only functional vessels, produced during angiogenesis, are involved in the transport of tumor cells from the primary tumor to lymph nodes or distant organs. Under this assumption, our results are less conflicting with Folkman’s concept. We found that higher MVDs in the nonmetastasized tumors were caused by the predominance of “microvessels” with a diameter smaller than 5 µm. It is unlikely that “microvessels” with a diameter smaller than 5 µm are functional vessels. More probable is that these structures include most of the migrating and sprouting endothelial cells. In addition, we found that microvessels with a diameter in the range of 10 to 15 µm predominated in the metastasized tumors. The latter finding gives support to the assumption that only functional vessels are involved in the process of metastasis, but this remains to be established by more appropriate techniques. Antibodies to CD34 and other panendothelial markers are unsuitable for this purpose, because they are incapable of selective visualization of functional vessels or other vascular structures preferentially used by tumor cells to metastasize. A perfusion marker to visualize functional vessels may be more appropriate in this respect.³³ In this study, anti-CD34 antibodies were used to visualize vessels instead of anti-CD31 antibodies proposed as standard for the visualization of vessels,²⁰ because the latter also stains plasma cells, often abundantly present in the inflammatory infiltrate of tongue carcinomas, hampering the automatic recognition of vessels by image analysis. CARD amplification enhanced the staining intensity, which seemed to be needed for the automatic recognition of vessels by an image analysis system but
also visualized more vessels than the standard procedure, especially in tumor in which the immunoreactivity was absent or the vessels were stained very weakly.\textsuperscript{26} In the metastasized tongue carcinomas, we found MVDs in the range of MVDs found by Gleich et al\textsuperscript{5,6} in squamous cell carcinomas of the tongue and floor of the mouth using antibodies directed against FVIII and CD31 (Table 4). In contrast to the latter studies we found significantly higher MVDs in the nonmetastasized tumors (Table 4). A possible explanation is that the use of monoclonal antibody directed against CD34 in combination with the CARD amplification step visualized more vascular elements of the prematuration phase of angiogenesis than standard immunohistochemical procedures using antibodies against CD31 and factor VIII without signal amplification widely used in studies on angiogenesis. Anti-CD34 antibodies generally react well with endothelial cells in large blood vessels, but their expression without CARD is diminished or absent from some microvessels in normal and many tumor tissues.\textsuperscript{34}

Metastasized and nonmetastasized tongue carcinomas differed most significantly for the percentage of microvessels with diameter smaller than 5 μm. This percentage was the only parameter selected by the stepwise logistic regression analysis, including all vascular parameters and the clinical T stage, to discriminate between metastasized and nonmetastasized tongue carcinomas. The univariate classifier resulted in 85% and 75% correct classifications in the group of metastasized and nonmetastasized tongue carcinomas, respectively. This classifier predicted the metastatic behavior independently of the tumor size, because the percentage of correct classifications for T1, T2, and T3 tumors were in the same range. Lower percentage of correct classifications in the group of nonmetastasized tumors could be expected, because micrometastases have been missed in the routine microscopic examination of the lymph nodes in neck dissection specimens. It was shown by molecular techniques that neoplastic cells could be identified in 6 of 28 lymph nodes (21%) that were initially negative by histopathologic assessment.\textsuperscript{35}

Although the results of this study regarding the prediction of metastatic behavior of tongue carcinomas were encouraging, the applicability of our logistic regression model is limited for use in treatment planning of the individual patients. In our opinion, an overall percentage of misclassification of 20% is still too high for use in clinical practice. When vessel parameters measured in biopsy specimens will be used in our logistic regression model to predict the metastatic behavior of the primary tumor at the time of diagnosis, higher percentages of misclassifications can be expected because of tissue sampling errors.

\textbf{Acknowledgment.} We thank Prof. D. J. Ruiter and Prof. D. Riediger for their helpful discussions, Ms J. H. Gemmink for her technical assistance, and Mr. V. M. J. I. Cuypers for performing the in duplo measurements.

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Angiogenesis in Tongue Carcinomas