Pergamon

Oral Oncol, Eur J Cancer, Vol. 30B, No. 2, pp. 82–87, 1994

0964–1955/94 $6.00 + 0.00

The Feasibility of Radioimmunotherapy of Head and Neck Cancer

Martijn Gerretsen, Jasper J. Quak, Ruud H. Brakenhoff, Gordon B. Snow and Guus A.M.S. van Dongen

Since the introduction of the hybridoma technology by Kohler and Milstein (*Nature* 1975, 256, 495–497), tremendous effort has been put in the realisation of Ehrlich's concept of the magic bullet, which was proposed as early as the beginning of the century. The first clinical studies for radioimmunoscintigraphy (RIS) and radioimmunotherapy (RIT) with radiolabelled antibodies were undertaken in the early 1980s. Since then, RIS has been performed on thousands of patients with various types of malignancies, like colon carcinoma, lung carcinoma, breast carcinoma, neuroblastoma, T-cell lymphoma and ovarian carcinoma. In addition, a substantial number of therapy trials with radiolabelled antibodies have been performed. The developments for head and neck squamous cell carcinoma (HNSCC) have only recently been able to catch up with these events to some extent. One of the main reasons for this slow progress has been the lack of monoclonal antibodies (Mab) with specificity for HNSCC. Although there are as yet no real tumour specific antigens known for HNSCC, which also holds true for the majority of malignancies arising from other tissues, we now have the availability of a number of Mab with high specificity for HNSCC and with a very restricted reaction pattern with normal tissues. Labelled with ^{131}I, these Mab have been shown to be highly capable to localise in HNSCC xenografts in nude mice. Based on these promising data, patient studies with one of these Mab, designated Mab E48, labelled with ^{99m}Tc, were started to evaluate the feasibility of RIS in patients with head and neck cancer. The first results of these studies indicated the capacity of ^{99m}Tc-labelled Mab E48 $F(ab')_2$ as well as IgG to detect metastatic and recurrent disease in these patients. These data justified further studies investigating the possibilities for RIT with this Mab. In preclinical experiments, the capacity of ^{131}I-labelled Mab E48 IgG to eradicate established HNSCC tumours in nude mice was shown. Following the latest developments in the field of radioimmunoconjugate chemistry and anticipating the need for more appropriate radionuclides for clinical applications, a technical protocol for the labelling of Mab with ^{186}Re was developed. Labelled with ^{186}Re, Mab E48 appears to be even better suited to eradicate established tumours than when labelled with ^{131}I. Based on these encouraging observations we are now making preparations for the first RIT studies with ^{186}Re-labelled Mab E48 in patients with head and neck cancer.
Oral Oncol, Eur J Cancer, Vol. 30B, No. 2, pp. 82–87, 1994.

INTRODUCTION

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 [1, 2]. Whereas fewer patients tend to die of uncontrolled locoregional disease, more patients are exposed to the risk of developing distant metastases and second primary tumours. This review will focus on the problem of distant metastases. The role of chemotherapy in patients with distant metastases is limited. Responses are observed but enhancement of survival is not obtained. Thus there is a need for more specific and more effective treatment of distant metastases. Radiolabelled monoclonal antibodies (Mab) may be particularly suitable for treatment of HNSCC metastases due to the intrinsic radiosensitivity of this tumour type [3].

So far, only a limited number of Mab to squamous cell carcinoma (SCC) have been described [4–11]. Most of these Mab however, show considerable cross reactivity with normal tissues. Among the few Mab reacting with SCC originating from various organs are Mab 17.13 [7], an IgM isotype antibody and less suited for *in vivo* immunolocalisation studies due to the unfavourable pharmacokinetics of these large immunoglobulins; Mab 3F8E3 [11] which is a low affinity antibody; and Mab 174H.64 [8], reacting with a cytoskeletal

Correspondence to G.A.M.S. van Dongen.
The authors are at the Department of Otolaryngology/Head and Neck Surgery, Free University Hospital, De Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands.
Received 20 Apr. 1993; accepted 21 Apr. 1993.

protein, which possibly prevents the binding of the Mab to the antigen because of the intracellular location.

We have developed a panel of Mab directed against HNSCC, amongst which is Mab E48. Mab E48 was raised against a SCC of the larynx, and shows strong and selective reactivity to squamous epithelia, like skin and oral mucosa, transitional epithelia of the bladder, and their neoplastic derivatives of various tissue sites [12]. So far tested, Mab E48 reacted with 90% of primary head and neck tumours ($n = 110$) and with the majority of the cells within these tumours. A comparable pattern was observed in 26 tumour infiltrated lymph nodes from neck dissection specimens.

Before a Mab can ultimately enter the clinic for radioimmunotherapy (RIT) studies, a number of preclinical as well as clinical criteria have to be met. First, immunohistochemical evaluation of normal as well as malignant tissues has to show the Mab to be selective for neoplastic tissue with minimal reaction on normal tissues, with special emphasis on cells of the reticuloendothelial lineage. Subsequently, the localisation characteristics of the Mab are investigated in athymic nude mice bearing human tumour xenografts. These mice are immunodeficient since they lack T-cells, thus permitting the growth of human tumour tissue when transplanted subcutaneously. When localisation in tumour tissue occurs in a satisfactory way, clinical studies will have to determine the localisation characteristics in patients, since biodistribution and pharmacokinetics of a murine antibody obviously will differ in man and mouse. Moreover, the absence of normal tissues in mice expressing the antigen recognised by the antibody under investigation is a clear disadvantage of this experimental model. Finally, the sensitivity of the tumour type for radiation will have to be investigated in the nude mouse. Only when all these criteria are met, are RIT studies in patients justified.

PRECLINICAL AND CLINICAL RIS STUDIES

Whereas a great number of radioimmunolocalisation studies in nude mice have been described for ovarian carcinoma, colorectal carcinoma, breast carcinoma, glioma, renal cell carcinoma and small cell lung carcinoma [13–15], there are only a limited number of studies evaluating the possibility of radioimmunolocalisation in nude mice bearing human squamous cell carcinoma xenografts. Wahl *et al.* [16] used the A9 Mab with moderate success to image HNSCC xenografts, obtaining tumour to blood ratios of 1.84 at day 7 after intravenous (i.v.) injection of ^{131}I-labelled Mab. Furthermore, this Mab shows reactivity with normal human endothelium, thus limiting its clinical application. In later studies it was shown that the A9 Mab is directed against the $\alpha_6\beta_4$ integrin, explaining the observed reactivity with the basement membrane of human endothelium [17]. In initial experiments evaluating the Mab E48 for radioimmunolocalization, Quak *et al.* [18] demonstrated preferential localisation of the Mab in tumour tissue, without any non-specific accumulation in non-tumour tissues. Localisation was analysed qualitative as well as quantitative, by imaging with a γ-camera and by counting activity in tissues after dissection, respectively. High percentages of the injected dose per gram (%ID/g), upto 18% at day 3, were obtained with high tumour to blood ratios, upto 7 at day 3. Images of xenografts could be obtained at day 3 and 7 without any background activity. In subsequent studies, Gerretsen *et al.* showed superior localisation characteristics of Mab E48 $F(ab')_2$ as compared with Mab E48 whole IgG. In this study, tumour to blood ratios were achieved at day 1 of 13.2 for $F(ab')_2$ and 1.2 for IgG, and reached a maximum at day 6 of 54.2 for $F(ab')_2$ and 6.4 for IgG [19]. These high tumour to blood ratios of $F(ab')_2$ allowed imaging of xenografts as soon as 24 h post injection, whereas xenografts in mice injected with IgG could not be visualised until day 3, despite the fact that IgG showed a higher maximum %ID/g than $F(ab')_2$, 14.6 at day 6 and 7.2 at day 1, respectively. An example of an image obtained in this way is shown in Fig. 1. In these studies the size of the tumours was approximately 350 mm^3. In studies using tumours with an approximate size of 70 mm^3, the maximum %ID/g reached values as high as 90% (unpublished data), confirming other studies in which the size of the tumour was shown to be an important parameter in determining the extent of immunoconjugate localisation.

In earlier studies, the use of Mab directed against the epidermal growth factor receptor and the carcinoembryonic antigen (CEA) in radioimmunoscintigraphy (RIS) of patients with verified head and neck cancer was evaluated [20, 21]. The value of these Mab in localising neck node metastases is not yet clear. In these studies, only a few patients showed regional neck node involvement, with tumours all larger than 2 cm in diameter. A limitation of these Mab may be that they are not specifically directed against HNSCC, which can result in false positive scintigrams as has been described for anti-CEA [22].

Based on the data obtained from preclinical experiments, in our department a phase I/II clinical trial, evaluating the safety and diagnostic accuracy of ^{99m}Tc-labelled Mab E48 $F(ab')_2$ for the detection of metastatic disease in patients with histologically proven HNSCC and with clinical evidence of cervical lymph node involvement, was started [23]. In this study, preoperative findings on lymph node status obtained by RIS was compared with computerised tomography (CT), magnetic resonance imaging (MRI), palpation and finally with the histopathological outcome of the neck dissection specimen. In 10 patients, all eight known tumours at the primary site were detected with RIS. Furthermore, RIS was correct in all 13 tumour involved neck sides and in 17 of 20 tumour involved lymph node levels. False negative observations comprised three levels containing tumour deposits smaller than 1 cm in diameter, two of which were not detected by any other diagnostic modality. In comparison, palpation, CT and MRI

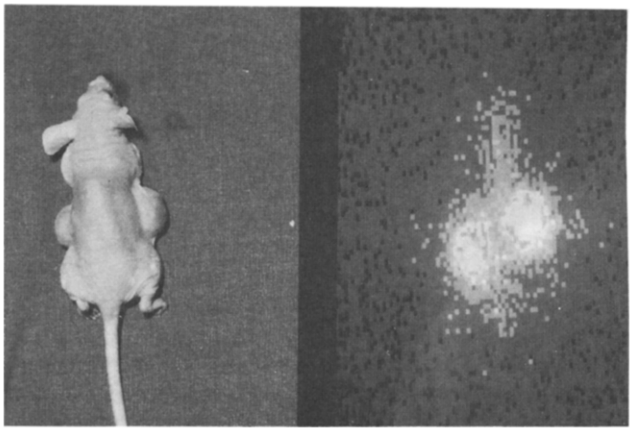

Fig. 1. On the left is shown a nude mouse bearing in the left and right flank a subcutaneously growing HNSCC xenograft. On the right images of such xenografts after injection of ^{131}I-labelled Mab E48, obtained with a γ-camera.

were correct in 13, 15 and 15 of the 20 tumour involved levels. There were two false positive observations with Mab E48 and three with palpation. No false positive detections were obtained with CT or MRI. In 2 of the patients, RIS provided clinically important information which was not provided by any other diagnostic method. In 1 patient, laryngeal carcinoma was observed at the primary site after previous radiotherapy. In another patient, bilateral instead of unilateral lymph node involvement became apparent. Figure 2(a and b) are examples of images of patients obtained after injection of ^{99m}Tc-labelled Mab E48. An unexpected finding in this study was the consistent uptake of activity in the adrenal glands. Until now, no clear explanation for this phenomenon had been found. Immunohistochemical examination of frozen sections of adrenal tissue so far has not shown any reactivity with Mab E48. Uptake in the adrenals does not seem to be related to the labelling technique used in this study, since RIS with the isotype matched (IgG1) monoclonal antibody 323A3 F(ab')$_2$ fragment, labelled with ^{99m}Tc in the same way as described for Mab E48, did not result in uptake in the adrenals. In addition to the uptake observed in the adrenal glands, uptake was observed in the nose and mouth region. The ^{99m}Tc-labelled 323A3 F(ab')$_2$ fragment, which is not reactive with normal oral mucosa, did not show any accumulation in this region. Therefore, it is thought that uptake of activity in the nose and mouth region as observed with Mab E48 F(ab')$_2$ is due to a specific antigen–antibody interaction in which the antibody has overcome the natural barriers formed by the capillary endothelium and the basement membrane of the mucosal epithelium. From following patient studies using Mab E48 IgG instead of F(ab')$_2$, preliminary data indicate that the use of IgG results in a decreased accumulation of activity in the adrenal glands as well as the nose and mouth region.

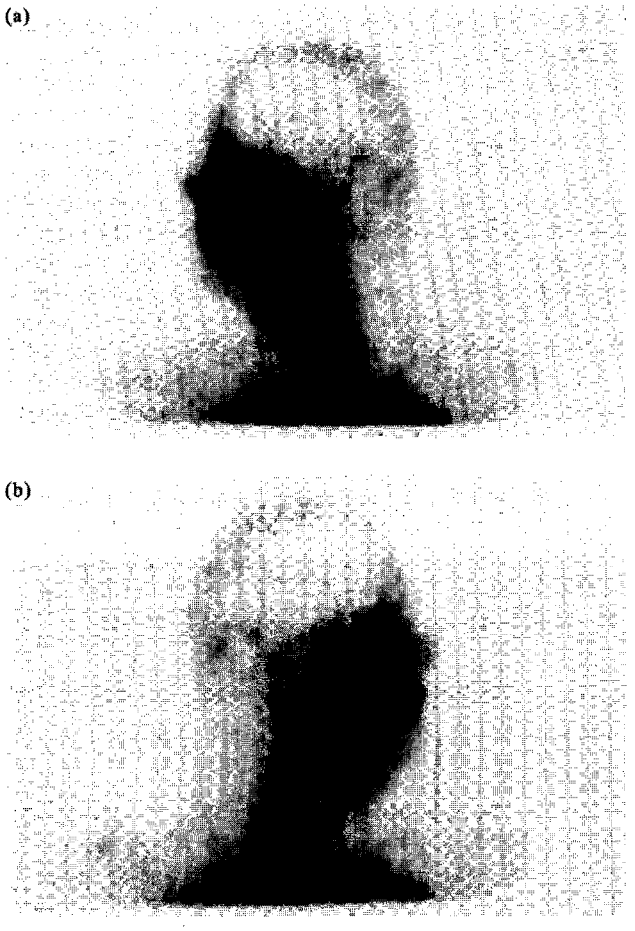

Fig. 2. Lateral images of the head and neck of a patient 21 h after injection of ^{99m}Tc-labelled Mab E48. (a) Left side, (b) right side. Note the uptake of activity in the primary tumour located on the floor of the mouth (arrow) and in the lymph node metastasis in the left side of the neck (arrowhead). (Republished from van Dongen *et al.*, 1992, with permission of the authors and publishers.)

The lack of anatomical structures obtained with RIS complicates the comparison to images obtained with CT and MRI. Each modality in its own way provides information uniquely important and complementary to each other. Further improvement of RIS might be achieved by the development of an accurate method to correlate different imaging modalities, like the fusion of images obtained by different diagnostic modalities. Another parameter limiting the present application of RIS for the detection of very small tumour deposits is not the use of Mab as a targeting vehicle, but is a direct consequence of the limited resolution of gamma cameras. In this respect, the developing field of positron emission tomography (PET), for which positron emitting radionuclides as ^{124}I or ^{18}F are coupled to Mab, seems to be very promising [24–26] and might result in a diagnostic modality with a very high resolving power, especially when incorporated into fused images. Moreover, PET provides a non-invasive method for an accurate and quantitative determination of radioisotope biodistribution, thus providing important information for dosimetry calculations and therapy scheduling.

In conclusion, RIS of HNSCC with radiolabelled Mab seems to be a feasible approach for the detection of metastatic and recurrent disease. However, when taking into account the relative substantial costs of RIS, the additional value of RIS over CT and MRI at the present does not seem high enough to consider this diagnostic modality for routine application in the clinic. More important with respect to the further perspectives for RIT, the accumulation of radioactivity in tumour tissue as proven in our clinical RIS trials does justify the development of radioimmunoconjugates for RIT of HNSCC. From this point of view, RIS can be regarded as a prelude to RIT.

PRECLINICAL RIT STUDIES

Encouraged by the findings in the RIS trials in our department, we are further pursuing the developments of radioimmunoconjugates for RIT, which of course is the ultimate goal of these studies. To this end, we started the preclinical evaluation of radiolabelled Mab E48 IgG for the eradication of established HNSCC xenografts in nude mice [27]. For these initial experiments, ^{131}I was chosen as the isotope with which to label Mab E48, since so far most clinical experience with RIT has been obtained with ^{131}I-labelled immunoconjugates and because of the ease with which Mab can be labelled with this isotope [28]. In these studies we demonstrated that a single bolus injection of ^{131}I-labelled Mab E48 IgG resulted in a dose-dependent growth delay, regression and complete remission of HNSCC xenografts. Comparison of the efficacy of RIT with the anti-tumour activity of a number of clinically used or experimental chemotherapeutic agents showed a significant higher tumour growth delay of RIT. Furthermore, no cures were observed with any of the chemotherapeutic agents, whereas in the highest dose used in

the RIT protocol, two out of seven tumours showed complete remission without regrowth during follow-up (>3 months). To our knowledge, this was the first study describing successful RIT of HNSCC in nude mice. Only one other study reports on RIT of HNSCC in nude mice, but in this study a polyclonal antibody preparation labelled with ^{90}Y was used [29]. With the dose range used in this study, severe toxicity was observed with the higher doses and no cures were observed. More important, the use of a polyclonal antibody implicates severe limitations with respect to the availability and reproducibility and excludes the possibility of humanisation or chimerisation, thus increasing the chances for the development of a human-anti-mouse-antibody (HAMA) response when using such a preparation in a multiple dose regimen in clinical trials. Formation of such HAMA no doubt will interfere with the biodistribution and pharmacokinetics of the immunoconjugate and furthermore can give rise to severe complications for patients upon repeated administration of the immunoconjugate. To prevent HAMA responses, reshaping the Mab molecule by recombinant DNA techniques will result in a mainly human Mab molecule, with only the variable antigen recognition site of murine origin, and this is a definite prerequisite for clinical RIT trials. Immunoglobulin proteins consist of two heavy and two light chains, each with a constant domain and a variable domain. These variable domains contain the antigen recognition site. The genes encoding for the human heavy and light constant domains have been cloned. By combining the murine genes encoding the variable domains of the immunoglobulin of interest with the human genes encoding the constant domains, a chimeric immunoglobulin is generated. Such a chimeric Mab, with only a limited part of murine origin, might be expected to be less immunogenic in the patient and thus decreases the chance of HAMA responses.

It is now generally accepted that ^{131}I is not the isotope of choice for clinical applications beause of the low percentage therapeutic β-emission (32%), the high percentage γ-emission (66%) and because of the rapid dehalogenation of ^{131}I-labelled conjugates. The high percentage γ-radiation, which has a long range, implicates limitations and very strict directions in the clinical use of this isotope to minimise radiation toxicity for the patient in the first place, but also for hospital personnel. One of the isotopes receiving a great deal of attention for the improvement of RIT is ^{186}Re. With a half life of 3.7 days, 9% γ-emission with ideal energy for imaging (137 KeV), 71% β-emission of 1.07 MeV and 21% β-emission of 0.94 MeV, ^{186}Re theoretically seems to be better suited for RIT than ^{131}I. ^{186}Re-labelled Mab have already been described in experimental tumour localisation and therapy studies [30, 31] as well as in phase I clinical trials [32]. Although several methods have been described for the labelling of Mab with ^{186}Re [30, 33, 34], there remained some doubt to the general applicability of these labelling techniques with respect to the stability of the conjugates and the specific activity that can be achieved with commercially available ^{186}Re [35]. Therefore, in our laboratory an efficient and reproducible technical protocol for aseptic labelling of stable [^{186}Re] Mab conjugates was developed [36]. Making use of a unique solid phase synthesis of MAG_3, the chelating agent used for ^{99m}Tc renal function measurement, conjugates with an isotope:Mab molar ratio of 7.3 can be generated, enabling preparation of ^{186}Re-conjugates for clinical purposes. In the nude mouse model ^{186}Re-labelled Mab E48 IgG in a dose range of 7.4–22.2 MBq resulted in 20–50% complete remissions of large established tumours [37]. A typical example of the anti-tumour effect of ^{186}Re is given in Fig. 3. In subsequent experiments with smaller tumours, 100% complete remissions were obtained with a single bolus injection of 22.2 MBq. In these tumours, a 6–7-fold increase in the percentage injected dose per gram of tumour was observed.

A final prerequisite before clinical RIT studies can be initiated is the chimerisation of the Mab molecule by recombinant DNA techniques to avoid or minimise the chance of HAMA responses. Although, until now in clinical RIS studies with Mab E48 no HAMA responses were observed after a single injection, after administration of higher doses of Mab or repeated injections HAMA responses may very well occur.

FUTURE PERSPECTIVES FOR RIT IN PATIENTS WITH HEAD AND NECK CANCER

So far, introduction of RIT into the clinic has not answered to the high expectations yet [38]. From the first clinical trials with tumour types as ovarian carcinoma, colon carcinoma, melanoma and hepatoma, it has become clear that the percentage injected dose taken up in large solid tumours is still an order of magnitude too low, and therapeutic ratios are still very low. At present, RIT is most successful in microscopic

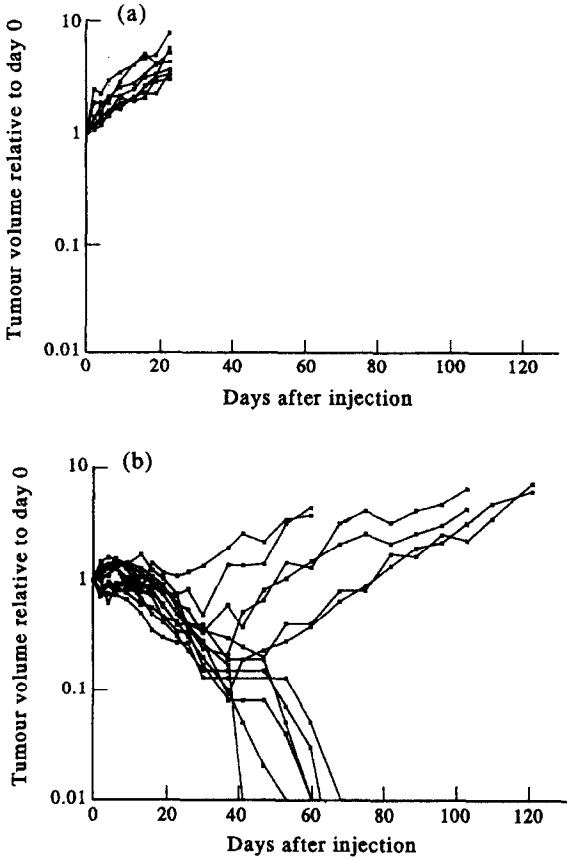

Fig. 3. Anti-tumour effect of ^{186}Re-labelled Mab E48 on the growth of HNSCC xenografts growing in nude mice, expressed as the tumour volume at any time point relative to the tumour volume at day 0. (a) Control group injected with a saline solution [number of mice (n)=4, number of tumours (t)=8]. (b) Group injected with 18.5 MBq ^{186}Re-labelled Mab E48 IgG (n=6, t=12). (Republished from Gerretsen *et al.*, 1993, with permission from the authors and publishers.)

residual disease. Most clinical trials with radiolabelled Mab for diagnosis or therapy of solid neoplasms have reported uptake in large tumours in the range of 0.001–0.01%ID/g. Preliminary data on the localisation of ^{99m}Tc-labelled Mab E48 IgG indicate accumulation of the conjugate in tumours of 0.5–4.0 cm diameter up to a mean of 0.03%ID/g at 44 h (range: 0.014–0.082, number of patients = 7). This looks very promising indeed when taking into account the higher accumulation in small tumour loads. Chatal *et al.* reported on the biodistribution of ^{111}In-labelled Mab OC125 intraperitoneally injected into patients with ovarian carcinoma, demonstrating low accumulation in large tumours (0.0014–0.0032%ID/g) but significantly higher accumulation in small tumour nodules (0.13 ± 0.08%ID/g) and malignant cell clusters (median 0.33 with a maximum 4.16%ID/g) [39]. Assuming that this size correlation also applies for head and neck tumours, which might be anticipated when reviewing biodistribution data in tumour bearing mice with large and small xenografts, and assuming that patients will tolerate a dose of 2.22 GBq/m^2 ^{131}I-labelled Mab E48 [40, 41], we previously stated that achieving radiation doses in tumour tissue enabling elimination of minimal residual disease lies within reach. As demonstrated by Breitz *et al.*, the first phase I clinical trials investigating the pharmacokinetics, toxicity and maximum tolerable dose (MTD) of a ^{186}Re-labelled Mab IgG and F(ab')$_2$ fragment in patients with colorectal, lung, ovarian, gastric or renal cancer showed that dose-limiting myelosuppression was observed at 4.44 GBq/m^2 for IgG and at 5.55 GBq/m^2 for F(ab')$_2$ in heavily pretreated patients. In patients with minimal treatment prior to entering this trial, no MTD for F(ab')$_2$ was reached at 7.4 GBq/m^2. At our department, preparations for a phase I clinical trial with ^{186}Re-labelled chimeric Mab E48 IgG in patients with metastatic (or recurrent) disease after conventional therapy are currently in progress. When this trial demonstrates that RIT is well tolerated, patients with locoregional disease who are at high risk for harbouring distant metastases will be treated with RIT in an adjuvant setting.

1. Cognetti EV, Pinnaro P, Carlini P, *et al.* Neoadjuvant chemotherapy in previously untreated patients with head and neck squamous cell cancer. *Cancer* 1988, **62**, 251–261.
2. Stell PM, Rawson NSB. Adjuvant chemotherapy in head and neck cancer. *Br J Cancer* 1990, **61**, 779–787.
3. Wessels BW, Harisiadis L, Carabell SC. Dosimetry and radiobiological efficacy of clinical radioimmunotherapy. *J Nucl Med* 1989, **30**, 827.
4. Zenner HP, Hermann IW. Monoclonal antibodies against surface antigens of laryngeal carcinoma cells. *Arch Otorhinolaryngol* 1981, **233**, 161–172.
5. Carey TE, Kimmel KA, Schwartz DR, Richter DE, Baker SR, Krause CJ. Antibodies to human squamous cell carcinoma. *Otolaryngol/Head and Neck Surg* 1983, **91**, 482–491.
6. Boeheim K, Speak JA, Frei E, Bernal SD. SQM-1 antibody defines a surface membrane antigen in squamous carcinoma of the head and neck. *Int J Cancer* 1985, **36**, 137–142.
7. Ranken R, White CF, Gottfried TG, *et al.* Reactivity of monoclonal antibody 17.13 with human squamous cell carcinoma and its application to tumor diagnosis. *Cancer Res* 1987, **47**, 5684–5690.
8. Samuel J, Noujaim AA, Willans DJ, Brzezinska GS, Haines DM, Longenecker BM. A novel marker for basal (stem) cells of mammalian stratified squamous epithelia and squamous cell carcinomas. *Cancer Res* 1989, **49**, 2465–2470.
9. Myoken Y, Moroyama T, Miyauchi S, Takada K, Namba M. Monoclonal antibodies against human oral squamous carcinoma reacting with keratin proteins. *Cancer* 1987, **60**, 2927–2937.
10. Inoue M, Nakanishi K, Sasagawa T, Tanizawa O, Inoue H, Hakura A. A novel monoclonal antibody against squamous cell carcinoma. *Jpn J Cancer* 1990, **81**, 176–182.
11. Tatake RJ, Amin KM, Maniar HS, Jambhekar NA, Srikhande SS, Gangal SG. Monoclonal antibody against human squamous-cell-carcinoma-associated antigen. *Int J Cancer* 1989, **44**, 840–845.
12. Quak JJ, Balm AJM, van Dongen GAMS, *et al.* A 22 Kd surface antigen detected by monoclonal antibody is exclusively expressed in stratified squamous and transitional epithelia. *Am J Pathol* 1990, **33**, 191–195.
13. Reilly RM. Radioimmunotherapy of malignancies. *Clin Pharm* 1991, **10**, 359–375.
14. Knox SJ, Goris ML, Wessels BW. Overview of animal studies comparing radioimmunotherapy with dose equivalent external beam irradiation. *Radiother Oncol* 1992, **23**, 111–117.
15. Wessels BW. Current status of animal radioimmunotherapy. *Cancer Res* 1990, **50** (suppl.), 970s–973s.
16. Wahl RL, Kimmel KA, Beierwaltes WH, Carey TE. Radioimmunodiagnosis of human derived squamous cell carcinoma. *Hybridoma* 1987, **60**, 111–119.
17. Van Waes C, Kozarsky KF, Warren AB, *et al.* The A9 antigen associated with aggressive human squamous carcinoma is structurally and functionally similar to the newly defined integrin $\alpha^6\beta_4$. *Cancer Res* 1991, **51**, 2395–2402.
18. Quak JJ, Balm AJM, Brakkee JGP, *et al.* Localization and imaging of radiolabeled monoclonal antibody against squamous-cell carcinoma of the head and neck in tumor bearing nude mice. *Int J Cancer* 1989, **44**, 534–538.
19. Gerretsen M, Quak JJ, Suh JS, *et al.* Superior localization and imaging of radiolabelled monoclonal antibody E48 F(ab')$_2$ fragment in xenografts of human squamous cell carcinoma of the head and neck and of the vulva as compared to monoclonal antibody E48 IgG. *Br J Cancer* 1991, **63**, 37–44.
20. Soo KC, Ward M, Roberts KR, *et al.* Radioimmunoscintigraphy of squamous carcinomas of the head and neck. *Head Neck Surg* 1987 **9**, 349–352.
21. Kairemo KJA, Hopsu EVM. Imaging of pharyngeal and laryngeal carcinomas with Indium-111-labeled monoclonal anti-CEA antibodies. *Laryngoscope* 1990, **100**, 1077–1082.
22. Kairemo KJA, Hopsu EVM. Imaging of tumours in the parotid region with indium-111-labelled monoclonal antibody reacting with carcinoembryonic antigen. *Acta Oncol* 1990, **29**, 539–543.
23. Van Dongen GAMS, Leverstein H, Roos JC, *et al.* Radioimmunoscintigraphy of head and neck cancer using ^{99m}Tc-labelled monoclonal antibody E48 F(ab')$_2$. *Cancer Res* 1992, **52**, 2569–2574.
24. Wilson CB, Snook DE, Dhokia B, *et al.* Quantitative measurement of monoclonal antibody distribution and blood flow using positron emission tomography and 124 Iodine in patients with breast cancer. *Int J Cancer* 1991, **47**, 344–347.
25. Garg PK, Garg S, Bigner DD, Zalutsky MR. Localization of Fluorine-18-labeled Mel-14 monoclonal antibody F(ab')$_2$ fragment in a subcutaneous xenograft model. *Cancer Res* 1992, **52**, 5054–5060.
26. Larson SM, Pentlow KS, Volkow ND, *et al.* PET scanning of Iodine-124-3F9 as an approach to tumor dosimetry during treatment planning for radioimmunotherapy in a child with neuroblastoma. *J Nucl Med* 1992, **33**, 2020–2023.
27. Gerretsen M, Schrijvers AHGJ, van Walsum M, *et al.* Radioimmunotherapy of human head and neck squamous cell carcinoma xenografts with ^{131}I-labelled monoclonal antibody E48 IgG. *Br J Cancer* 1992, **66**, 496–502.
28. Haisma HJ, Hilgers J, Zurawski VR. Iodination of monoclonal antibodies for diagnosis and therapy using a convenient one vial method. *J Nucl Med* 1986, **27**, 1890–1895.
29. Shikani AH, Richtsmeier WJ, Klein JL, Kopher KA. Radiolabeled antibody therapy for squamous cell carcinoma of the head and neck. *Arch Otolaryngol/Head Neck Surg* 1992, **118**, 521–525.
30. Goldrosen MH, Biddle WC, Pancook J, *et al.* Biodistribution, pharmacokinetics and imaging studies with ^{186}Re-labeled NR-LU-10 whole antibody in LS174T colonic tumor-bearing mice. *Cancer Res* 1990, **50**, 7973–7978.
31. Beaumier PL, Venkatesan P, Vanderheyden J-L, *et al.* ^{186}Re radioimmunotherapy of small cell lung carcinoma xenografts in nude mice. *Cancer Res* 1991, **51**, 676–681.
32. Breitz HB, Weiden PL, Vanderheyden J-L, *et al.* Clinical experience with rhenium-186-labeled monoclonal antibodies for

radioimmunotherapy: results of phase I trials. *J Nucl Med* 1992, **33**, 1099–1109.
33. Najafi A, Alauddin MM, Sosa A, *et al.* The evaluation of ^{186}Re-labeled antibodies using N2S4 chelate *in vitro* and *in vivo* using tumour-bearing nude mice. *Int J Radiat Appl Instrum Part B* 1992, **19**, 205–212.
34. John E, Thakur ML, DeFulvio J, McDevitt ML, Damjanov I. Rhenium-186-labeled monoclonal antibodies for radioimmunotherapy: preparation and evaluation. *J Nucl Med* 1993, **34**, 260–267.
35. Goldenberg DM, Griffiths GL. Radioimmunotherapy: arming the missiles. *J Nucl Med* 1992, **33**, 1110–1112.
36. Visser GWM, Gerretsen M, Herscheid JDM, Snow GB, van Dongen GAMS. Labelling of monoclonal antibodies with ^{186}Re using the MAG_3 chelate for radioimmunotherapy of cancer: a technical protocol. *J Nucl Med* (in press).
37. Gerretsen M, Visser GWM, van Walsum M, Meijer CJLM, Snow GB, van Dongen GAMS. ^{186}Re-labelled monoclonal antibody E48 IgG mediated therapy of human head and neck squamous cell carcinoma xenografts. *Cancer Res* (in press).
38. Britton KE, Buraggi GL, Bares R, *et al.* A brief guide to the practice of radioimmunoscintigraphy and radioimmunotherapy in cancer. Report of the European Association of Nuclear Task Group on the clinical utility of radiolabeled antibodies. *Int J Bio Markers* 1989, **4**, 106–118.
39. Chatal J-F, Saccavini J-C, Thedrez P, *et al.* Biodistribution of Indium-111-labeled OC125 monoclonal antibody intraperitoneally injected into patients operated on for ovarian carcinomas. *Cancer Res* 1989, **49**, 3087–3094.
40. Ward B, Mather S, Shepherd J, *et al.* The treatment of intraperitoneal malignant disease with monoclonal antibody guided ^{131}I therapy. *Br J Cancer* 1988, **58**, 658–662.
41. Rosen ST, Zimmer AM, Goldman-Leikin R, *et al.* Radioimmunodetection and radioimmunotherapy of cutaneous T cell lymphomas using an ^{131}I-labeled monoclonal antibody: an Illinois Cancer Council Study. *J Clin Oncol* 1987, **5**, 562–573.