Radioimmunoscintigraphy and Biodistribution of Technetium-99m-labeled Monoclonal Antibody U36 in Patients with Head and Neck Cancer

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ABSTRACT

So far, mAb E48 is the most promising antibody described for specific targeting of head and neck squamous cell carcinoma (HNSCC) in patients. On the basis of its more homogeneous reactivity pattern on HNSCC, the novel mAb U36 may be even better suited for targeting. In this study the biodistribution of mAb U36 was evaluated by radioimmunoscintigraphy (RIS) and biopsy measurements in 10 patients who were suspected of having neck lymph node metastases from a histologically proven HNSCC and who had been scheduled to undergo resection of the primary tumor and neck dissection. Patients received 1.8–53.0 mg mAb U36 IgG labeled with 756 ± 95 MBq technetium-99m i.v.

Preoperatively, palpation, computerized tomography, magnetic resonance imaging, and RIS were performed. RIS images included planar and single-photon emission computed tomography images of the head and neck and planar images of the whole body. The diagnostic findings were recorded per side as well as per lymph node level of the neck and compared to the histopathological outcome. Radioactivity in blood samples and biopsies from the surgical specimens were measured.

All 10 primary tumors were visualized by RIS. All diagnostic modalities were correct in 7 of 14 tumor-involved lymph node levels. The missed lymph node metastases comprised micrometastases, small tumor-involved nodes (<9 mm), and tumor-involved nodes with much necrosis, keratin, or fibrin. There were no false-positive observations with mAb U36. Besides activity uptake in tumor tissue, only a slight accumulation of activity was observed in the mouth, lungs, liver, spleen, kidneys, and scrotal area. Biopsies from the surgical specimen showed a high tumor uptake of 20.4 ± 12.4% of the injected dose/kg (range, 8.0–43.0% of injected dose/kg), 44 h postinjection. An increase in the mAb dose did not influence uptake of activity in tumor tissue. The mean tumor:nontumor ratio at this point time was 2.3 for mucosa, 2.8 for blood, 3.0 for bone marrow aspirate, 12.9 for fat, and 13.0 for muscle tissue.

The present clinical study shows that technetium-99m-labeled U36 IgG accumulates selectively and to a high level in HNSCC. The tumor-targeting results for U36 IgG are comparable to those previously described for E48 IgG. On the basis of the results of ongoing biodistribution studies in which both mAbs E48 and U36, labeled with different iodine isotopes, are simultaneously evaluated for tumor uptake and retention in HNSCC patients, one of these mAbs will be selected for future adjuvant radioimmunotherapy trials.

INTRODUCTION

Squamous cell carcinoma represents about 90% of all head and neck cancers. Worldwide, the incidence of HNSCC is about 500,000. Despite intensive efforts in prevention, screening, and therapy, in the last decades the 5-year survival rates have not improved substantially. This is mainly due to the development of locoregional recurrences and distant metastases caused by the persistence of minimal residual disease after surgery (1, 2). Patients with advanced stage HNSCC and particularly those with multiple lymph node involvement are at risk for failure at primary and distant sites (3, 4). It is obvious that these patients need adjuvant therapy. Unfortunately, chemotherapy is only of value for palliation of HNSCC (2). Since HNSCC has a high intrinsic sensitivity for irradiation (5), we focus on the use of mAbs labeled with radionuclides for RIT.

For this purpose we produced a panel of mAbs directed against HNSCC (6–9). Two of these mAbs, designated E48 and U36, are exclusively reactive with normal and malignant squamous and transitional epithelia. mAb E48 recognizes a Mr 16,000 outer cell surface antigen probably involved in cell-cell adhesion (10). mAb E48 was shown to be highly capable for selective tumor targeting in HNSCC patients (11–13). The high and selective tumor uptake of radiolabeled mAb E48 is promising for the application of mAb E48 in RIT (13–14). However, a limitation of mAb E48 is the heterogeneity of the E48 antigen expression in 30% of HNSCCs (15). As a consequence, not all HNSCC patients will be eligible for RIT with mAb E48. For this reason we recently developed a new mAb designated U36. mAb U36 recognizes a Mr 200,000 antigen which is homogeneously expressed in 96% of HNSCCs (15). In HNSCC xenograft-
bearing nude mice, radiolabeled mAb U36 showed high uptake and good retention in tumor tissue (9).

In this study the potential of mAb U36 for targeting HNSCC in patients is evaluated. To allow for comparison with mAb E48, we investigated 99mTc-labeled mAb U36, using RIS and measurements of activity in blood samples and biopsies obtained from surgical specimens, in the same way as we previously described for mAb E48 (11-13).

PATIENTS AND METHODS

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Patient Study. The protocol was approved by the Dutch Health Council and the Institutional Review Board of the Free University Hospital. Informed consent was obtained from all participants.

Ten patients, who were suspected of having neck lymph node metastasis from a histologically proven HNSCC and were scheduled to undergo a resection of the primary tumor and a neck dissection, participated in this study. The primary tumor and the status of neck lymph nodes were classified according to the TNM system of the International Union Against Cancer, the Union Internationale Contre le Cancer (16). See Table 1 for patient and tumor characteristics and injected mAb dose. Prior to enrollment, a biopsy of the primary tumor had to show a positive immunoperoxidase staining with mAb U36. Semiquantitative evaluation of these stainings revealed reactivity of mAb U36 with 75-100% of the cells within these tumors. Prior and up to 7 days after administration of 99mTc-labeled mAb U36, urine and blood were obtained for chemical analysis and assessment of mAb pharmacokinetics. Electrolytes, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, lactate dehydrogenase, urea nitrogen, creatinine, and uric acid were determined in serum. Hematological determinations included hemoglobin, hematocrit, platelet count, WBC count and differentiation, and sedimentation rate. Skin tests were not performed. Vital signs were recorded before and up to 3 h after injection. Before and 6 weeks after injection, serum samples were obtained from patients for the assessment of HAMA development with a mAb U36-related HAMA assay.

Patients received 2.4 ± 0.5 mg U36 IgG radiolabeled with 99mTc (mean, 756 ± 95 MBq) by i.v. injection in 5 min. As a bridging study between RIS and RIT, two patients (patients 6 and 7) received simultaneously 10 mg unlabeled U36 IgG and three patients (patients 8-10) received additionally 50 mg unlabeled U36 IgG.

mAb U36. mAb U36 was derived after immunization of mice with viable cells of the HNSCC cell line UM-SCC-22B. The antigen recognized by mAb U36 is a M, 200,000 protein located at the outer cell surface (9). The mAb U36-defined antigen is expressed by 99% of the primary HNSCCs (n = 196). A comparable reactivity pattern was observed in 28 lymph nodes metastases (15).

Antibody Preparation. The U36 IgG used in this study was supplied by Centocor, Inc. (Leiden, the Netherlands). U36 IgG was purified from a concentrated tissue culture supernatant by affinity chromatography on a protein A-Sepharose column. For virus inactivation, IgG from the protein A eluate was treated for at least 6 h with Tween 80 and tri-n-butylphosphate. The protein A-purified IgG was further purified on Q-Sepharose.

Preparation of 99mTc-labeled U36 IgG. All radiolabeling procedures were performed under aseptic conditions in a shielded laminar flow hood. All glassware, plastics, and solutions were sterile and pyrogen free. For labeling mAb U36 IgG with 99mTc, a modification of the multistep procedure as described by Fritzberg et al. (18) was followed, using a S-benzoylmercaptoethylglycylglycine chelator (19) which was a gift from Mallinckrodt Medical B.V. (Petten, the Netherlands). A mean of 98.2 ± 1.2% of the 99mTc was bound to IgG as determined by chromatography on ITLC-SG strips (Gelman Sciences, Ann Arbor, MI) with 0.1 M citrate buffer, pH 5.0. Every radiolabeled U36 IgG preparation was assayed for immunoreactivity by measuring the binding to gluteraldehyde-fixed cells of the HNSCC cell line UM-SCC-14C (9). As determined by a modified Lineweaver-Burk plot, the immunoreactive fraction of 99mTc-labeled U36 IgG at infinite antigen excess was 74.7 ± 9.0%. The affinity constant for U36 IgG was 3.5 × 1010 M⁻¹ as determined by the Scatchard plot.

Imaging Studies. All patients were examined by palpation, CT, MRI, and RIS of the neck prior to surgery. Preoper-
ative palpation was performed by the same experienced head and neck surgeon. CT scans were obtained with a fourth generation Siemens Somaton Plus (Siemens AG, Erlangen, Germany) after i.v. administration of contrast medium (Ultravist 300 mg iodine/ml; Schering AG, Berlin, Germany). Contiguous axial 5–6-mm scanning planes were used. MRI examinations were done on a 0.6-tesla imaging system (Teslacon; Technicare-General Electric, Milwaukee, WI) using a partial volume coil. Axial T1-weighted spin echo and gadolinium-diethylenetri-aminepentaacetic acid (Magnevist; Schering AG) enhanced T1-weighted gradient-recalled echo images were made in all patients without claustrophobia. Slice thickness varied from 3 to 5 mm, with an interslice gap of 50% as described by Van den Brekel et al. (20). Criteria for the optimal assessment of cervical lymph node metastases by CT or MRI, as defined in our institute, were used. At CT and MRI, neck levels were considered malignant if nodes with central necrosis were depicted, or if the minimal diameter in the axial plane of a node was 11 mm or more for nodes located in level II (subdigastric) and 10 mm or more for all other nodes, or if groups of three or more borderline lymph nodes (1 or 2 mm smaller) were seen (21).

RIS was performed with a large-field view gamma camera (Dual Head Genesys Imaging System; ADAC Laboratories, Milpitas, CA) equipped with low-energy parallel hole collimators and connected to a computer (Pegasys; ADAC Laboratories). Planar anterior and posterior images of the head and neck and the whole body were acquired immediately, at 16 h, and at 21 h after injection. SPECT images of the head and neck were acquired 16 h after injection, while lateral scans of the head and neck were obtained 21 h after injection. Planar images included the following acquisition parameters: matrix size $128 \times 128$ (head and neck) or $256 \times 256$ (whole body) and at least 100 kilocounts were obtained per view during 5–20 min. Acquisition data for SPECT imaging were: 64 angles were recorded, 30-s acquisition/angle, 360-degree circular orbit, and matrix size $64 \times 64$. Interpretation of the images was based on asymmetry and retention of activity, especially on late images.

CT, MRI, or RIS examinations were each scored by one experienced examiner. All examiners were blinded to the results of other examinations and the pathological outcome. They only were informed about the site of the primary tumor. All patients had neck dissections performed 2 days after administration of the radioimmunoconjugate. After fixation, all palpable and visible lymph nodes were dissected from the surgical specimen and cut into 2–4-mm-thick slices for microscopic examination. The size of the lymph nodes does not change by fixation (21). The different slices of one lymph node were examined by a pathologist, and the percentage of tumor involvement was estimated. The outcome of the histopathological examination of the neck dissection specimens was used as "gold standard."

For topographical evaluation the findings were recorded per side as well as per lymph node level according to the Memorial Sloan-Kettering Cancer Center Classification (22). Level I includes the contents of the submental and the submandibular triangles. Levels II, III, and IV include the lymph nodes adjacent to the internal jugular vein and the lymph nodes contained within the fibroadipose tissue located medial to the sternocleidomastoid muscle. This area is arbitrarily divided into three equal parts, level II being the highest and level IV the lowest level. Level V includes the contents of the posterior cervical triangle.

**Pharmacokinetics.** Blood samples were obtained from the arm opposite the injection site for determination of activity up to 44 h p.i. Aliquots of blood samples were measured for $^{99m}$Tc activity in a well counter (1282 Compugamma; LKB Wallac, Turku, Finland), compared to an aliquot retained from the conjugate preparation, and corrected for decay. Blood activity was expressed as the %ID/kg. HPLC analysis of the serum samples up to 21 h p.i. revealed that more than 95% of the radioactivity was bound to the mAb. The pharmacokinetics was analyzed modeling a time versus radioactivity curve for each infusion. A MW/Pharm program (MediWare; Groningen, the Netherlands) was used for nonlinear Bayesian estimation of pharmacokinetic parameters. One-, two-, and three-compartment models were fit to the data. The peeling algorithm was used to estimate initial parameters. A Bayesian least-square method was used to estimate the final parameters: the initial ($t_{1/2}$ $\alpha$) and final half-lives ($t_{1/2}$ $\beta$).

**Biodistribution.** In all patients biopsies of the primary tumor and several other tissues were taken from the surgical specimen. In these patients blood and bone marrow aspiration and biopsy were taken under general anesthesia just before surgery. All biopsies were weighed and the amount of $^{99m}$Tc was measured in a well counter. The effect of self-absorption by volume effects was corrected by comparison of the sample with a set of reference samples, prepared by diluting an equal amount of the standard in different volumes of saline. All data were corrected for decay, scattered radiation, and converted to %ID/kg tissue. Tumor:nontumor ratios were calculated using matched uptake values of one patient. If in a patient several biopsies of one kind of tissue were taken, the mean uptake in this tissue was calculated and used for further analysis. After counting, all biopsies were assessed histopathologically to determine the presence or absence of HNSCC.

**Statistical Analysis.** Student's $t$ test for paired and unpaired data was used to test the statistical significance of the differences between the uptake of U36 IgG in different tissues and at different mAb doses.

**RESULTS**

No adverse reactions were observed which could be related to the injection of the antibody, and no significant changes were noted in blood and urine analysis. Patient 3 (receiving 2.2 mg U36 IgG) and patient 6 (receiving 13.0 mg U36 IgG) developed a HAMA response.

All 10 primary tumors were visualized by RIS. Whole-body images up to 21 h p.i. showed blood pool activity with the visualization of liver, lungs, heart, spleen, kidneys, and nose. Uptake of activity was also seen in the scrotal area and sometimes in the intestine and gallbladder at 16 and 21 h p.i. Besides this, slight accumulation was observed in the mouth. For representative images, see Fig. 1.

All patients underwent unilateral neck dissection. A total of 50 levels were histopathologically examined. All 10 operated sides contained metastases of HNSCC in a total of 14 levels. One patient refused MRI examination because of claustropho-
Fig. 1 A, planar anterior image of the head and neck of a patient with a carcinoma of the right inferior alveolar processus 16 h after injection of $^{99m}$Tc-labeled U36 IgG. Increased uptake is seen in the mouth on the right side. Only one spot can be distinguished (arrow). B, a sagittal SPECT slice of the same patient shows two separate spots, representing the primary tumor in the lower jaw (arrow) and the subdigastric lymph node metastasis (arrowhead). C, anterior, and D, posterior whole-body images 16 h after injection. Note the clear visualization of the primary tumor (arrow).

The findings on palpation, CT, MRI, RIS, and histopathology are summarized in Tables 2 and 3. Findings which were evaluable were analyzed per neck side (Table 2) and per lymph node level (Table 3) and correlated with histopathological results. RIS detected lymph node metastases in 7 of 14 (sensitivity, 50%) levels and in 6 of 10 sides (sensitivity, 60%). In addition to the planar images, SPECT images provided extra information (Fig. 1). There were no false-positive RIS findings. Seven levels and four sides were scored false negative. Interpretation of RIS was correct in 43 of 50 levels (accuracy, 86%) and in 6 of 10 sides (accuracy, 60%). Accuracy of palpation, CT, and MRI was per level 86%, 83%, and 87%, respectively, and per side 60%, 80%, and 67%, respectively.

Of the 14 tumor-containing levels, 7 were missed by RIS. The paraffin slides of the missed metastatic lymph nodes were reexamined histopathologically. The missed lymph node metastases ($n = 14$) proved to be all small lymph nodes (less than 9 mm in diameter, $n = 5$), lymph nodes containing small tumor deposits (micrometastasis, $n = 5$), or lymph node metastases containing a large proportion of necrosis ($n = 3$), keratin ($n = 1$), or fibrin ($n = 1$) deposits.

Pharmacokinetics. The time versus radioactivity curve of U36 IgG best fit a two-compartment model. The mean initial ($t_{1/2 \alpha}$) and final half-lives ($t_{1/2 \beta}$) for U36 IgG at the 2-mg dose...
accumulates selectively and to a high level in tumor tissue. Should be efficiently targeted. Thus, a mAb is required which should recognize an antigen located at the outer surface of tumor selection of mAbs to be used for this purpose. Ideally, the mAb should be preferentially of the IgG class rather than of the IgM class since these latter is restricted in the penetration into tumor nodules due to its large size. The antigen should be expressed by all tumors and by all tumor cells within these tumors, but not by normal tissues, especially not those which are easily accessible for mAbs. Besides favorable immunohistochemical characteristics, the mAb has to show tumor selectivity in tumor-targeting studies in patients. Data presented in this article and a previous article (9) indicate that mAb U36 may be better suited for targeting head and neck cancer than any other mAb described until now.

Most of the mAbs directed against HNSCC do not fulfill the aforementioned criteria (23–38). Some of these mAbs are very poorly characterized (23, 29, 35), some are directed against antigens which are predominantly localized intracellularly (31, 33, 36), some show a very heterogenous reactivity pattern on tumors (34, 36, 38), while others are of the IgM class (25–27, 30, 36–38). Besides this, some mAbs meet limitations for tumor targeting due to their reactivity with normal tissues, like mAb G10 with blood group antigen (24), mAbs A9 and SQM1 with normal blood vessels (25, 26), mAb LuCa2 with thyroid, kidney, colon, and other epithelia (28), and mAb B10 with connective tissue, striated muscle, and nerve fiber (31). Cross-reactivity with normal tissues also limits the use of so-called pan-carcinoma mAbs, mAbs reactive with several types of tumors (11, 15, 39–43). Among these are pan-carcinoma mAbs which in clinical RIS studies showed extensive accumulation at nontumor sites, thus hampering their applicability for therapy (15, 44). Better results have been obtained with mAbs selectively reactive with HNSCC. Baum et al. (43) recently reported on a RIS study in which 99mTc-labeled mAb 174H.64 showed good tumor targeting. Unfortunately, tissue uptake levels were not determined for this mAb. The internal localization of the 174H.64 antigen especially may be a serious problem for RIT. The most extensively studied mAb for targeting of head and neck cancer is mAb E48. Our group already showed the good tumor-targeting capacity of mAb E48 using RIS and biopsy measurements (13). A limitation of this mAb is its heterogeneous reactivity with about 30% of the head and neck tumors, a reason to develop new mAbs more specifically may be a serious problem for RIT. The most extensively studied mAb for targeting of head and neck cancer is mAb E48. Our group already showed the good tumor-targeting capacity of mAb E48 using RIS and biopsy measurements (13). A limitation of this mAb is its heterogeneous reactivity with about 30% of the head and neck tumors, a reason to develop new mAbs more homogeneously reactive with HNSCC (9). Another reason to search for improved mAbs at that time was the high uptake of mAb E48 in the adrenals as observed in RIS studies using low doses (1–2 mg) of mAb. Later studies indicated, however, that adrenal uptake was strongly diminished when a higher mAb dose was administered to HNSCC patients (11, 12, 15, 39–43). Among these are pan-carcinoma mAbs which in clinical RIS studies showed extensive accumulation at nontumor sites, thus hampering their applicability for therapy (15, 44). Better results have been obtained with mAbs selectively reactive with HNSCC. Baum et al. (43) recently reported on a RIS study in which 99mTc-labeled mAb 174H.64 showed good tumor targeting. Unfortunately, tissue uptake levels were not determined for this mAb. The internal localization of the 174H.64 antigen especially may be a serious problem for RIT. The most extensively studied mAb for targeting of head and neck cancer is mAb E48. Our group already showed the good tumor-targeting capacity of mAb E48 using RIS and biopsy measurements (13). A limitation of this mAb is its heterogeneous reactivity with about 30% of the head and neck tumors, a reason to develop new mAbs more homogeneously reactive with HNSCC (9). Another reason to search for improved mAbs at that time was the high uptake of mAb E48 in the adrenals as observed in RIS studies using low doses (1–2 mg) of mAb. Later studies indicated, however, that adrenal uptake was strongly diminished when a higher mAb E48

were 6.7 ± 2.6 h and 50.9 ± 22.7 h, respectively. An increase in the mAb dose did not influence the clearance from the blood: $t_{1/2\alpha}$ and $t_{1/2\beta}$ were for 99mTc-labeled U36 IgG at 10 mg 5.1 ± 1.1 h and 50.3 ± 9.4 h at and mlg 4.5 ± 2.3 h and 50.8 ± 24.2 h, respectively.

**Biodistribution.** The uptake values and tumor:nontumor ratios of 99mTc-labeled U36 IgG in biopsies from the surgical specimen as well as in blood and bone marrow are shown in Table 4. Activity uptake was the highest in tumor tissue: 20.4 ± 12.4 (mean ± SD); range, 8.0–43.0%ID/kg. High 99mTc activity was also seen in normal mucosa (10.1 ± 6.4%ID/kg), but was significantly ($P < 0.05$) lower than in tumor tissue. Tumor-negative lymph nodes contained significantly ($P < 0.01$) more 99mTc activity than tumor-negative lymph nodes: 4.2 ± 1.7 and 1.7 ± 0.4%ID/kg, respectively, with a mean ratio of 3.2. Low activity was seen in bone marrow biopsies: 3.0 ± 1.1%ID/kg. Bone marrow aspiration showed a mean 99mTc activity of 7.2 ± 1.4%ID/kg, while the activity in blood was slightly higher (7.8 ± 1.7%ID/kg, mean bone marrow:blood ratio: 0.9 ± 0.0). The activity in the bone marrow aspirate was mainly located in the plasma (supernatant). The mean plasma activity of the bone marrow aspirate and blood plasma were similar (12.3 ± 3.0 and 12.5 ± 2.6%ID/kg, respectively; mean bone marrow plasma: blood plasma ratio 1.0 ± 0.1). Mean tumor:nontumor ratios varied between 2.3 ± 1.5 for mucosa and 14.3 ± 9.9 for tumor-negative lymph nodes (Table 4). For muscle, fat, blood, and bone marrow aspirate, these ratios were 13.0 ± 8.7, 12.9 ± 5.0, 2.8 ± 1.9, and 3.0 ± 2.0, respectively.

**Mean uptake of 99mTc-labeled U36 was also calculated at low (2 mg) and high (12–52 mg) mAb dose separately. A higher mAb dose did not influence the tumor uptake of 99mTc-labeled U36 IgG at 2 mg 24.8 ± 15.4%ID/kg and at 12–52 mg 16.1 ± 5.6%ID/kg ($P > 0.2$). Also the uptake in other tissues and the tumor:nontumor ratios were not influenced by an increase of the mAb dose.**

**DISCUSSION**

Radioimmunotherapy is a challenging option for adjuvant therapy of HNSCC, especially because of the intrinsic sensitivities of HNSCC for irradiation (5). For effective adjuvant radioimmunotherapy, it can be anticipated that all tumor deposits, including malignant cell clusters and small tumor nodules, should be efficiently targeted. Thus, a mAb is required which accumulates selectively and to a high level in tumor tissue.

On the basis of this assumption, criteria can be set for the selection of mAbs to be used for this purpose. Ideally, the mAb should recognize an antigen located at the outer surface of tumor cells. The mAb should be preferentially of the IgG class rather than of the IgM class since these latter is restricted in the penetration into tumor nodules due to its large size. The antigen should be expressed by all tumors and by all tumor cells within these tumors, but not by normal tissues, especially not those which are easily accessible for mAbs. Besides favorable immunohistochemical characteristics, the mAb has to show tumor selectivity in tumor-targeting studies in patients. Data presented in this article and a previous article (9) indicate that mAb U36 may be better suited for targeting head and neck cancer than any other mAb described until now.

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Because of these drawbacks only a few mAbs have been administered to HNSCC patients (11, 12, 15, 39–43). Among these are pan-carcinoma mAbs which in clinical RIS studies showed extensive accumulation at nontumor sites, thus hampering their applicability for therapy (15, 44). Better results have been obtained with mAbs selectively reactive with HNSCC. Baum et al. (43) recently reported on a RIS study in which 99mTc-labeled mAb 174H.64 showed good tumor targeting. Unfortunately, tissue uptake levels were not determined for this mAb. The internal localization of the 174H.64 antigen especially may be a serious problem for RIT. The most extensively studied mAb for targeting of head and neck cancer is mAb E48. Our group already showed the good tumor-targeting capacity of mAb E48 using RIS and biopsy measurements (13). A limitation of this mAb is its heterogeneous reactivity with about 30% of the head and neck tumors, a reason to develop new mAbs more homogeneously reactive with HNSCC (9). Another reason to search for improved mAbs at that time was the high uptake of mAb E48 in the adrenals as observed in RIS studies using low doses (1–2 mg) of mAb. Later studies indicated, however, that adrenal uptake was strongly diminished when a higher mAb E48
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Table 4  Uptake and tumor:nontumor ratios of 99mTc-labeled U36 IgG in biopsies obtained from the surgical specimens 44 h p.i.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Mean ± SD (Uptake (%ID/kg))</th>
<th>Mean ± SD (Tumor:nontumor ratio)</th>
<th>Range</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td>20.4 ± 12.4</td>
<td>8.0-43.0</td>
<td>2.3 ± 1.5</td>
<td>0.8-5.7</td>
</tr>
<tr>
<td>Positive lymph node</td>
<td>10.1 ± 6.4</td>
<td>4.6-28.0</td>
<td>7.6 ± 4.6</td>
<td>1.8-17.3</td>
</tr>
<tr>
<td>Negative lymph node</td>
<td>4.2 ± 1.7</td>
<td>1.7-6.4</td>
<td>14.3 ± 9.9</td>
<td>4.9-33.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.7 ± 0.4</td>
<td>1.0-2.2</td>
<td>13.0 ± 8.7</td>
<td>4.0-32.8</td>
</tr>
<tr>
<td>Fat</td>
<td>1.4 ± 0.6</td>
<td>0.6-2.3</td>
<td>12.9 ± 5.0</td>
<td>5.6-23.3</td>
</tr>
<tr>
<td>Submandibular gland</td>
<td>3.9 ± 2.7</td>
<td>1.2-9.7</td>
<td>8.8 ± 5.8</td>
<td>2.2-16.4</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>7.1 ± 4.0</td>
<td>2.1-11.7</td>
<td>9.0 ± 8.6</td>
<td>0.7-20.8</td>
</tr>
<tr>
<td>Vein</td>
<td>3.7 ± 1.5</td>
<td>1.8-7.4</td>
<td>6.0 ± 4.0</td>
<td>2.2-14.7</td>
</tr>
<tr>
<td>Cartilage</td>
<td>2.5 ± 1.7</td>
<td>0.9-5.6</td>
<td>14.2 ± 17.6</td>
<td>3.6-49.3</td>
</tr>
<tr>
<td>Bone marrow biopsy</td>
<td>3.0 ± 1.1</td>
<td>1.6-5.0</td>
<td>6.4 ± 3.5</td>
<td>2.8-13.8</td>
</tr>
<tr>
<td>Total bone marrow aspiration</td>
<td>7.2 ± 1.4</td>
<td>5.4-10.5</td>
<td>3.0 ± 2.0</td>
<td>1.1-7.5</td>
</tr>
<tr>
<td>Supernatant bone marrow aspiration</td>
<td>12.3 ± 3.0</td>
<td>9.7-20.1</td>
<td>1.7 ± 1.1</td>
<td>0.6-4.1</td>
</tr>
<tr>
<td>Sediment bone marrow aspiration</td>
<td>2.5 ± 0.5</td>
<td>2.0-3.8</td>
<td>8.8 ± 5.8</td>
<td>2.4-20.1</td>
</tr>
<tr>
<td>Blood</td>
<td>7.8 ± 1.7</td>
<td>5.9-11.9</td>
<td>2.8 ± 1.9</td>
<td>1.0-7.0</td>
</tr>
<tr>
<td>Plasma</td>
<td>12.5 ± 2.6</td>
<td>9.9-19.1</td>
<td>1.7 ± 1.1</td>
<td>0.6-4.0</td>
</tr>
</tbody>
</table>

a Number of patients from whom biopsies were obtained.

b Primary tumor:lymph node metastases ratio.

dose of 12–52 mg was used (13). These data indicate that mAb E48 may be suitable for RIT although not for all HNSCC patients.

As a result of our renewed effort to develop mAbs suitable for in vivo tumor targeting, mAb U36 was developed. As stated above, mAb U36 may have superior qualities for tumor targeting. mAb U36 is of the IgG1 subclass and shows high-affinity binding with a M̄ 200,000 surface antigen expressed by normal squamous epithelia and squamous cell carcinoma. Up to 96% of the 196 stained HNSCC tumors showed strong reactivity (>50% of the tumor cells stained). mAb U36 shows a more extensive reactivity with HNSCC than other mAbs for which such quantitative values are available (25, 45, 46). For example mAb E48, evaluated on the same panel of tumors as mAb U36, showed a similar strong reactivity in 70% of the tumors. Most other mAbs used for clinical targeting of other tumor types show a more heterogeneous reactivity pattern (47–49).

The present study demonstrates that mAb U36 indeed harbors potential for selective tumor targeting in HNSCC patients. All primary tumors were visualized with 99mTc-labeled U36 IgG on planar anterior views of the head and neck. For the detection of lymph node metastases, RIS with mTc-labeled U36 IgG was as reliable as the other diagnostic modalities: palpation, CT, and MRI. However, in this small group of patients RIS could not detect lymph node metastases which were also detected by the other diagnostic modalities. Thus, RIS did not improve the diagnosis of the neck. Analysis of the missed lymph nodes revealed the presence of only a small amount of viable tumor cells. Furthermore, mAb U36 was bound to these cells as was demonstrated immunohistochemically by using peroxidase-labeled rabbit anti-mouse IgG (data not shown). Therefore, tumor-containing lymph nodes were missed by RIS probably due to the limited amount of antigen accessible for the mAb and maybe due to the limited sensitivity of a gamma camera. Other disadvantages of RIS limiting routine application are the complexity, high costs, and lack of anatomical structures for orientation as compared to CT and MRI. Therefore, the use of radiolabeled mAb U36 for diagnostic purposes seems to be limited, as was previously also found for mAb E48 (11, 12).

Another, more challenging application of radiolabeled mAbs is RIT. In RIS, 99mTc-labeled U36 IgG showed selective tumor targeting. Tumor uptake of 99mTc-labeled U36 IgG, as assessed in biopsies from the surgical specimens, was high as compared to the uptake of other mAbs in other tumors (13). Uptake in tumor tissue and tumor-positive lymph nodes was significantly higher than in normal mucosa and tumor-negative lymph nodes, respectively. Activity uptake in tumors was also higher than in other normal tissues, as is reflected in the mean tumor:nontumor ratios ranging from 6.4 for bone marrow biopsy to 13.0 for muscle. The activity in bone marrow aspirate was almost the same as in blood and was mainly based on plasma activity as became apparent upon centrifugation. This is particularly important since, in general, bone marrow is the dose-limiting organ in RIT. mAb U36 did not show uptake in the adrenal glands as mAb E48 did at a low mAb dose of 1–2 mg. Whether mAb U36 is better suited for RIT than mAb E48 with respect to tumor uptake levels and tumor:nontumor ratios is not clear as yet. Tumor uptake levels and tumor:nontumor ratios of radiolabeled U36 IgG (44 h p.i.) are similar to those previously found for 1–2 mg radiolabeled E48 IgG [e.g., mean tumor uptake mAb U36: 24.8 ± 15.4%ID/kg (n = 5), mean tumor uptake mAb E48: 25.8 ± 17.9%ID/kg (n = 15)]. However, tumor uptake levels of mAb E48 IgG increased 2-fold when the mAb dose was increased to 10–50 mg, a phenomenon not observed with mAb U36 IgG. This discrepancy cannot be explained by alterations in mAb pharmacokinetics since these pharmacokinetics remained the same for both upon mAb dose escalation (13). Because mAb U36 and mAb E48 uptake levels were measured in different and small groups of patients, these differences should be interpreted with caution. Moreover, the variance of tumor load in biopsies may influence this comparison as well. To make direct comparisons possible, we recently started a study in which U36 IgG and E48 IgG labeled with different radionuclides are coinjected in HNSCC patients. On
the basis of the results from this study, one of these mAbs will be selected for adjuvant clinical RIT trials.

Besides this, we will also focus on the use of unconjugated mAbs for adjuvant therapy of head and neck cancer. The feasibility of this approach was recently demonstrated in a randomized trial in which murine mAb 17–1A (IgG2a) was used in adjuvant therapy of resected Duke’s C colorectal carcinoma (50). After a mean follow-up of 5 years antibody treatment reduced the overall death rate by 30%. Treatment with mAb 17–1A prolonged distant relapse while no effect was seen on local relapse. Antitumor effects with mAb 17–1A were also observed in tumor-bearing nude mice, and in these studies evidence was found for a mechanism of ADCC (51).

Recently, we finished the chimerization of the murine mAb E48 (IgG1) to a mouse/human IgG1. This cmAb E48 was shown to be highly capable for mediation of ADCC in vitro. We also constructed a cmAb U36 IgGi which is currently being evaluated for its ADCC capacity. Unconjugated cmAb U36 and cmAb E48, either alone or in combination, will be evaluated in an adjuvant therapy trial in a group of patients at high risk for developing local recurrences and distant metastases.

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