Radioimmunotherapy of human head and neck squamous cell carcinoma xenografts with $^{131}$I-labelled monoclonal antibody E48 IgG

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Summary  Monoclonal antibody (MAB) E48 reacts with a 22kD antigen exclusively expressed in squamous and transitional epithelia and their neoplastic counterparts. Radiolabelled with $^{99}$Tc, MAB E48 is capable of targeting metastatic and recurrent disease in patients with head and neck cancer. In this study, the capacity of $^{131}$I-labelled MAB E48 to eradicat xenografts of human squamous cell carcinoma of the head and neck (HNSCC) in nude mice was examined. Experimental groups received a single i.v. bolus injection of 400$\mu$Ci MAB E48 IgG (number of mice (n) = 6, number of tumours (t) = 9) or 800$\mu$Ci MAB E48 IgG (n = 5, t = 7), whereas control groups received either diluent (n = 3, t = 5), unlabelled MAB E48 IgG (n = 4, t = 5) or 800$\mu$Ci $^{131}$I-labelled isotype-matched control MAB (n = 6, t = 9). A 4.1-fold increase in the median tumour volume doubling time and regression of two out of ten tumours (20%) was observed in mice treated with 400$\mu$Ci. In mice treated with 800$\mu$Ci, two out of seven tumours (29%) showed complete remission without regrowth during follow-up (>3 months). Median tumour volume doubling time in the remaining five tumours was increased 7.8-fold. No antitumour effects were observed in mice injected with diluent, unlabelled MAB E48 or $^{131}$I-labelled control MAB. In the same xenograft model, chemotherapy with doxorubicin, 5-fluorouracil, cisplatin, bleomycin, methotrexate or 2',2'-difluorodeoxyctydine yielded a less profound effect on tumour volume doubling time. Increases in tumour volume doubling time with these chemotherapeutic agents were 4, 2.2, 2.1, 1.7, 0, and 2.6 respectively. Moreover, no cures were observed with any of these chemotherapeutic agents. From the tissue distribution of 800$\mu$Ci MAB E48, the absorbed cumulative radiation doses of tumour and various organs were calculated using the trapezoid integration method for the area under the curve. To tumour xenografts, 12,170cGy was delivered, blood received 2,984cGy, whereas in every other tissue the accumulated dose was less than 6% of the dose delivered to tumour. These data, describing the first radiolabelled MAB with therapeutic efficacy against HNSCC, suggest radioimmunotherapy with MAB E48 to be a potential therapeutic modality for the treatment of head and neck cancer.

Despite an increase in the locoregional control of head and neck squamous cell carcinoma (HNSCC), due to improved surgery and radiotherapy, current therapy regimens have failed as yet to increase the 5-year survival rate of patients with head and neck cancer (Choksi et al., 1988; Cognetti et al., 1988). Whereas fewer patients tend to die because of uncontrolled locoregional disease, there is an increase in the number of distant metastases and second primary tumours. The role of chemotherapy in these patients is limited. Responses are often observed but enhancement of survival is not obtained. These facts justify the search for more specific and effective therapeutic methods. Since HNSCC have an intrinsic sensitivity for radiation (Wessels et al., 1989a), we focus on the use of monoclonal antibodies labelled with radioisotopes for radioimmunotherapy (RIT). RIT of human tumours in experimental and/or clinical settings has already been described for various types of cancer, including colorectal carcinomas (Esteban et al., 1990; Lee et al., 1990; Schlom et al., 1991; Blumenthal et al., 1991), malignant gliomas (Colapinto et al., 1990; Lee et al., 1985; Lee et al., 1988b; Williams et al., 1990), ovarian carcinoma (Stewart et al., 1989; Ward et al., 1988), small cell lung carcinoma (Smith et al., 1990; Smith et al., 1991; Beaumier et al., 1991), mammary carcinoma (Senekowitsch et al., 1989), renal cell carcinoma (Wessels et al., 1989b; Chioi et al., 1988), and cutaneous T cell lymphoma (Rosen et al., 1987; Mulshine et al., 1991). Thus far however, no HNSCC-specific MABs have been available to test the efficacy of RIT to eradicate HNSCC xenografts in an experimental setting. Therefore, we have developed a panel of MABs, among which MAB E48, raised against HNSCC (Quak et al., 1990a; Quak et al., 1990b; Quak et al., 1992). MAB E48 recognises a 20–22kD antigen, on normal tissues selectively expressed on stratified squamous epithelia and transitional epithelium of the bladder. On tumours, reactivity is restricted to malignancies arising from these tissues. The MAB E48 defined antigen is involved in the structural organisation of squamous epithelia, possibly at the level of cell-cell adhesion (Schrijvers et al., 1991). Biodistribution and imaging studies with tracer amounts of $^{131}$I-labelled E48 IgG and F(ab')2 fragments already demonstrated the capacity of MAB E48 for specific delivery of radioisotope to HNSCC xenografts (Quak et al., 1989; Gerretsen et al., 1991). Recent data from an ongoing phase I/II trial with intravenously administered $^{99}$Tc labelled MAB E48 F(ab')2, and IgG in patients with HNSCC indicate that MAB E48 is highly capable of detecting metastatic and recurrent disease (van Dongen et al., 1992). In the present study we demonstrate a dose dependent growth delay, regression and complete remission of established tumours by injection of single doses $^{131}$I-labelled MAB E48 in nude mice bearing HNSCC xenografts. In this experimental model, the efficacy of RIT was compared to the antitumour activity of a number of clinically used or experimental chemotherapeutic agents (Braakhuis et al., 1991).

Material and methods

Monoclonal antibodies

Monoclonal antibody E48 was raised against a SCC of the larynx (Quak et al., 1990). Affinity-purified MAB E48 IgG and control MAB Myoscin® IgG, raised against myosin, were obtained from Centocor Europe Inc., Leiden, The Netherlands. Both are murine MABs of the IgG1 subclass.

Xenografts

Female nude mice (NMRI, 25–32g Harlan Olac CPB, Zeist, The Netherlands) were 8–10 weeks old at the time of the experiments. The head and neck SCC xenograft line HNX-
HN was established by subcutaneous implantation of tumour fragments measuring 3 x 3 x 1 mm, in the lateral thoracic region on both sides of nude mice. Thereafter, the xenograft line was maintained by serial transplantation (Braakhuis et al., 1989). The tumour from which the HNX-HN line originates was a T4N2M0 squamous cell carcinoma of the base of the tongue from a 54-year-old female patient. As determined by indirect immunoperoxidase staining, the expression pattern of the MAb E48 defined antigen in the HNX-HN line was comparable to the pattern of the majority of human HNSCC tumours (Gerretsen et al., 1991). During experiments, food and water, with potassium iodide added to the water to prevent thyroid accumulation of 131I, were available ad libitum.

Iodine-131 labelling

Iodination of MAb IgG was performed essentially as described earlier (Gerretsen et al., 1991). MAb IgG in phosphate buffered saline, pH 7.4, and 131I were mixed in a ratio of approximately 1 mg MAb:10 mCi 131I in a vial coated with Iodogen (Pierce). After 10 min incubation at room temperature, a sample was removed to determine the amount of incorporated iodine by TCA precipitation. To the reaction mixture, 1 ml AG1-X-8 resin (BioRad) in PBS, 1% BSA was added to absorb free iodine. To remove the resin and to stabilize the product, the reaction mixture was filtered through a 0.22 μm filter.

Quality control of 131I-labelled MAb E48 IgG

After labelling, the immunoreactive fraction was at least 85% in all experiments. Incorporated 131I was higher than 90% in all experiments as determined by TCA precipitation. Specific activity of the radioimmunoconjugate varied between 5 and 10 mCi mg⁻¹.

MAb IgG in vitro binding assay

The binding characteristics of radiolabelled MAb E48 IgG were analysed in an immunoreactivity assay, essentially as described earlier (Gerretsen et al., 1991). In short, cells of the squamous cell carcinoma cell line UM-SCC 22B, a gift from Dr T.E. Carey (Ann Arbor, MI, USA), were fixed in 1% paraformaldehyde and five serial dilutions, ranging from 5 x 10⁴ cells/tube to 3.1 x 10⁵ cells/tube, were made with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS). To the tubes, 10,000 cpm of the labelled MAb IgG was added and incubated 120 min at room temperature. To a duplicate of the last sample, excess unlabelled MAb IgG was added to determine non-specific binding. Cells were spun down and radioactivity in the pellet and supernatant was determined in a gamma counter and the percentage bound and free radiolabelled MAB was calculated (LKW-Wallac 1218 CompuGamma). Data were graphically analysed in a modified Lineweaver–Burke plot and the immunoreactive fraction was determined by linear extrapolation to conditions representing infinite antigen excess.

Toxicity studies

The maximum tolerated dose of each of the chemotherapeutic agents, corresponding to a weight loss between 5 and 15%, was determined as described earlier (Braakhuis et al., 1989; Braakhuis et al., 1991). In the same way, the maximum tolerated dose of 131I-labelled MAb E48 was determined. Nude mice without xenografts were injected with diluent (PBS) or with increasing doses of 131I-labelled MAb E48 and was determined. Nude mice without xenografts were injected with diluent (PBS) or with increasing doses of 131I-labelled MAb E48. Total body dose was determined in a dose calibrator. Total accumulated radiation dose was calculated as described in the section 'Dosimetry calculations'. The weight of the mice was measured daily over a period of 4 weeks, at which timepoint no radioactivity could be detected.

In vivo biodistribution studies

Biodistribution studies with tracer dose 131I-labelled MAb E48 IgG in nude mice bearing HNX-HN xenografts have previously been described (Gerretsen et al., 1991; Quak et al., 1989). To compare the biodistribution of a therapeutic dose with a tracer dose, 28 mice bearing xenografts of a size comparable with the tracer dose study were injected i.v. with 800 μCi 131I-labelled MAb E48 IgG. At the time of injection the estimated xenograft volume was 323 ± 244 mm³ as determined by measuring the tumour in three dimensions with calipers ((L x W x H)/2) (versus 352 ± 207.5 in the tracer dose study). Mice were killed, and dissected 2, 5 and 8 h and 1, 3, 7, 10, 14, 21, 28 and 35 days after i.v. injection. Organs were immediately removed, placed in 5 ml plastic tubes and weighed. Samples were taken from blood, urine, tumour, liver, spleen, kidney, heart, stomach, ileum, colon, bladder, sternum, muscle, lung, skin and tongue. After weighing, radioactivity in all organs and tumours was counted in a gamma counter. The antibody uptake in the tumour and other tissues was calculated as the percentage of the injected dose per gram of tissue (% ID·g⁻¹).

Radioimmunotherapy

Mice bearing 1 or 2 xenografts with a volume between 50 and 250 mm³ were given a single intravenous injection of 400 (n = 6, t = 9) or 800 (n = 5, t = 7) μCi 131I-labelled MAb E48 IgG. Control groups were given diluent (n = 3, t = 5), unlabelled MAb E48 IgG (n = 4, t = 5; amount equivalent to 800 μCi 131I-labelled MAb E48) or 800 μCi 131I-labelled control MAb IgG (n = 6, t = 9). Groups were randomised for initial tumour volume, for diluent 90 ± 68 (mean ± s.e.m.), for unlabelled MAb E48 96 ± 26, for 800 μCi 131I-labelled control MAb 122 ± 106, for 400 μCi 131I-labelled MAb E48 93 ± 40, and for 800 μCi 131I-labelled MAb E48 118 ± 32. At day 1, 2, and 3, cages were cleaned to remove excreted radioactivity and thereafter this was done weekly. During the first week mice were weighed daily and tumour size was determined daily as described earlier. After the first week weight and tumour size were determined twice a week. At the same timepoints whole body dose was measured in a dose calibrator. Mice were sacrificed when tumour size exceeded 1000 mm³.

Dosimetry calculations

Dosimetry calculations were performed using the data of the biodistribution of 800 μCi 131I-labelled MAb E48 IgG. The absorbed cumulative radiation dose for tumour and various organs was calculated using the trapezoid integration method for the area under the curve (Badger et al., 1986). Due to the therapeutic effect of the dose, tumours at day 35 had almost completely regressed and were thus not included in the calculations. The final segment of the area under the curve was calculated based on the biological half-life: dose of last segment = dose previous segment (day 21-day 28) x 0.693 (t in previous segment)⁻¹. cGy were further calculated by multiplying the μCi·g⁻¹ by the g·C·d·Gy·(μCi·h·g⁻¹)⁻¹ factor published by the Medical Internal Radiation Dose committee for 131I of 0.4313 (Dilman, 1969).

Chemotherapy

All drugs were injected at the maximum tolerated dose level (5–15% weight loss). Schedules were based on results of experiments performed in previous studies (Braakhuis et al., 1989; Braakhuis et al., 1991). Mean number of mice and experiments in all schedules was 5 and 7, respectively. The volume of the tumours at the time of injection ranged between 50–150 mm³. The following doses and injection schedules were applied: Doxorubicin (DOX, Farmitalia, Bournonville–Pharma, Almere, The Netherlands) at 8 mg·kg⁻¹ i.v. at day 0 and 8; dFdC (2',2'-difluorodeoxycytidin, Gemcitabine, LY 188011, Lilly...
Tumour bearing mice were treated with RIT or chemotherapy when most tumours reached a volume of at least 50 mm³ (range 50–250 mm³). Tumours smaller than 50 mm³ at the time of injection were not included in the determination of the tumour volume doubling time because of inaccuracy in measuring these tumours. Tumour growth was expressed as the tumour volume at each timepoint relative to the tumour volume at day 0. Efficacy of RIT as well as chemotherapy was expressed by means of the tumour growth delay factor (GDF), defined as (TD₅₀-TDₓ)/TDₓ (TD₅₀ = median tumour volume doubling time of treated mice, TDₓ = median tumour volume doubling time of control mice). Prolonged survival (survival defined as the time period between day 0 and the time point of sacrifice, being when tumour size exceeded 1000 mm³) was determined by comparing experimental groups with treatment groups using the Mann–Whitney U-test.

Evaluation of therapeutic efficacy

Figure 2 Biokdistribution of 800µCi 131I-labelled MAB E48 in nude mice bearing HNX-HNx xenografts. Mice were bled, killed and dissected 2, 5 and 8h and 1, 3, 7, 10, 14, 21, 28 and 35 days after injection and the percentage injected dose per gram (%IDg⁻¹) was calculated and plotted versus time. Tumour (○), blood (■) and lung (▲) are shown.

and reached a maximum of 19.4 ± 2.9% IDg⁻¹ after 3 days. Activity is retained in the tumour up to 8.1 ± 3.5% IDg⁻¹ at day 28. No specific accumulation is observed in any other tissue.

Dosimetry calculations

The absorbed cumulative radiation dose for tumour and various organs is shown in Figure 3. Based on the area under the curve of the biokdistribution data of 800µCi 131I-labelled MAB E48 IgG the absorbed radiation dose to tumours in the group receiving 800µCi was 12,170 cGy, whereas blood received only 2,984 cGy. Other tissues received the following dose: lung: 662 cGy; kidney: 607 cGy; spleen: 581 cGy; bladder: 571 cGy; heart: 543 cGy; colon: 424 cGy; ileum: 405 cGy; sternum: 403 cGy; muscle: 276 cGy; stomach: 251 cGy.

Evaluation of therapeutic efficacy

Tumour growth expressed as the tumour volume at each timepoint relative to the tumour volume at day 0 for control and treatment groups is shown in Figure 4. Tumours in the groups receiving unlabelled MAB E48 IgG (Figure 4a), 800µCi 131I-labelled control MAB IgG (Figure 4b) and diluent (Figure 4c) all showed exponential growth. Median tumour volume doubling times in the group receiving diluent or unlabelled MAB E48 IgG was 5.5 days. Median tumour volume doubling time in the group receiving 800µCi 131I-labelled control MAB showed a minimal, statistically insignificant increase. All tumours in the group receiving 400µCi 131I-labelled MAB E48 IgG (Figure 4d) showed delay of growth with a median tumour volume doubling time of 22.6 days, while two out of nine tumours showed regression. All tumours in the group receiving 800µCi 131I-labelled MAB E48 IgG (Figure 4e) showed regression, with a median tumour volume doubling time of 43 days. Moreover, in this group, two out of seven tumours showed complete remission without regrowth during follow-up (>3 months). After sacrificing these animals, no evidence of tumour could be detected at the site of implantation. The tumour growth delay factor calculated for the 400 and 800µCi groups was 3.1 and 6.8, respectively. Weight loss in experimental groups did not exceed 15% at any timepoint. When compared with the tumour growth delay factor of chemotherapeutic agents like Adriamycin (3.0), fluorouracil (1.2), cisplatin (1.1), bleomycin (0.7), methotrexate (0) and 2,2'-difluorodeoxyxytidine (1.6), established in the same HNX-HNx xenograft, RIT shows a very high therapeutic efficacy (Figure 5). No cures were observed with chemotherapeutic agents. Pro-
The image contains scientific text that describes the effects of radiolabelled MAb E48, a therapeutic strategy in experimental radioimmunotherapy. The text discusses the use of labelled MAb E48 in experiments with different MAb concentrations and dosimetry calculations. The text also mentions the use of various chemotherapeutic agents, such as doxorubicin (Adr), 5-fluorouracil (5-FU), cisplatin (CP), bleomycin (Bleo), and methotrexate (MTX), to treat xenografts and evaluate their efficacy in combination with radiolabelled MAb E48.

**Discussion**

Therapeutic efficacy of radiolabelled MAb in nude mice model has been described for several tumour types. Although clinical radioimmunoscintigraphy studies for the detection of HNSCC have been reported with 111In-labelled anti-epidermal growth factor receptor (Soo et al., 1987) and with 111In-labelled anti-carinoembryonic antigen (Kairemo & Hopsu, 1990; Kairemo & Hopsu, 1990), no reports are available on therapy experiments of HNSCC xenografts with radiolabelled MAb. Here we present the first data on RIT of HNSCC. As a first approach to assess the potential of radiolabelled MAb E48 in eradicating HNSCC xenografts, therapy experiments, consisting of single bolus injection of two different doses, were designed in a straightforward manner. Dosimetry calculations were based on the biodistribution of a therapeutic dose, since continued tumour growth in biodistribution experiments with tracer dose may well result in underestimation of the radiation dose up to 35–52% (Lee et al., 1990; Badger et al., 1986). In our studies, no differences in biodistribution between tracer and therapeutic doses were observed. In tracer dose studies however, no data were available during the first 12h and after day 7, whereas in this study data were obtained from 2h p.i. up to 35 days, allowing more accurate dosimetry calculations. A remarkable good retention of MAb E48 was observed with 8.1 ± 3.5%IDg−1 at day 28 after injection.

Although numerous reports with 111In-labelled MAb IgG or F(ab')2 have been described with anti-tumour effects, only few studies achieve complete remissions after single bolus injections. Wessels et al. reported complete remissions of renal cell carcinoma xenografts after single bolus injection of 600μCi 131I-labelled MAb IgG (Wessels et al., 1989), whereas Sharkey et al. observed no regrowth of colon carcinoma xenografts after a single injection of 1mCi MAb IgG (Sharkey et al., 1987). Lee et al. obtained apparent cures of mice with intracranial glioma xenografts after a single injection of 1.25mCi MAb IgG (Lee et al., 1988). Complete ablation of highly radiation sensitive neuroblastoma xenografts was achieved with a single injection of 1mCi IgG by the group of Cheung et al. (Cheung et al., 1986). Buchegger et al. completely eradicated xenografts of colon carcinomas with single injections of 2,200–2,800μCi, but instead of IgG, pooled F(ab')2 fragments of three different anti-CEA MAbS were used (Buchegger et al., 1989). Other successful studies applied fractionated protocols (Smith et al., 1991; Senekowitsch et al., 1989; Schlolm et al., 1990; Buchegger et...
In our study, single injections of 400 or 800 μCi 131I-labelled E48 MAB IgG showed pronounced anti-tumour effects, resulting in complete remissions of two out of seven tumours in the group receiving 800 μCi 131I-labelled MAB E48 IgG. No remnant tumour could be detected when mice were sacrificed after 3 months follow-up. These cures might very well be due to the intrinsic sensitivity of head and neck tumours for radiation (Wessels et al., 1989a). In addition, the accumulated dose, 12,170 cGy, in tumour tissue as a result of a single bolus injection 800 μCi 131I labelled MAB E48 was very high, reflecting the excellent targeting and retention characteristics of MAB E48 in this experimental model.

Therapeutic efficacy of RIT has been found to be inversely correlated with tumour size (Scholm et al., 1991; Lee et al., 1988; Sharkey et al., 1987). Accordingly, RIT has the potential to be the most useful in adjuvant therapy when minimal disease is present (Sharkey et al., 1987; Langmuir & Sutherland, 1988). In the case of head and neck cancer this would apply to patients with stage III and IV disease. In these patients local recurrences occur in 50–60%, while 15–25% develop distant metastases after surgery and/or radiotherapy (Choksi et al., 1988). Unfortunately, no relevant metastatic model for HNSCC is available. In our study, the correlation between tumour size and therapeutic effect could not be demonstrated in the selected tumours.

In several studies, an increase in therapeutic efficacy combined with a decrease in toxicity has been observed when total dose was given in multiple fractions (Buchegger et al., 1990; Buchegger et al., 1989; Colapinto et al., 1990; Smith et al., 1991; Scholm et al., 1990). Therefore, the efficacy of RIT with MAB E48 with respect to growing, established HNX-HN xenografts will be further investigated comparing single injection regimen to multiple injection regimens. Furthermore, since 131I is not the isotope of choice in clinical applications because of the low percentage therapeutic β-emission (32%) and the high percentage damaging γ-emission (66%), and because of the rapid dehalogenation of 131I-labelled conjugates, we have developed a MAB E48 radioimmunoconjugate labelled with 99mTc, an isotope with a high percentage β-emission (90%) and low percentage γ-emission (8%). MABs labelled with this isotope have already been described in tumour localisation and tumour therapy studies (Beaumier et al., 1991; Goldrozen et al., 1991). MAB E48 labelled with this isotope will be tested in the HNX-HN xenograft model.

Thusfar, clinical results with chemotherapy have been disappointing with respect to the effect on 5-year survival of patients, despite the number of trials over the past 10 years (Choksi et al. 1988; Snow, 1991). In our HNX-HN xenograft model, a number of conventional drugs, known to produce remissions in patients with head and neck cancer, and one experimental chemotherapeutic agent have been evaluated (Braakhuis et al., 1991) unpublished data. In the dose schedules described, none of the chemotherapeutic agents caused tumour growth delay factors higher than those obtained with either 800 μCi or 400 μCi 131I-labelled MAB E48 IgG. Furthermore, no cures were observed with these chemotherapeutic agents.

One of the limitations of the nude mouse xenograft model for RIT studies with radiolabelled MABs is the absence of antigen expression in normal tissues. The presence of the MAB E48 defined antigen in normal tissues in the clinical setting would obviously influence the pharmacokinetics and biodistribution of radiolabelled MAB E48. In clinical radioimmunoscintigraphy studies using 99mTc-labelled MAB E48 (Fab'), fragment we observed uptake of radioactivity in normal oral mucosa and adrenal glands (van Dongen et al., 1992). Uptake in these tissues seems to be diminished when using whole IgG. Most clinical trials with radiolabelled MAB for diagnosis or therapy of solid neoplasms have reported MAB uptake in large tumours in the range of 0.001–0.01%IDg⁻¹ (Goldenberg, 1981; Epenotos & Kosmas, 1989). Preliminary data on the localisation of 99mTc-labelled MAB E48 IgG indicate accumulation of the conjugate in tumours of 0.5–4.0 cm diameter up to a mean %IDg⁻¹ of 0.03 at 4 h (range: 0.0143–0.0823, number of patients = 7). This looks very promising indeed, when taking into account the higher accumulation of MABs in small tumour loads. Chatal et al. reported on the biodistribution of 131I-labelled MAB OC125 intraperitoneally injected into patients with ovarian carcinoma, demonstrating low accumulation in large tumours (0.0014–0.0032 %IDg⁻¹) but significantly higher accumulation in small tumour nodules (0.13 ± 0.08 %IDg⁻¹) and malignant cell clusters (median 0.33 with a maximum of 4.16 %IDg⁻¹) (Chatal et al., 1989). Assuming that this size correlation also applies for head and neck tumours and assuming that patients will tolerate a dose of 100 mCi of 131I-labelled MAB E48 (or an equivalent dose of 99mTc-labelled MAB E48) (Rosen et al., 1987; Ward et al., 1988), achieving radiation doses in tumour tissue enabling elimination of minimal disease lies within reach.

Our data, showing the capacity of a single bolus injection 131I-labelled MAB E48 to eradicate HNSCC xenografts in nude mice, present the first successful RIT results for head and neck squamous cell carcinoma. Together with data from an ongoing phase I clinical trial in our hospital, showing the capacity of 99mTc-labelled MAB E48 (Fab')₂ fragment and IgG in detecting metastatic and recurrent disease, this indicates the potential of radiolabelled MAB E48 for radioimmunotherapy of patients with head and neck cancer.

This work was supported in part by Centocor Europe Inc., Leiden, The Netherlands.

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