Initial Experience with High-Dose Radioimmunotherapy of Metastatic Medullary Thyroid Cancer Using ^{131}I-MN-14 F(ab)$_2$ Anti–Carcinoembryonic Antigen MAb and AHSCR

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This phase I study was initiated to determine the toxicity and therapeutic potential of high-dose ^{131}I-MN-14 F(ab)$_2$ anti–carcinoembryonic antigen monoclonal antibody (MAb) combined with autologous hematopoietic stem cell rescue (AHSCR) in patients with rapidly progressing metastatic medullary thyroid cancer. **Methods:** Twelve patients were entered into the study. Dose escalation was based on prescribed radiation doses to critical organs (i.e., kidney, lung, and liver). Starting doses were 900 cGy to the kidney and no more than 1200 cGy to the lung and liver, with dose increments of 300 cGy until the maximum tolerable dose is determined. Tumor targeting was assessed by external scintigraphy, toxicity was assessed according to the common toxicity criteria of the National Cancer Institute, and therapy responses were assessed by CT, serum carcinoembryonic antigen, and calcitonin. **Results:** One patient received 9.95 GBq ^{131}I-MN-14 F(ab)$_2$, for an initial dose of 656 cGy to critical organs, 8 received 900 cGy (8.69–17.98 GBq), and 3 received 1200 cGy (15.17–17.69 GBq). The MAb scans of all patients showed positive findings. Autologous hematopoietic stem cells were given to all patients 1–2 wk after therapy, when the total body radiation exposure was less than 5.2×10^{-7} C/kg/h. Dose-limiting toxicity, defined as grade 3 or 4 nonhematologic toxicity, was not seen in the patient who received the 656-cGy dose, and only 1 of the 8 patients treated at the 900-cGy dose level had grade 3 toxicity, which was gastrointestinal and reversible. No dose-limiting toxicity was seen in the 3 patients treated at the 1200-cGy dose level. Except for the instance of grade 3 gastrointestinal toxicity, nonhematologic toxicity was relatively mild, with only grade 1 or 2 toxicity observed in 9 patients. No renal toxicity was seen. Of the 12 patients, 1 had partial remission for 1 y, another had a minor response for 3 mo, and 10 had stabilization of disease lasting between 1 and 16 months. **Conclusion:** The results show the safety of administering high myeloablative doses of ^{131}I-MN-14 F(ab)$_2$ with AHSCR in patients with metastatic medullary thyroid cancer. The antitumor responses in patients with aggressive, rapidly progressing disease are encouraging and warrant further research to optimize the effectiveness of this new treatment.

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Few therapies are available for unresectable or metastatic medullary thyroid cancer (*1,2*). Chemotherapy has had limited success, and external beam radiation may control only local disease (*2,3*). Our group recently used a third modality, namely radioimmunotherapy with radiolabeled monoclonal antibodies (MAbs) against carcinoembryonic antigen, for the treatment of this disease. The rationale for this approach lies in the strong expression of carcinoembryonic antigen (*4–6*), in addition to calcitonin, by medullary thyroid cancer and our prior excellent results for targeting metastatic medullary thyroid cancer sites with radiolabeled anticarcinoembryonic antigen MAbs (*7,8*). The results of pilot radioimmunotherapy trials using nonmyeloablative doses of ^{131}I-NP-4 and MN-14 anticarcinoembryonic antigen MAbs and their fragments have been reported (*7*). Although encouraging antitumor effects were observed in these trials, the responses seen were limited because the patients had advanced disease and the radiation absorbed at most tumor sites was moderate—usually less than 3000 cGy. Nevertheless, the results suggested that medullary thyroid cancer might be a suitable target for radioimmunotherapy if higher doses of radioactivity can be given to these patients without excessive organ toxicity. Because the administration of substantially higher doses of radioactivity is immediately hampered by severe, life-threatening myelotoxicity and potential bone marrow ablation (*9*), the most logical approach was to use autologous hematopoietic stem cell rescue (AHSCR) in combination with high-dose therapy (*9*). Also important was selection of the ideal candidate from the murine anticarcinoembryonic antigen MAbs then available. Of the NP-4 and MN-14 MAbs and their bivalent fragments, MN-14 F(ab)$_2$ appeared to be optimal for radioimmuno-

therapy of medullary thyroid cancer. The bivalent fragments, similar to intact MN-14, had a 10-fold higher affinity (1×10^9 L/mol) than the first-generation NP-4 MAbs (*10*). Moreover, an imaging sensitivity of 99% has been shown with ^{131}I-MN-14 F(ab)$_2$ in patients with established medullary thyroid cancer (*8*). In addition to these favorable characteristics, ^{131}I-MN-14 F(ab)$_2$ was shown to be less immunogenic than ^{131}I-MN-14 immunoglobulin G (IgG) and to have higher tumor-to-marrow, -lung, and -liver radiation absorbed dose ratios, albeit similar tumor-to-kidney ratios (*11*). ^{131}I-MN-14 F(ab)$_2$ appeared, therefore, to be the most suitable agent to test as potential therapy for medullary thyroid cancer, both at nonmyeloablative and myeloablative doses. We report the results of a phase I study of high-dose ^{131}I-MN-14 F(ab)$_2$ combined with AHSCR in patients with rapidly progressing metastatic medullary thyroid cancer.

MATERIALS AND METHODS

Antibody Preparation and Radioiodination

MN-14 (Immu-14; Immunomedics, Inc., Morris Plains, NJ) was purified by protein-A and ion-exchange chromatography (Q-Sepharose; Pharmacia, Piscataway, NJ), and the final purity was tested by immunoelectrophoresis, polyacrylamide gel electrophoresis using reducing and nonreducing conditions, and size-exclusion high-performance liquid chromatography. The F(ab)$_2$ of MN-14 was prepared by papain digestion. Undigested IgG was removed with protein A, followed by repeated ultrafiltration (Amicon Corp., Beverly, MA). The final product was tested for sterility, pyrogen content, and general safety according to recommended methods (*12*). Radioiodination with Na^{131}I (New England Nuclear–DuPont, North Billerica, MA) was by the chloramine-T or iodogen method (*13*). The specific activity was between 444 and 666 MBq/mg. Binding of the radioiodinated MN-14 F(ab)$_2$ to a carcinoembryonic antigen immunoadsorbent was more than 80%, and by size exclusion high-performance liquid chromatography (GF-250; DuPont, Wilmington, DE), 70%–95% of the activity was native-size F(ab)$_2$, with between 5% and 15% Fab fragments, less than 7% aggregation, and less than 2% unbound iodine.

Patient Selection and Registration

Patients with histologically proven medullary thyroid cancer, both familial and sporadic, were studied. Patients had to have evidence of distant metastases (liver, lung, or bone) or unresectable gross primary or locoregional disease with clear evidence of disease progression either by conventional radiologic methods (CT, MRI, or bone scanning) or by tumor markers (calcitonin or carcinoembryonic antigen). Table 1 lists patient characteristics including age, sex, medullary thyroid cancer form (sporadic or familial), prior therapy, the periods elapsed from primary surgery and from the last therapy (surgery, radiation, or chemotherapy) to the MAb therapy, and calcitonin and carcinoembryonic antigen levels at the time of MAb treatment.

Twelve patients were studied (3 women, 9 men; age range, 20–64 y). Nine had sporadic and 3 had familial medullary thyroid cancer. All but 2 had undergone total thyroidectomy. Nine had undergone, in addition, a unilateral radical or modified radical neck dissection, and 2 had undergone bilateral dissection. Seven had received cervical (with or without upper mediastinal) radiation, delivering doses ranging from 10 to 60.5 Gy, 1 had received external beam radiation (30 Gy) to his right scapula, and another had received external beam radiation (30 Gy) to the right femur. Two had received ^{131}I therapy (5.55 and 5.88 GBq) intended to eradicate any residual thyroid tissue, and 1 had received prior low-dose radioimmunotherapy with 5.85 GBq ^{131}I-MN-14 F(ab)$_2$ 1 y before the current therapy. Four had received prior chemotherapy, 2 with doxorubicin, dacarbazine, cyclophosphamide, and vincristine; 1 with paclitaxel; and 1 with melphalan, vinblastine, doxorubicin, 5-fluorouracil cyclophosphamide, and mitoxantrone. The median time between primary surgery and MAb therapy was 2.15 y (range, 9 mo to 16 y), and the median time between the last therapy, including any surgical intervention, and MAb therapy was 1 y (range, 1.5 mo to 15.7 y). The patients' carcinoembryonic antigen and calcitonin values at the time of study entry ranged from 24 to 6723 ng/mL (estimated median, 214 ng/mL) and from 89 to 601,000 pg/mL (estimated median, 36,134 pg/mL), respectively.

To be eligible for this trial, patients had to be at least 4 wk beyond any major surgery, radiation, or chemotherapy and must have recovered from any prior treatment-induced toxicity. The patients had a performance status of at least 70 on the Karnofsky scale (Eastern Cooperative Oncology Group score, 0–2) and a minimal life expectancy of 3 mo; no severe anorexia, nausea, or vomiting; normal hepatic and renal function; a white blood cell count of at least 3000/mm^3 or a granulocyte count of at least 1500/mm^3, and a platelet count of at least 100,000. Patients were excluded from treatment if they were pregnant or had received extensive irradiation to more than 25% of their red marrow. All patients were mentally responsible and signed an informed consent form. The therapy trial was conducted under an investigational new drug application (BB-IND-5673) from the Food and Drug Administration, and the protocol was approved by the institutional review board of each participating institution.

Radiologic correlative studies, such as CT, MRI, and bone scanning, were performed within 4 wk before antibody imaging, with follow-up studies performed at a minimum of 1 and 3 mo. If tumor regression or disease stabilization was seen at 3 mo, additional CT scans were obtained at 6 and 12 mo, or later, when indicated. Circulating calcitonin was measured, whenever possible using the same laboratory, within 1–2 wk before treatment and in 1- to 2-mo intervals for 1 y or more. Carcinoembryonic antigen was determined using a human antimouse antibody (HAMA)–resistant assay (Dianon Systems, Stratford, CT) for all patients within 1 wk of treatment and, whenever possible, at monthly intervals for 1 y or more thereafter.

Trial Design and Antibody Infusions

Before receiving therapy, all patients underwent harvesting of autologous hematopoietic stem cells. In the first 4 patients and the tenth patient, autologous bone marrow was harvested; in subsequent patients, peripheral blood stem cells were harvested. Autologous bone marrow or peripheral blood stem cells were reinfused in all patients once the total body radiation exposure was less than 5.2×10^{-7} C/kg/h, according to the practice of Press et al. (*9*).

Dose escalation was based on prescribed radiation dose estimates to the critical organs (kidneys, liver, and lung), as determined by a diagnostic–dosimetric study performed 1 wk before the therapy infusion. The diagnostic–dosimetric infusion consisted of 0.3 GBq ^{131}I-MN-14 F(ab)$_2$ labeled to a particular amount of MAb protein, depending on the plasma carcinoembryonic antigen level. On the basis of our prior experience, a certain minimal MAb protein dose was chosen to neutralize any adverse effect of

TABLE 1
Patient Characteristics

Patient			Medullary thyroid		Time from primary surgery to MAb	Time from last therapy to MAb	Carcinoembryonic	Calcitonin
No.	Age (y)	Sex	cancer form	Prior therapy	treatment	treatment	antigen (ng/mL)	(pg/mL)
1	53	M	Sporadic	Total thyroidectomy with right modified neck and upper mediastinal dissection, left modified neck dissection	9 mo	5 mo	2415	103,240
2	34	M	Sporadic	Total thyroidectomy with right radical neck dissection, cervical radiation (60.5 Gy), chemotherapy with doxorubicin, dacarbazine, cyclophosphamide, and vincristine (4 cycles)	6.5 y	13 mo	238	601,000
3	44	M	Sporadic	Total thyroidectomy with right radical neck and mediastinal dissection, cervical and mediastinal radiation (10 Gy), right upper femur radiation (30 Gy)	1 y	11 mo	24	22,750
4	66	M	Sporadic	Total thyroidectomy with right radical neck dissection, cervical radiation (55.8 Gy)	4.5 y	4.25 y	6723	34,567
5	64	M	Sporadic	Cervical and mediastinal radiation (40 Gy), total thyroidectomy, chemotherapy with paclitaxel (3 cycles)	1.5 y	1.0 y	190	29,500
6	20	F	Familial	Right thyroid lobectomy with right modified neck and mediastinal dissection, left thyroid lobectomy	10 mo	8 mo	136	8,085
7	31	F	Familial	Total thyroidectomy with right neck dissection, ^{131}I therapy (5.88 GBq), low-dose radioimmunotherapy with 5.85 GBq ^{131}I-MN-14 $F(ab)_2$	1.5 y	1.0 y	750	37,702
8	51	M	Sporadic	Right thyroid lobectomy with right radical neck dissection, cervical radiation (60 Gy), ^{131}I therapy (5.55 GBq)	16 y	15.7 y	3800	326,270
9	45	F	Familial	Total thyroidectomy, bilateral modified radical neck dissection, cervical and mediastinal radiation (56 Gy), mediastinal dissection	13.2 y	10.7 y	500	75,136
10	51	M	Sporadic	Total thyroidectomy and left functional neck dissection, oral anticancer drug therapy	2.8 y	1.5 mo	54	3,638
11	35	M	Sporadic	Total thyroidectomy with right neck and mediastinal dissection, cervical radiation (54 Gy), chemotherapy with cyclophosphamide, doxorubicin, vincristine, and dacarbazine (5 cycles)	1.5 y	2 mo	55	89
12	54	M	Sporadic	Left thyroidectomy with limited left neck dissection, radiation to right scapula (30 Gy), chemotherapy with melphalan (14 cycles), melphalan and vinblastine (5 cycles), doxorubicin (10 cycles), 5-fluorouracil, cyclophosphamide, mitoxantrone (7 cycles)	15 y	3 y	180	86,000

circulating plasma carcinoembryonic antigen on the radiolabeled MAb clearance and tumor targeting by minimizing the level of MAb complexation with plasma carcinoembryonic antigen (i.e., to ≤20%) (*11*). Hence, approximately 1 mg protein was given to patients with a plasma carcinoembryonic antigen level of ≤25 ng/mL; 10 mg, to patients with a level of 25–250 ng/mL; and 20 mg, to patients with a level of >250–2000 ng/mL. In patients with a level of >2000 ng/mL, 1 mg protein was given for every 100 ng/mL of plasma carcinoembryonic antigen (e.g., in a patient with a level of 5000 ng/mL, at least 50 mg protein were given). The MAb protein doses given in the therapy infusion were those needed to obtain the prescribed dose of radioactivity when labeling at a specific activity of 444–666 MBq/mg but were chosen to be at least as high as those given in the diagnostic study. With this protein dose schedule, the amount of radiolabeled MAb complexation with plasma carcinoembryonic antigen was small, both during the diagnostic infusion and during the therapy infusion. Moreover, few differences in the MAb blood and total body clearance rates were seen between the diagnostic and therapy studies in the same patient or between patients.

Table 2 lists the therapeutic infusions of ^{131}I-MN-14 F(ab)$_2$ in the 12 patients entered into the study, including both the critical organ radiation absorbed dose level and radioactivity amounts. In accord with recommendations by the Food and Drug Administration, the starting dose was set at 900 cGy for the kidneys and did not exceed 1200 cGy for the lung and liver. The radiation dose was then escalated in 300-cGy increments until the maximum tolerated dose had been determined. The first patient to whom we planned to administer the 900-cGy dose to the kidneys was actually treated at a dose of only 656 cGy, because the calculated dose at the 900-cGy level to the kidney exceeded the maximum radioactivity (9.25 GBq ± 20% of ^{131}I) that our affiliated hospital then allowed. Therefore, this patient received only 995 GBq instead of the 13.65 GBq that he could have received without this limitation, which was later removed. A cohort of 3 additional patients was then treated at the 900-cGy dose level to the kidneys (not exceeding 1200 cGy to the lungs and liver). Because the radiation absorbed dose to the kidneys was intentionally set to be lower than that to the lungs or liver, and because the kidneys usually had the highest radiation absorbed dose estimate on a cGy/MBq basis, the radiation dose to the kidneys ultimately determined the amount of radioactivity to be administered in 11 of 12 patients. The only patient in whom the lung dose (1200 cGy, as set for the first cohort of patients) determined the amount of radioactivity administered was 1 (patient 2) with diffuse bilateral pulmonary metastases. In this patient, the lung dose in centigrays was substantially higher than the liver and kidney doses; in fact, the dose to the kidneys was only 592 cGy.

TABLE 2
Antibody Infusions of ^{131}I-MN-14 F(ab)$_2$

Patient no.	Injection no.	Weeks	Antibody infusions GBq	Antibody infusions mg	Dose level entered (cGy)
1	1	0	0.30	28.8	
	2	1	9.95	23.3	900/1200*
2	1	0	0.30	10.0	
	2	1	8.69	17.1	900/1200
3	1	0	0.30	0.6	
	2	1	8.60	15.7	900/1200
4	1	0	0.30	40.7	
	2	1	15.39	40.9	900/1200
5	1	0	0.30	10.0	
	2	1	16.98	25.4	900/1200
6	1	0	0.30	10.0	
	2	1	12.47	27.0	900/1200
7	1	0†	9.40	20.0	900/1200
8	1	0	0.31	50.0	
	2	1	15.35	50.0	900/1200
9	1	0	0.30	20.0	
	2	3	17.98	41.9	900/1200
10	1	0	0.30	10.0	
	2	1	15.17	31.8	1200/1500
11	1	0	0.30	10.1	
	2	1	17.46	37.2	1200/1500
12	1	0	0.30	10.0	
	2	1	17.69	44.2	1200/1500

*This patient was given reduced dose of only 656 cGy to kidneys, because calculated millicurie amount at 900-cGy level to kidney exceeded maximum allowable radioactivity (9.25 GBq ± 20% of ^{131}I) that could be given at our affiliated hospital at that time.

†This patient did not have diagnostic–dosimetric study performed 1 week before therapy. Instead, study from her first low-dose radioimmunotherapy, performed 1 y earlier, was used.

Because our intent was for this trial to be myeloablative, severe, albeit transient, hematologic toxicity was expected; therefore, only nonhematologic grade 3 or 4 toxicity was considered to be dose limiting. After 3 patients completed 8 wk of treatment at a given dose level without any dose-limiting toxicity, patient entry proceeded to the next dose level. The 8-wk window was chosen because we thought this represented the most likely period during which nonhematologic toxicity would occur (*9*). If dose-limiting toxicity occurred at any dose level, 3 additional patients were entered at that dose so that we could further evaluate the toxicity. Only after 5 of the 6 patients at that dose level completed 8 wk of treatment without any dose-limiting toxicity did escalation proceed to the next higher dose level. If 1 or more of the 3 additional patients experienced dose-limiting toxicity, the maximum tolerated dose was considered to have been exceeded. The maximum tolerated dose was defined as the dose at which fewer than a third of the patients (i.e., 0/6 or 1/6) experienced dose-limiting toxicity.

All antibody infusions were given intravenously, proceeding slowly over the first 5 min and then at a more rapid rate, with the infusion completed within 15–45 min. All patients received potassium perchlorate (200 mg orally, twice per day) in advance, to decrease gastric uptake of radioiodine. Because almost all patients had undergone total thyroidectomy, we did not administer nonradioactive iodine (Lugol's or saturated solution of potassium iodide [SSKI]) to the first 4 patients entered into the study. In later patients, however, we administered 5 drops orally, 3 times per day, because 1 patient had experienced severe gastrointestinal toxicity. Subsequent biodistribution studies in mice suggested that pretreatment with Lugol's solution decreased gastric and intestinal uptake of ^{131}I-MN-14 F(ab)$_2$ by about 50%. Hence, Lugol's solution or SSKI was given to minimize the radiation dose to the gastrointestinal tract from free radioiodine released after MAb breakdown.

Pharmacokinetic Analysis, Imaging, and Dosimetry

Total-body clearance rates for the diagnostic infusions were determined by whole-body scanning data obtained 3–6 times during a 7-d period starting as early as 2–6 h after the radiolabeled antibody infusion. Whole-body clearance after each therapy dose

was determined by a handheld rate meter, with measurements taken immediately after the infusion and repeated twice daily until the end of the study. Whole-blood samples taken during this same period provided data for determining blood clearance rates. Three to 5 blood samples were taken during the first 24 h, and an additional 4 samples were taken over the next 6 d. Total-body activity was expressed as a monoexponential rate of clearance, whereas blood clearance was fit to either a mono- or biexponential function. On the basis of these curves, the biologic half-life in blood and body was determined. Estimates of the slopes of the distribution (α) and elimination (β) phases, and their respective intercepts, were then used in a nonlinear, least-squares, curve-fitting program to generate both monophasic and biphasic clearance curves. If the biphasic curve significantly improved the sum of the squares, this curve was selected as the best fit; otherwise, the monophasic curve was used to define blood clearance.

Planar imaging (500 kcts per view) consisting of anterior and posterior scans of the head, chest, abdomen, and pelvis was performed using DS-X Sophy cameras (Sopha Medical Systems, Columbia, MD) or a dual-head Solus camera (ADAC Laboratories, Milpitas, CA) equipped with a high-energy collimator. Images were obtained at 4 h and then daily for 4–7 d after infusion of a diagnostic dose of ^{131}I-MN-14-F(ab)$_2$, using a 128 × 128 matrix. After the therapy doses of ^{131}I-MN-14 F(ab)$_2$ were administered, imaging was initiated when the level of activity fell below 5 mR/h at a distance of 1 m, usually 5–7 d after antibody infusion, for at least 3 d. SPECT studies (64 × 64 matrix) of the chest, abdomen, and pelvis were performed on at least 1 occasion 24 h or more after the diagnostic infusion (or 5–7 d after the therapy infusion) to better identify the site of tumor by improved contrast resolution.

To calculate the organ and tumor radiation absorbed doses, we used for the γ camera an activity quantification technique based on buildup factor methodology (*14*). Anterior and posterior planar images of the chest, abdomen, and pelvis were obtained in at least 5 imaging sessions (usually days 1 through 5) after the diagnostic study. Tumor volumes were primarily measured by CT. However, our previously validated SPECT volume program was used to measure the volume of occult lesions detected by the MAb scan but not by CT (*15*). The standard human weights given by the MIRD committee were used to estimate the volume of normal organs (*16*). The organ and tumor time–activity data were then fit to either an exponential function by a nonlinear, least-squares, curve-fitting routine or by a trapezoidal modeling method and then integrated to obtain the cumulated activity. The cumulated activity in the red marrow was calculated from the blood by assuming a marrow-to-blood activity concentration ratio of 0.36, as suggested by Sgourous (*17*). The mean dose in centigrays (rads) to the various target organs, with the exception of the tumors, was then obtained according to the MIRD schema with correction for the remainder of the body activity, as described previously (*14*). Also, the mean dose in centigrays to the tumors was obtained by the method reported previously (*14*).

Toxicity and Tumor Response

Toxicity was graded according to the common toxicity criteria of the National Cancer Institute. We monitored all patients for toxicity by performing complete peripheral blood cell counts weekly. Organ toxicity was assessed at 4, 8, 12, and 24 wk after therapy. Thereafter, follow-up tests were planned for every 6 mo, whenever possible, up to 5 y, to monitor any chronic toxicity. Renal and hepatic function was assayed by biochemical profiles; pulmonary toxicity, by pulmonary function tests, including lung volumes and diffusion capacity; and cardiac toxicity, by blood pool scanning. Neurologic and gastrointestinal toxicity was assessed by physical examination and by notation of any system-related symptoms during the follow-up period. Tumor responses were assessed at 1 and 3 mo after treatment, and if a response was observed, additional monitoring was performed. CT was the primary method used to assess therapeutic response. However, MRI or bone scanning was also used in some patients, particularly those with negative or equivocal CT findings. Both tumor markers in the blood (calcitonin and carcinoembryonic antigen) were assessed at 1- to 2-mo intervals until 1 y after therapy. A reduction in tumor markers of at least 25% for at least 1 mo was considered indicative of an antitumor effect, and a more than 25% increase was considered indicative of biochemical progression. Complete remission was defined as complete disappearance of all detectable disease for a minimum of 4 wk, a partial response was defined as a reduction of at least 50% in the sum of the products of the longest perpendicular diameters of all measurable lesions for a minimum of 4 wk, and disease progression was defined as an increase of at least 25% in the diameter of lesions or the appearance of new lesions. Responses were considered to be minor when the reduction in disease was between 25% and 50%.

HAMA Determination

A blood HAMA titer was determined for all patients at baseline and at a minimum of 1, 2, 4, 8, and 12 wk. HAMA was monitored by the ImmuSTRIP HAMA IgG assay (Immunomedics), which has a normal titer of less than 74 ng/mL

RESULTS

Pharmacokinetics

Table 3 lists the pharmacokinetic parameters of the diagnostic and therapeutic doses of ^{131}I-MN-14 F(ab)$_2$ in the 12 patients. The average half-lives in blood and whole body for the diagnostic infusions were 22.4 ± 5.7 and 60.0 ± 11.2 h, respectively, compared with 20.9 ± 4.1 and 54.3 ± 13.5 h, respectively, for the therapy infusions. In general, agreement was good between the blood and total body half-lives determined from the diagnostic–dosimetric studies and those obtained from the therapy studies (r = 0.79 for both the blood and the total-body half-lives). For all patients, the whole-body clearance was best defined by a monoexponential function whereas the blood clearance was best defined by a biexponential function. The average α and β half-lives in the blood for the diagnostic infusions were 10.4 ± 6.4 and 67.3 ± 46.3 h, respectively, compared with 16.5 ± 4.3 and 107.2 ± 111.2 h, respectively, for the therapy infusions (not statistically significant). The average total-body and red marrow doses determined using the diagnostic–dosimetric studies were 0.02 ± 0.004 and 0.036 ± 0.01 cGy/MBq, respectively, compared with 0.017 ± 0.004 and 0.031 ± 0.009 cGy/MBq, respectively, for the therapy infusions. Correlation was excellent between the blood and total-body radiation absorbed doses (in cGy/mCi) obtained from the diagnostic–dosimetric studies and those obtained from the therapy studies (r = 0.97 for both the blood and the total body doses).

TABLE 3
Pharmacokinetic Parameters for ^{131}I-MN-14 F(ab)$_2$ Infusions

Patient no.	Injection no.	Half-life (h)	
		Blood	Total body
1	1	17.3	60.1
	2	15.1	62.3
2	1	23.0	60.8
	2	24.6	56.7
3	1	17.1	53.6
	2	16.8	36.0
4	1	24.8	79.8
	2	22.6	63.1
5	1	19.2	57.3
	2	26.9	41.4
6	1	23.8	58.4
	2	20.9	52.9
7	1*	17.9	32.3
8	1	35.3	80.8
	2	26.5	82.7
9	1	14.4	58.2
	2	17.3	57.1
10	1	26.8	59.8
	2	ND	49.7
11	1	23.1	43.6
	2	17.9	60.0
12	1	21.1	52.0
	2	23.5	57.8

*This patient did not have diagnostic–dosimetric study performed 1 wk before therapy.

ND = not determined.

Half-life is time required for 50% of injected activity to be cleared from blood or body. Blood clearance curves were described by biexponential model, and total-body clearance curves were described by single exponential model.

Tumor Targeting

The diagnostic antibody scans showed positive findings for all 12 patients, and all lesions seen on the diagnostic studies were also seen on post-therapy images. However, some lesions that were not detected or not clearly delineated on the diagnostic studies were detected or better delineated on the post-therapy images, probably because their later acquisition after the therapy infusion (days 6–9) improved their quality. Overall, a total of 93 of 96 known or suspected disease sites (97%) were visualized. The only false-negative findings were a diffuse cervical spine involvement seen by MRI in 1 patient and a focal skull lesion seen by bone scanning in another patient, However, in general, targeting of bone and bone marrow in these patients was excellent, often better than that seen by CT or bone scanning, as later proven by MRI or bone marrow biopsy.

Organ and Tumor Dosimetry

The average radiation absorbed doses (in cGy/MBq) to the lungs, liver, and kidneys determined using the diagnostic–dosimetric studies were 0.063 ± 0.035, 0.059 ± 0.029, and 0.068 ± 0.012, respectively. Interestingly, the organ radiation doses determined by the therapy infusions were not significantly different. The average lung, liver, and kidney radiation absorbed doses were 0.046 ± 0.033, 0.052 ± 0.033, and 0.060 ± 0.019, respectively. The correlation coefficients for the lung, liver, and kidney doses determined by both measurements were 0.91, 0.73, and 0.77, respectively.

Tumor radiation dose was estimated in 7 patients with 22 well-defined tumor masses (mass range, 1.8–155.0 g; mean mass, 109 ± 292 g; median mass, 33.7 g). The mean tumor dose in cGy/MBq was 0.63 ± 1.01 (range, 0.073–4.78; median, 0.32), and the mean tumor dose in centigrays delivered was 8574.6 ± 15577.7 (range, 1,104–73,640; median, 3,862.5). These doses were approximately 19.0-, 11.8-, 8.3-, and 9.2-fold higher than those to the red marrow, lung, liver, and kidney, respectively, and 32.9-fold higher than that to the whole body. The slightly lower tumor-to-liver ratio compared with the tumor-to-kidney ratio was caused by an overestimation of the "actual" normal liver doses in some patients because of small multifocal hepatic involvement.

Dose Escalation and Toxicity

Table 4 summarizes nonhematologic toxicity, including grade, time of onset, and duration, observed in all 12 patients. The first patient, to whom we administered a lower dose than planned, did not have evidence of treatment-related nonhematologic toxicity. Of the next 3 patients entered at the 900-cGy dose level, dose-limiting toxicity developed in only 1 (patient 4). The toxicity, which was manifested by grade 3 nausea that began 10 d after therapy and lasted approximately 5 mo, and grade 3 vomiting that began 30 d after therapy and lasted 2 wk, left no residual effects. Because grade 3 gastrointestinal toxicity was considered dose limiting, we decided to enter at least 2 additional patients at the 900-cGy level to obtain a cohort of at least 6. In fact, 5 additional patients were entered at this level to confirm its safety at this critical phase of the trial. In none of these additional 5 patients did dose-limiting toxicity develop, including 1 patient (patient 7) who had received low-dose radioimmunotherapy (5.85 GBq) of the same MAb 1 y earlier. Aside from the grade 3 gastrointestinal toxicity seen in the first patient, toxicity at the 900-cGy dose level included transient grade 1 or 2 nausea or vomiting in 5 patients, grade 1 pulmonary toxicity (alteration in pulmonary function tests) in 1 patient, grade 2 cardiac toxicity (decrease in left ventricular ejection fraction without symptoms of congestive heart failure) in 2 patients, grade 1 elevation in transaminases in 1 patient, and grade 2 neurologic toxicity (numbness and generalized weakness) in 1 patient. No renal toxicity occurred. On the basis of the lack of nonhematologic dose-limiting toxicity in this group of patients, we escalated to the next dose level—1200 cGy. Three patients were treated at this dose. In none of the 3 did dose-limiting toxicity develop. Two patients had grade 1 pulmonary toxicity, and 2 had grade 1 or 2 nausea, vomiting, or diarrhea. Because none of the 3 patients treated at the 1200-cGy dose level experienced dose-limiting toxicity,

TABLE 4
Nonhematologic Toxicities in Treated Patients

Patient no.	Therapy injection (GBq)	Renal			Hepatic			Gastrointestinal			Cardiac			Pulmonary			Neurologic		
		Grade	Time of onset of toxicity	Duration of toxicity	Grade	Time of onset of toxicity	Duration of toxicity	Grade	Time of onset of toxicity	Duration of toxicity	Grade	Time of onset of toxicity	Duration of toxicity	Grade	Time of onset of toxicity	Duration of toxicity	Grade	Time of onset of toxicity	Duration of toxicity
1	9.95	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
2	8.69	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
3	8.58	0	NA	NA	0	NA	NA	0	NA	NA	2	55	ND*	0	NA	NA	0	NA	NA
4	15.39	0	NA	NA	0	NA	NA	3	10	158	0	NA	NA	0	NA	NA	0	NA	NA
5	16.98	0	NA	NA	0	NA	NA	2	1	2	0	NA	NA	0	NA	NA	0	NA	NA
6	12.47	0	NA	NA	0	NA	NA	2	0	7	2	56	330+	1	27	29	0	NA	NA
7	9.40	0	NA	NA	0	NA	NA	1	0	5	0	NA	NA	0	NA	NA	0	NA	NA
8	15.35	0	NA	NA	1	39	85+†	2	1	4	0	NA	NA	1	39	ND‡	2	0	559
9	17.98	0	NA	NA	0	NA	NA	1	8	12	0	NA	NA	0	NA	NA	0	NA	NA
10	15.17	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	1	24	ND	0	NA	NA
11	17.46	0	NA	NA	0	NA	NA	2	1	3	0	NA	NA	0	NA	NA	0	NA	NA
12	17.69	0	NA	NA	0	NA	NA	1	2	2	0	NA	NA	1	56	56	0	NA	NA

*Patient refused further follow-up tests and went on to receive several doses of chemotherapy without any reported cardiac toxicity.
†Patient did not have follow-up biochemical tests performed until 5 mo after this date, at which time the transaminases normalized.
‡Patient was not able to undergo further follow-up scanning because of denial of insurance coverage.
NA = not applicable; ND = not determined.
Time of onset and duration of toxicity are measured in days.

dose escalation could have proceeded to the next level. However, we elected to discontinue this trial and use the newly available humanized form of the antibody. All except 1 of the 12 patients were followed up for more than 6 mo after treatment (range, 12–30 mo; median, 22 mo) to check for chronic organ toxicity. This was found in only 1 (patient no. 6), in whom the cardiac ejection fraction decreased from 55% 6 months after therapy to 46% 1 y after therapy. Thus, at this relatively early time, preliminary data indicate little chronic toxicity.

Severe, albeit transient, hematologic toxicity was expected in this myeloablative trial. However, we did not see engraftment failure in any patient. In 10 of 12 assessable patients, the expected grade 3 or 4 leukopenia or thrombocytopenia developed approximately 2–5 wk after therapy. The time until grade 3 or 4 white blood cell and neutrophil toxicity ranged from 9 to 34 d (median, 22 d), whereas that for platelet toxicity ranged from 16 to 29 d (median, 21 d). The duration of grade 3 or 4 leukopenia was 1–14 d (median, 5 d), whereas that of grade 3 or 4 thrombocytopenia was 1–7 d (median, 3 d). Only 1 incident of neutropenic sepsis was seen, and 1 other patient had a mild infection. Bleeding, which was manifested as mild epistaxis or bruising, was seen in only 2 patients.

HAMA

HAMA developed in 7 of the 11 antibody-naive patients treated, who had normal HAMA levels (i.e., <74 ng/mL) at baseline. However, in all these patients, abnormal HAMA titers were seen only 3 wk after the first MAb infusion or 2 wk after the therapy infusion. The peak HAMA titers were usually low, ranging from 88 to 766 ng/mL (median, 323 ng/mL), with the peak occurring approximately 4–8 wk after therapy. In patient no. 7, who started with an abnormal HAMA level of 183 ng/mL (still within the eligibility criteria) at the time of therapy, higher levels of HAMA (1122 ng/mL) developed 2 wk after therapy, and a relatively high peak value of 25,315 ng/mL was reached 8 wk after therapy.

Antitumor Effects

Table 5 summarizes the radiologic and biochemical antitumor effects observed after therapy in the 12 patients. The partial remission experienced by 1 patient for 1 y was quite significant because he apparently had rapidly progressing disease before therapy, with a massive tumor burden in the liver and an extremely high carcinoembryonic antigen level of 6723 ng/mL. In fact, the patient's CT scan obtained 2 mo after therapy showed increased disease compared with a scan obtained 2 wk before therapy (carcinoembryonic antigen also increased to 8490 ng/mL), probably indicating disease progression in the 2 wk before therapy began. However, tumor lesions gradually regressed starting 4.5 mo after therapy, indicating a protracted response of medullary thyroid cancer. In addition, the patient's carcinoembryonic antigen decreased to 4810 ng/mL. Tumor targeting in the liver of this patient was exquisite, with calculated radiation doses ranging from 5088 to 73,640 cGy delivered to tumors. Figure 1 shows this targeting and the 2 CT scans obtained at 2 and at 9 mo, with the latter indicating marked regression of even the large tumors. In addition to the interesting and protracted response of this patient, another patient had a minor response (regression of diffuse pulmonary metasta-

TABLE 5
Antitumor Effects Observed

Patient no.	Dose injected (GBq)	Dose to tumor (cGy)	Duration of antitumor effects
1	9.95	1,999–3,876	Radiologically stable disease (1 mo)
2	8.69	11,630	Minor response (3 mo); 89% decease in calcitonin at 2 mo (3 mo); stable carcinoembryonic antigen (7 mo)
3	8.60	1,399–5,176	Radiologically stable disease (3 mo); stable carcinoembryonic antigen and calcitonin levels (4 mo)
4	15.39	5,088–73,640	Partial remission (12 mo)
5	16.98	1,900–4,177	Radiologically stable disease (16 mo); 58% decrease in calcitonin at 4 mo (8 mo); 44% decrease in carcinoembryonic antigen at 7 mo (5 mo)
6	12.47	ND	Radiologically stable disease (13+ mo); stable carcinoembryonic antigen and calcitonin (12+ mo)
7	9.40	ND	Radiologically stable disease (12+ mo); stable carcinoembryonic antigen (6 mo); stable calcitonin (10 mo)
8	15.35	1,104	Radiologically stable disease (8 mo); stable carcinoembryonic antigen (7+ mo); 90% decrease in calcitonin at 1 mo (7+ mo)
9	17.98	ND	Radiologically stable disease (13+ mo); stable carcinoembryonic antigen (3+ mo); increased calcitonin by 73% at 3.5 mo
10	15.17	5,297–7,191	Radiologically stable disease (4 mo); stable calcitonin (4 mo); stable carcinoembryonic antigen (2+ mo)
11	17.46	ND	Radiologically stable disease (6 mo); 86% decrease in calcitonin at 5 mo; 68%+ (still ongoing) decrease in carcinoembryonic antigen at 6 mo
12	17.69	ND	Radiologically stable disease (13+ mo); stable carcinoembryonic antigen (7 mo)

ND = not determined.

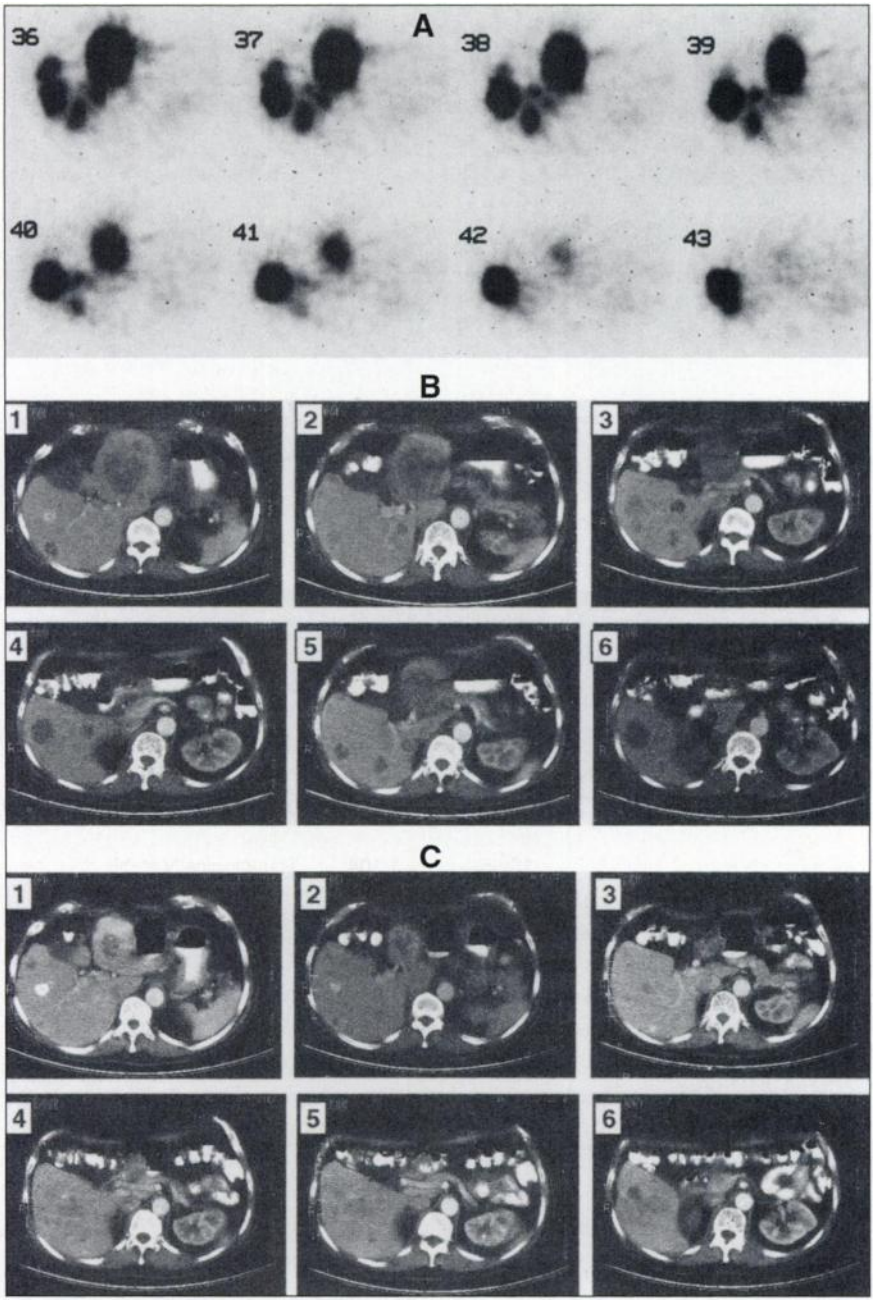

FIGURE 1. (A) Transverse abdominal SPECT images obtained 2 d after injection of 0.3 GBq (8 mCi) ^{131}I-MN-14 F(ab)$_2$ show excellent targeting of multiple liver metastases in right and left lobes. Metastases correspond to those seen on CT scans obtained 2 (B) and 9 mo (C) after radioimmunotherapy with 15.39 GBq (416 mCi) ^{131}I-MN-14 F(ab)$_2$. In comparison with 2-mo scan, 9-mo scan shows marked regression of even large tumor lesions in left liver lobe.

ses) for 3 mo accompanied by a significant, 3-mo decrease in plasma calcitonin levels (89%) 2 mo after therapy. Serum carcinoembryonic antigen remained relatively stable in this patient for 7 mo after therapy. Radiologic methods (primarily CT) showed the disease of the other 10 patients to stabilize for periods ranging from 1 to 16 months. Of these patients, the disease of 4 remained stable for between 12+ and 13+ months (median, 12.5+ months), whereas the disease of the other 6 progressed (Table 5). Figure 2 shows CT scans, obtained before and after therapy, of a patient with stable disease. The scans show improvement in some lesions. This patient had stable disease for 16 months after therapy.

DISCUSSION

We believe that this report is the first to describe use of high-dose radioimmunotherapy with AHSCR for metastatic medullary thyroid cancer and is one of only a few about high-dose radioimmunotherapy of solid tumors (*18–20*).

Although relatively few patients were included in the study and they were treated at only the first 2 dose levels planned, several important observations can be made. First, dose escalation of radioimmunotherapy with AHSCR is feasible in patients with medullary thyroid cancer, with only moderate nonhematologic toxicity. Only 1 instance of dose-limiting toxicity occurred (grade 3 gastrointestinal); other nonhematologic toxicity was grade 1 or 2 and usually

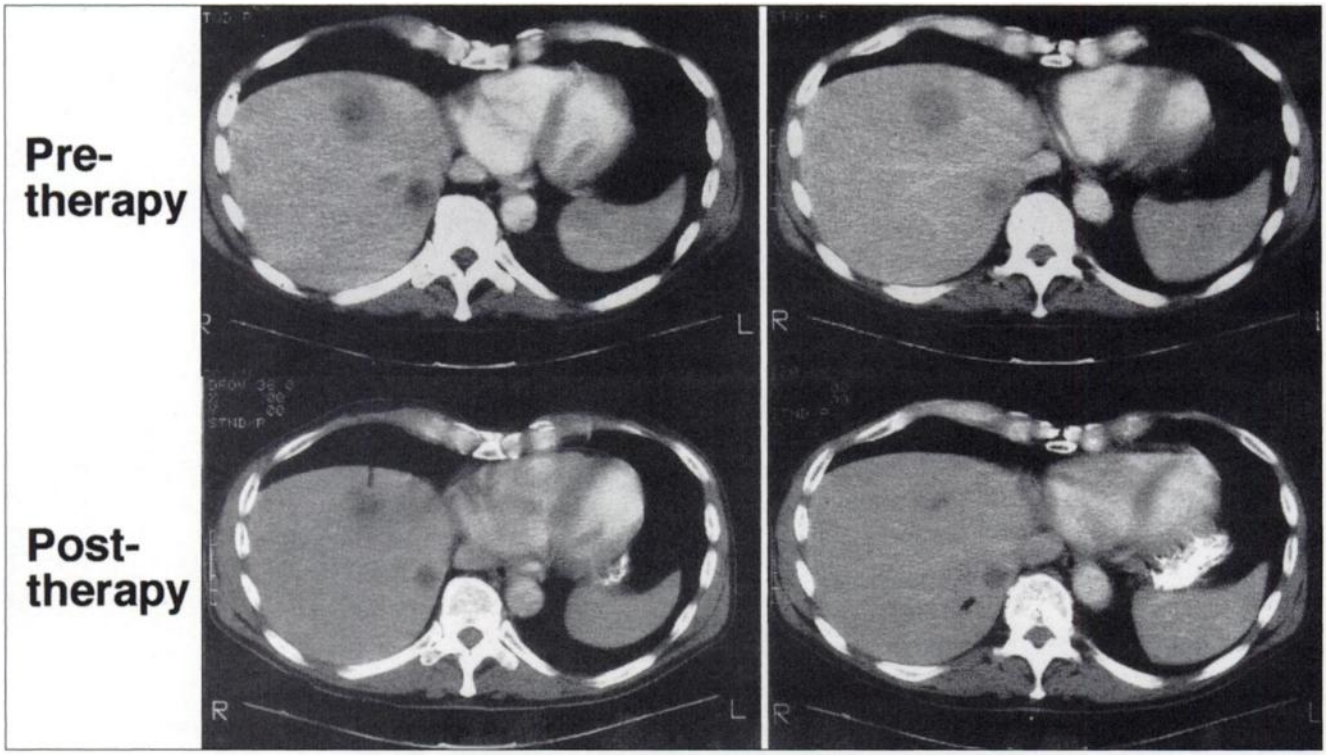

FIGURE 2. Abdominal CT scans of patient whose disease stabilized after radioimmunotherapy with 16.98 GBq (459 mCi) ^{131}I-MN-14 F(ab)$_2$. In comparison with scans obtained before therapy, scans obtained 7 mo after therapy show improvement of some lesions.

transient. Moreover, the instance of dose-limiting toxicity occurred when Lugol's solution or SSKI was not given. After we reinstituted a rigorous schedule of SSKI administration, we did not observe gastrointestinal toxicity greater than grade 2. Second, although still only moderate, the nonhematologic toxicity observed is clearly more frequent and more intense than that seen in nonmyeloablative radioimmunotherapy trials, with the potential for delayed or more chronic toxicity, as was observed in 1 patient in whom the cardiac ejection fraction decreased from 55% 6 mo after therapy to 46% 1 y after therapy. Thus, special caution should be exercised in such trials, and more frequent, longer term follow-up appears warranted. Third, this trial shows that it is possible to deliver much higher radiation doses to tumors than in nonmyeloablative radioimmunotherapy trials. In some instances these doses exceeded 6000 cGy, which is similar to doses delivered to local disease sites with external beam radiation. These higher doses are more likely to result in significant antitumor responses. Press et al. (*9,21*), using a different antibody (^{131}I-B1 anti-CD20 MAb), found the expected maximum tolerated dose to critical organs to be 2700 cGy. Although the treatment doses given to our patients presumably corresponded to only 30%–40% of that maximum, encouraging antitumor effects were seen. One patient experienced partial remission for 1 y, another had a minor response for 3 mo, and the remaining 10 had radiologic evidence of disease stabilization for periods ranging from 1 to 16 months. Of these 10 patients, 4 continue to be stable at a median duration of 12.5+ months. The disease stabilization is meaningful, because only patients with clearly evident disease progression, in some cases quite rapid, were enrolled. Also noteworthy is the more than 50% decrease in calcitonin or carcinoembryonic antigen levels in 4 of 12 patients.

Although we established that the 1200-cGy dose to critical organs was safe, thus allowing further escalation beyond this level, we elected to discontinue the trial in favor of using the newly available humanized form of MN-14. The humanized form has the advantage of reduced immunogenicity compared with the murine form and the potential for eliciting an immune effector function with potentially additive antitumor effects (*22*). As the doses are escalated further, using this radiolabeled humanized MAb either alone or in combination with chemotherapy, the prospects of achieving better antitumor effects will increase, and the role of high-dose radioimmunotherapy in the management of patients with metastatic medullary thyroid cancer and other solid tumors may then be defined.

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