Radioimmunotherapy for the Intensification of Conditioning Before Stem Cell Transplantation: Differences in Dosimetry and Biokinetics of ^{188}Re- and ^{99m}Tc-Labeled Anti–NCA-95 MAbs

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A new concept is the intensification of preparative regimens for patients with advanced leukemia using monoclonal antibodies (MAbs) with an affinity for β emitter–labeled bone marrow. ^{188}Re is a high-energy β emitter that has therapeutic promise. Our first aim was to clarify whether the therapeutic application of ^{188}Re-MAb against nonspecific cross-reacting antigen 95 (NCA-95) can be predicted from biokinetic data derived from ^{99m}Tc-labeled NCA-95. Our second aim was to show that a radiation absorbed dose of ≥12 Gy in the bone marrow can be achieved using ^{188}Re-MAb. **Methods:** Dosimetric data were obtained for both radiotracers from multiple planar whole-body scans (double-head γ camera), blood samples, and urine measurements from 12 patients with advanced leukemia. Radiation absorbed doses were calculated using MIRDOSE 3 software. **Results:** Radiation absorbed doses to bone marrow, liver, spleen, lung, and kidney were 2.24, 0.50, 1.93, 0.05, and 0.90 mGy/MBq, respectively, using ^{99m}Tc-MAb and 1.45, 0.43, 1.32, 0.07, and 0.71 mGy/MBq, respectively, using ^{188}Re-MAb. These differences were statistically significant for bone marrow, spleen, and kidney. The main differences were less accumulation of ^{188}Re-MAb in bone marrow (31% ± 13% compared with 52% ± 13%) and faster elimination through urine (25% ± 3% compared with 15% ± 5% after 24 h). On the basis of these data, a mean marrow dose of 14 ± 7 Gy was achieved in 12 patients suffering from leukemia after application of approximately 10 ± 2 GBq ^{188}Re-MAb. **Conclusion:** Myeloablative radiation absorbed doses can easily be achieved using ^{188}Re-MAb. ^{99m}Tc- and ^{188}Re-MAb showed similar whole-body distributions. However, direct prediction of radiation absorbed doses from the ^{99m}Tc-MAb, assuming identical biokinetic behavior, is not valid for the ^{188}Re-MAb in a single patient. Therefore, individual dosimetry using ^{188}Re-MAb is needed to calculate therapeutic activity.

Key Words: ^{188}Re; monoclonal antibody against nonspecific cross-reacting antigen 95; dosimetry; radioimmunotherapy; bone marrow transplantation

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Bone marrow transplantation is an effective and widely used treatment for leukemia. However, patients with advanced disease have a high relapse rate despite the high-dose chemotherapy used in most conditioning regimens (*1*). An increased dose of total body irradiation (TBI) significantly reduces the probability of relapse but, because of increased mortality from toxicity, does not improve survival (*2,3*). Radioimmunotherapy can selectively deliver radiation to bone marrow while sparing the other organs. This concept was introduced by Scheinberg et al. (*4*) and Appelbaum et al. (*5*) using ^{131}I-labeled monoclonal antibodies (MAbs) against antigens on hematopoietic cells such as CD45 and CD33 (*4–7*). The success of this approach appeared to be limited by low CD33 expression on target cells and by rapid deiodination and release of ^{131}I from the target tissue because of internalization of the antibody–antigen complex. This experience suggests that results can be improved using MAbs with a different specificity and different β emitters.

One of the most promising high-energy β emitters is ^{188}Re, which can be obtained daily from a ^{188}W/^{188}Re radionuclide generator in high specific volume (*8*). ^{188}Re decays by β emission (E_{max} = 2.11 MeV) followed by emission of 155 keV γ photons with a 15% probability, which is sufficient for γ camera imaging.

Labeling procedures for rhenium have been reported for various MAbs (*9–11*). Of special interest is the labeling of the MAb BW 250/183 (Anti-Granulocyte; CIS Medipro SA, Geneva, Switzerland) against nonspecific cross-reacting antigen 95 (NCA-95) (*12*). This MAb is well characterized with respect to biokinetic data (*13*), clinical application in bone marrow scintigraphy, and localization of infection (*14–18*). Although Breitz et al. (*9*) reported that biokinetic data of ^{99m}Tc- and ^{186}Re-MAbs (NR-LU-10) agreed well, this study was undertaken to clarify whether biokinetic data derived from ^{99m}Tc-labeled Anti-Granulocyte can be used to predict the therapeutic application biokinetics of ^{188}Re-labeled Anti-Granulocyte. Moreover, we wanted to

show that a radiation absorbed dose of approximately 12 Gy can be deposited in bone marrow using ^{188}Re-MAb without jeopardizing the other organs.

MATERIALS AND METHODS

Radionuclides and Radiolabeling

^{188}Re was obtained from a ^{188}W/^{188}Re radionuclide generator as a solution of sodium perrhenate in 0.9% chloride (Oak Ridge National Laboratory, Oak Ridge, TN). Full details of generator performance have been published elsewhere (*8,19*). The radioactivity was concentrated to a small volume (>3.0 GBq/mL) by an anion exchange column (*8*). Activity was counted using a rate meter (CRC-120; Capintec, Inc., Ramsey, NJ), and radiochemical impurities were <0.001% (*20*). Labeling of Anti-Granulocyte has been described in detail elsewhere (*12*). In short, reduction of MAbs was performed by treatment with Tris-(2-carboxyethyl)phosphine over 20 min at room temperature under N_2 protection. Reduced MAb was purified from excess phosphine using a Sephadex G-25 PD10 column (Pharmacia, Uppsala, Sweden) and incubated with ^{188}Re-perrhenate for 2 h at 37°C. Quality control included high-pressure liquid chromatography (HPLC) with simultaneous monitoring of protein (ultraviolet absorption at 280-nm wavelength) and radioactivity (NaI γ detector) to characterize the labeled product (Fig. 1) as well as instant thin-layer chromatography to determine the amount of colloids. ^{188}Re incorporation was >95% in the final product, with <3% unbound ^{188}Re perrhenate and <2% colloid.

In Vitro Stability of the Radiotracers

Labeled MAbs were measured in competition with human serum albumin (HSA). Radiolabeled MAbs were incubated in 5% HSA at 37°C in a closed vial and analyzed at times ranging from 1 to 24 h by HPLC to determine the chemical form of the radiolabel. To investigate the effect of the protectant 2,5-dihydroxybenzoic acid, gentisic acid (Aldrich, Milwaukee, WI), on in vitro stability of the radiolabel, we added 2 mg gentisic acid to the preparation. The solution was prepared freshly in sterile acetic buffer at a pH of 4.0. Radiolabeled MAbs were incubated in 5% HSA at 37°C in a closed vial and analyzed by HPLC as described.

Phantom Studies

Phantom studies were undertaken to check the performance of the γ camera, the attenuation of the γ photons, and the dosimetry calculation program (MIRDOSE3) for ^{99m}Tc and ^{188}Re (*21*). Imaging was performed at the same intervals as for patient studies with a double-head γ camera (Bodyscan; Siemens, Erlangen, Germany). ^{99m}Tc and ^{188}Re were imaged with a low-energy general-purpose collimator (peak, 140 keV; window, 15%) and a high-energy collimator (peak, 155 keV; window, 20%), respectively. The sensitivities of this system for ^{99m}Tc and ^{188}Re were 5.06 and 0.92 cpm/kBq, respectively. The transmission of counts from ^{99m}Tc and ^{188}Re in water was compared in a Jaszczack phantom (Physikalisch-technische Werkstätten Dr. Pychlau GmbH, Freiburg, Germany). Dead-time loss was estimated using a 250-mL tissue culture flask filled with 2 GBq ^{188}Re and performing sequential imaging over 120 h (*9*).

Patient Imaging Studies and Dosimetric Calculations

For dosimetry, patients received 1.0 mg Anti-Granulocyte labeled with 500–800 MBq ^{99m}Tc. Six whole-body scintigraphy studies were performed at 30 min; at 1, 2, 4, and 16 h; and at 1 d

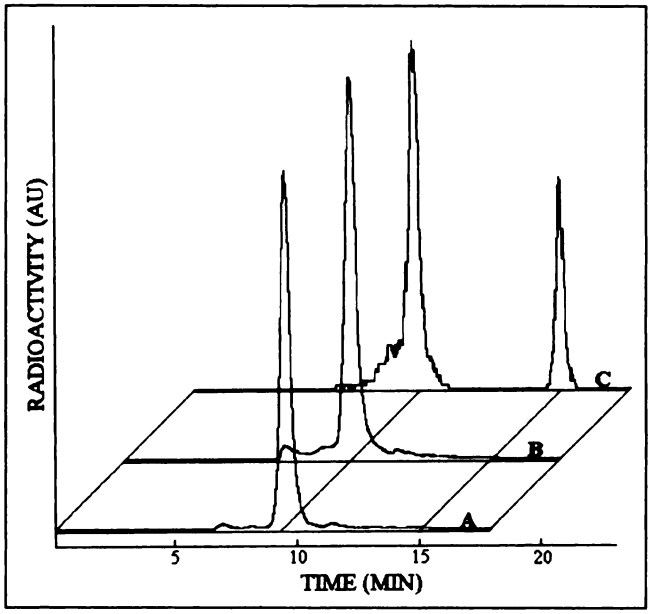

FIGURE 1. HPLC analysis of in vitro stability of ^{188}Re anti-NCA-95 MAb before incubation in HSA (A), after 4 h HSA incubation (B) ($^{188}ReO_4^-$ < 5%), and after 24 h HSA incubation (C) ($^{188}ReO_4^-$ = 30%). First fraction represents high-molecular-weight complexes (i.e., labeled MAb), and second fraction represents low-molecular-weight free ^{188}Re-perrhenate. However, in presence of gentisic acid, in vitro stability increased and no significant amounts of free perrhenate could be detected after 24 h.

after injection to evaluate the distribution and elimination of the radiotracer. For ^{188}Re dosimetry, the procedure was extended to 2 d. Blood samples and urine were collected over the same interval to determine radiotracer clearance using a well counter (Autogamm 5500; Canberra Packard, Dreieich, Germany). The calibration factor between the rate meter and the well counter yielded 10,803 cpm/kBq and 47,700 cpm/kBq for ^{188}Re and ^{99m}Tc, respectively. Scanning speed was varied to adjust for physical decay. A geometric mean image of the anterior and posterior whole-body scans was generated for each patient and each measurement. The count distribution within this geometrically averaged image was assumed to be a proportional estimate of the activity distribution inside the body, and no further attenuation correction was performed (*22,23*). A region-of-interest (ROI) analysis was performed to determine the organs' counting rate (i.e., liver, spleen, kidneys, bone marrow, and background). The ROIs were drawn individually on each scan of each patient with respect to similar ROI size. A rectangular whole-body ROI was used to measure the total activity in the body. The whole-body counts were normalized to percentage injected dose by subtraction of the amount excreted in urine from the amount injected: $\text{Activity}_{\text{organ}} = \text{Activity}_{\text{total body}}\ (\text{Counts}_{\text{organ}}/\text{Counts}_{\text{total body}})$ and $\text{Activity}_{\text{total body}} = \text{Activity}_{\text{injected}} - \text{Activity}_{\text{urine}}$. The bone marrow count was approximated with rectangular ROIs as illustrated in Figure 2E. To obtain the counts for the remainder of the body, the sum of the organ counts was subtracted from the whole-body counts.

Decay-corrected time–activity points for each source organ or the remainder of tissue were fitted with up to 3 exponential functions (Fig. 3). The residence times were obtained using these results and the physical decay constant of ^{188}Re. The radiation

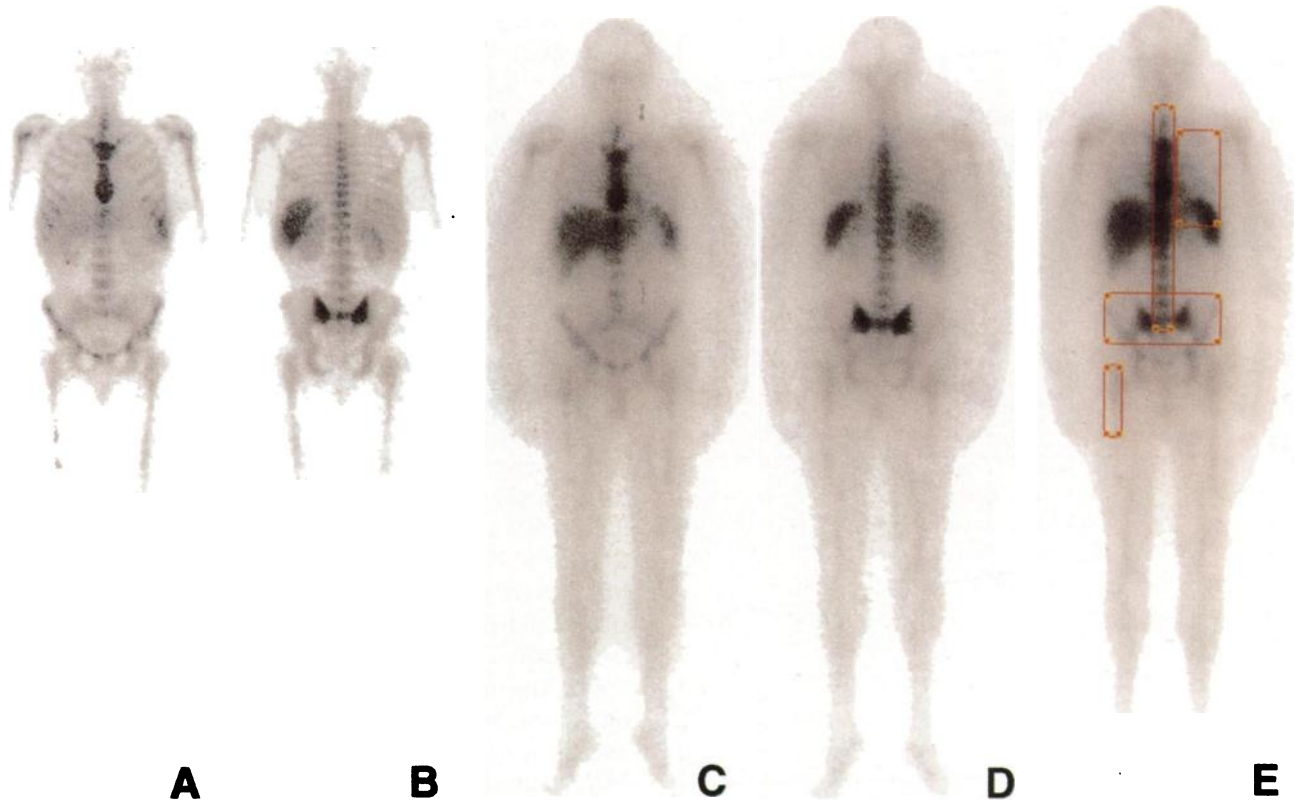

FIGURE 2. Planar whole-body imaging after application of ^{99m}Tc-MAb (A and B) and ^{188}Re-MAb (C and D) in same patient (1 wk difference) reveals similar radiotracer distribution. More inhomogeneous image presentation of ^{188}Re scan was caused by scatter from high-energy photons (478 keV and 633 keV) and could not be avoided completely even using medium-energy collimator. (E) Bone marrow size was approximated by rectangular ROIs. Bone marrow uptake was estimated from lower lumbar spine. Mean ± SD of 3 estimations was 4.7% ± 2.5% (range, 0.5–8.7), which was highly reproducible.

exposure was calculated using the MIRDOSE3 software (*21*). To predict dosimetry for ^{188}Re therapy from ^{99m}Tc-MAb, the biologic half-lives derived from ^{99m}Tc data with the physical properties of ^{188}Re were used for MIRDOSE3.

Clinical Applications

With the approval of the Ulm University Hospital Ethics Committee, 12 patients referred for bone marrow transplantation for leukemia were eligible for this study. Table 1 presents their characteristics. All patients gave written informed consent. The indication of intensified treatment was a high risk of relapse because of remission status (e.g., >first complete remission, relapse, nonresponder) or the presence of high-risk cytogenetic features in patients experiencing a first remission, such as Philadelphia positivity in patients with acute lymphoblastic leukemia.

For radioimmunotherapy, patients were pretreated with sodium perchlorate (Irenat; Bayer, Leverkusen, Germany) to block the thyroid and gastric mucosa (800 mg 3 times per day, initiated 24 h before the first MAb infusion and continued up to 1 week after the last radiotracer application). This pretreatment was shown to effectively protect against ^{188}Re-perrhenate (*24*). ^{188}Re-MAb was administered within 1 week after the ^{99m}Tc-MAb imaging study. The initial dose of ^{188}Re-MAb yielded approximately 3.3 GBq (range, 1.0–4.7 GBq) and was infused slowly over 10 min and followed by sequential imaging for up to 48 h. Therapy was completed with further ^{188}Re-MAb applications every second day depending on the eluted activity from the ^{188}W/^{188}Re generator.

The conditioning therapy included TBI with 12 Gy in an anteroposterior setup and chemotherapy with cyclophosphamide in a total of 120 mg/kg body weight. One patient received additional thiotepa at 10 mg/kg, and busulfan in a total of 16 mg/kg replaced TBI in 2 patients receiving a second transplant after prior TBI (Table 1). Except for 1 patient receiving bone marrow, all patients received allogeneic peripheral blood stem cells (Table 1). For prophylaxis against graft-versus-host disease, the transplants were depleted of T cells either by incubation of the leukapheresis with the MAb CAMPATH 1H (Therapeutic Antibody Centre, Headington, Oxford, United Kingdom) (anti-CD52) (5 patients with identical family donors) or by CD34 selection and CD3 depletion (Seprate SC column [CellPro Inc., Bothell, WA] + MACS beads [Miltenyi Biotec, Bergisch Gladbach, Germany] or Clinimacs [Miltenyi Biotec]).

RESULTS

In Vitro Stability of the Labeled MAb

Samples of ^{99m}Tc-MAb incubated in 5% HSA at 37°C showed high stability by means of HPLC. The MAb-bound radioactivity was approximately 100% after 24 h. However, loss from MAb because of reoxidation was significantly higher for rhenium than for technetium. Quality control showed that a separation of MAb and radiolabel of about 5% after 6 h increased to 30% after 24 h. Unbound rhenium

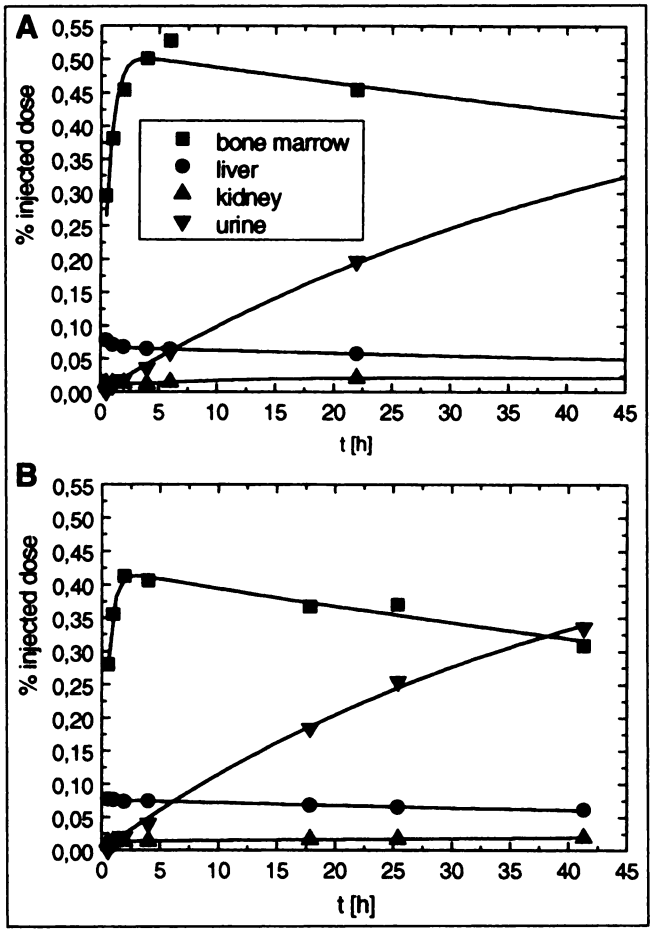

FIGURE 3. Typical time–activity curves of relevant organs and urinary excretion normalized to injected dose of ^{99m}Tc-MAb (A) and ^{188}Re-MAb (B) as calculated from sequential whole-body images in single patient (patient 7). t = time.

appeared as perrhenate, as shown by an HPLC peak at 15.1 min (Fig. 1). No other radiochemical components could be analyzed through HPLC.

The protectant gentisic acid significantly increased in vitro stability after 24 h. The HPLC revealed that the bulk of radioactivity (approximately 90%) was still associated with the protein fraction. At 6 min, a high-molecular-weight oligomer peak of approximately 9% could be analyzed through HPLC, but no perrhenate was present.

γ Camera Imaging

Dead-time loss in γ camera imaging increased for more than 370 MBq in the field of view. Assuming a typical whole-body distribution of ^{188}Re-MAb, after radiotracer application of 1.0 GBq this limit was never reached. The attenuation derived by the Jaszczak phantom yielded 0.1153/cm and 0.0563/cm for ^{99m}Tc and ^{188}Re, respectively.

Bone marrow uptake of ^{99m}Tc-MAb reached 52.4% ± 12.6% (decay corrected) 2 h after injection and declined to 48.5% ± 12.2% after 24 h. Whole-body and blood half-lives were 21.2 ± 0.9 and 5.2 ± 1.0 h, respectively. Urinary excretion was 15.4% ± 4.6% within the first day. Significant bowel activity could not be detected on serial images. The bone marrow uptake of ^{188}Re-MAb was 30.8% ± 13.4% 2 h after injection and fell to 24.6% ± 11.4% and 22.7% ± 7.9% after 24 and 48 h, respectively. Whole-body and blood half-lives were 19.3 ± 0.6 and 6.0 ± 1.4 h, respectively. Urinary excretion was 25.3% ± 3.3% and 36.4% ± 4.2% after 24 and 48 h, respectively. Significant bowel activity could not be detected on serial images within 2 d. The maximum uptake into the bone marrow was lower and the elimination was faster (resulting in shorter residence times of the bone marrow) for ^{188}Re-MAb than for ^{99m}Tc-MAb (Tables 2 and 3). However, bone marrow uptake of the 2 radiotracers did not correlate.

Radiation Absorbed Dose Calculations

The radiation absorbed doses (mGy/MBq), derived from the biologic data of ^{99m}Tc-MAb and the physical data of ^{188}Re, delivered to the total body, red marrow, lung, liver, spleen, and kidneys were 0.14 ± 0.02, 2.24 ± 0.62, 0.05 ± 0.05, 0.50 ± 0.36, 1.93 ± 1.39, and 0.90 ± 0.25, respectively. For ^{188}Re-MAb, the radiation absorbed doses (mGy/MBq) delivered to the total body, red marrow, lung, liver, spleen, and kidneys were 0.14 ± 0.02, 1.45 ± 0.71, 0.07 ± 0.02, 0.43 ± 0.21, 1.32 ± 0.99, and 0.71 ± 0.17, respectively. The differences were statistically significant for the total body, red marrow, spleen, and kidneys ($P < 0.05$, paired t test), primarily because of lower uptake of ^{188}Re-MAb to the bone marrow combined with faster elimination.

Clinical Application of the ^{188}Re-MAb

Based on the latter dosimetric values, a median bone marrow dose of 14.0 ± 7.2 Gy was achieved after administration of 9.7 ± 1.5 GBq ^{188}Re-MAb. Therapy was well tolerated, with the exception of mild-to-moderate nausea (5/12 patients). Allergic and pharmacologic reactions did not occur. Rapid and stable engraftment was observed in all patients. One patient died from veno-occlusive disease, one from CMV pneumonia, one from septicemia, and one from relapse of acute myelogenous leukemia. The other 8 patients are in good clinical condition with no evidence of disease (mean follow-up, 8 mo).

DISCUSSION

Advanced leukemia has a high relapse rate despite high-dose chemotherapy and TBI. Increasing the dose of TBI reduces the probability of relapse but increases mortality from toxicity. However, selective radiation delivery to the bone marrow is possible using specific MAbs labeled with a β emitter, as shown by Scheinberg et al. (*4*) using ^{131}I-MAbs reacting with a glycoprotein found on myeloid leukemia blasts (CD33). The intention is not to kill the labeled cell directly but to irradiate the environment of the tagged cell within a mean range of approximately 1 mm. Unspecific binding to the reticuloendothelial system of the liver and spleen predicts high radiation absorbed doses, but rapid deiodination actually decreases the radiation dose in these organs. The use of highly specific MAbs as well as a β emitter that does not accumulate in any organ can reduce these side effects. A promising radioisotope for various

TABLE 1
Characteristics of Patients Undergoing Radioimmunotherapy for Conditioning of Advanced Leukemia

Patient no.	Age (y)	Sex	Diagnosis	Conditioning therapy	GvHD-proph.	Stem cell source	Risk factor	Previous transplantation	Donor
1	38	F	AML 4.CR	Bu/Cy	TCD	PB	>CR1	Allo-BMT	Family mismatch (1 locus)
2	52	M	AML CR2	TBI/Cy	TCD	PB	>CR1		Sibling identical
3	44	M	AML PR2	TBI/Cy	TCD	BM	Relapse		Sibling identical
4	49	M	CML Ph− CP2	TBI/Cy	TCD	PB	Extramedullary blast crisis		MUD identical
5	34	M	CML Ph+ CP2	Bu/Cy	TCD	PB	relapse, >CP1	Auto-PBSCT	MUD identical
6	18	F	ALL CR2	TBI/Cy	TCD	PB	>CR1		Family mismatch (1 locus)
7	44	M	ALL Ph+ CR1	TBI/Cy	TCD	PB	Ph+		Sibling identical
8	27	F	AML PD after PR1	TBI/Cy/TT	TCD	PB	No CR		Family mismatch (2 loci)
9	49	M	AML CR2	TBI/Cy	TCD	PB	>CR1		Sibling identical
10	35	F	AML CR2	TBI/Cy	TCD	PB	>CR1		MUD identical
11	32	F	AML PR1	TBI/Cy	TCD	PB	PR		Family mismatch (2 loci)
12	55	M	B-CLL	TBI/Cy	TCD	PB	PR		Sibling identical

GvHD-proph. = graft-versus-host disease prophylaxis; AML = acute myelogenous leukemia; 4.CR = fourth complete remission; Bu = busulfan, 4 × 4 mg/kg; Cy = cyclophosphamide, 2 × 60 mg/kg; TCD = T cell depletion; PB = peripheral blood; CR1 = first complete remission; Allo-BMT = allogeneic bone marrow transplantation; CR2 = second complete remission; PR2 = second partial remission; BM = bone marrow; CML = chronic myelogenous leukemia; Ph− = Philadelphia chromosome–negative; CP2 = second chronic phase; MUD = matched unrelated donor; Ph+ = Philadelphia chromosome–positive; CP1 = first chronic phase; Auto-PBSCT = autologous peripheral blood stem cell transplantation; ALL = acute lymphoblastic leukemia; PD = progressive disease; PR1 = first partial remission; TT = thiotepa, 10 mg/kg; CR = complete remission; CLL = chronic lymphoblastic leukemia.

therapy procedures (e.g., pain palliation of bone metastases or endovascular brachytherapy), including radioimmunotherapy, is the high-energy β emitter ^{188}Re.

^{188}Re-perrhenate can be obtained daily from the ^{188}W/^{188}Re radionuclide generator. Perrhenate has a chemical form similar to that of pertechnetate, resulting in similar labeling techniques (*25*). Labeling procedures for MAbs using ^{188}Re have been reported (*9–11*). ^{99m}Tc-MAb against nonspecific cross-reacting antigen 95 (NCA-95) binds to granulocytes and other myeloid precursors (*14–16*). Approximately 55% of the injected dose is taken up from bone marrow within 1 h, and the radiotracer is slowly eliminated from there with a biologic half-life of 44 h (*13*). In contrast to other available MAbs (anti-CD33 and anti-CD45), this kit is commercially available and easy to label with ^{99m}Tc. Important modifications in the labeling procedure have been undertaken to enhance the number of binding sites for labeling with ^{188}Re (*12*). However, the different specific activity (e.g., 30-fold more molecules need to be bound for therapy with ^{188}Re perrhenate than for diagnostic use of ^{99m}Tc-MAb) required a comparison of ^{99m}Tc- and ^{188}Re-MAb biodistribution.

TABLE 2
Radiation Absorbed Doses

Patient no.	Total body	Liver	Spleen	Lung	Kidney	Bone marrow	Small intestine	Upper large intestine	Lower large intestine	Ovary	Testis
1	0.15 (0.17)	0.75 (0.76)	1.53 (3.12)	0.09 (0.20)	0.84 (1.29)	1.09 (2.04)	0.09 (0.05)	0.09 (0.05)	0.09 (0.05)	0.09 (0.05)	
2	0.12 (0.12)	0.35 (0.34)	0.57 (0.70)	0.07 (0.03)	1.03 (0.99)	1.05 (2.42)	0.07 (0.03)	0.07 (0.03)	0.07 (0.03)		0.07 (0.02)
3	0.12 (0.13)	0.33 (0.24)	1.03 (1.17)	0.06 (0.05)	0.93 (1.24)	1.46 (1.92)	0.06 (0.05)	0.06 (0.05)	0.06 (0.05)		0.05 (0.05)
4	0.12 (0.13)	0.48 (0.39)	1.65 (3.43)	0.07 (0.04)	0.79 (0.93)	0.97 (2.21)	0.08 (0.04)	0.08 (0.04)	0.07 (0.04)		0.07 (0.03)
5	0.13 (0.14)	0.93 (1.52)	1.11 (3.23)	0.09 (0.03)	0.63 (0.82)	0.27 (1.67)	0.09 (0.03)	0.09 (0.03)	0.09 (0.03)		0.09 (0.03)
6	0.16 (0.17)	0.23 (0.43)	0.50 (1.49)	0.11 (0.06)	0.85 (1.28)	1.14 (2.17)	0.11 (0.06)	0.11 (0.06)	0.11 (0.06)	0.11 (0.06)	
7	0.10 (0.10)	0.34 (0.19)	1.34 (0.76)	0.03 (0.03)	0.56 (0.83)	1.60 (1.69)	0.03 (0.03)	0.03 (0.03)	0.03 (0.03)		0.03 (0.03)
8	0.17 (0.17)	0.55 (0.50)	1.21 (1.49)	0.04 (0.01)	0.59 (0.64)	2.78 (3.43)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	
9	0.12 (0.12)	0.39 (0.34)	1.25 (1.56)	0.06 (0.04)	0.63 (0.70)	1.58 (1.97)	0.06 (0.04)	0.06 (0.04)	0.06 (0.04)		0.05 (0.04)
10	0.15 (0.15)	0.28 (0.52)	0.69 (0.64)	0.08 (0.05)	0.62 (0.76)	1.58 (2.01)	0.08 (0.05)	0.08 (0.05)	0.08 (0.05)	0.08 (0.05)	
11	0.16 (0.17)	0.33 (0.24)	0.74 (0.64)	0.04 (0.02)	0.58 (0.66)	2.72 (3.54)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	0.04 (0.02)	
12	0.12 (0.13)	0.25 (0.53)	4.23 (4.88)	0.06 (0.04)	0.52 (0.68)	1.13 (1.84)	0.06 (0.04)	0.06 (0.04)	0.06 (0.04)		0.06 (0.04)
Mean	0.14 (0.14)	0.43 (0.50)	1.32 (1.93)	0.07 (0.05)	0.71 (0.90)	1.45 (2.24)	0.07 (0.04)	0.07 (0.04)	0.07 (0.04)	0.07 (0.04)	0.06 (0.03)
SD	0.02 (0.03)	0.21 (0.36)	0.99 (1.39)	0.02 (0.05)	0.17 (0.25)	0.71 (0.62)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.02)	0.02 (0.01)

Values are mGy/MBq and are derived from ^{188}Re-MAb. Values derived from ^{99m}Tc-MAb and predicted to ^{188}Re-MAb by exchange of physical properties are given in brackets parentheses.

TABLE 3
Kinetic Data of ^{188}Re-MAb

Patient no.	Bone marrow uptake 2 h	24 h	48 h	Biologic half-life (h)	Urinary excretion 24 h	48 h
1	0.36 (0.49)	0.22 (0.42)	ND	43 (69)	0.26 (0.14)	ND
2	0.13 (0.64)	0.16 (0.72)	0.12	71 (ND)	0.28 (0.06)	0.36
3	0.24 (0.43)	0.22 (ND)	0.20	277 (95)	0.29 (ND)	0.41
4	0.24 (0.66)	0.20 (0.52)	0.20	77 (69)	0.25 (0.19)	0.36
5	0.12 (0.42)	0.04 (0.37)	ND	18 (107)	0.22 (0.16)	ND
6	0.17 (0.61)	0.11 (0.45)	0.10	54 (44)	0.27 (10.8)	0.39
7	0.29 (0.40)	0.22 (0.42)	0.21	60 (274)	0.19 (0.13)	0.28
8	0.46 (0.81)	0.39 (0.72)	0.31	59 (166)	0.22 (0.19)	0.35
9	0.34 (0.45)	0.31 (0.45)	0.22	98 (146)	0.27 (0.20)	0.35
10	0.56 (0.42)	0.43 (0.42)	0.33	60 (329)	0.31 (0.23)	0.43
11	0.34 (0.49)	0.31 (0.44)	0.28	96 (147)	0.25 (0.16)	0.36
12	0.42 (0.48)	0.35 (0.41)	0.30	65 (99)	0.25 (0.14)	0.35
Mean	0.31 (0.52)	0.25 (0.49)	0.23	82 (140)	0.25 (0.15)	0.36
SD	0.13 (0.13)	0.11 (0.12)	0.08	65 (89)	0.03 (0.05)	0.04

ND = not determined.
Values are % injected dose. Values derived from ^{99m}Tc-MAb are given in parentheses.

Breitz et al. (*9*) could not show a significant difference in biodistribution of ^{99m}Tc- and ^{186}Re-MAb. However, Juweid et al. (*11*) showed that ^{188}Re-MN-14 (an immunoglobulin G anti–carcinoembryonic antigen MAb) had a biologic half-life shorter than that previously reported for ^{131}I-MN-14 (8.2 for ^{188}Re-MN-14 compared with 27.3 h for ^{131}I-MN-14). They assumed that the shorter blood half-life of ^{188}Re-NM-14 may have been related, at least in part, to a relative in vivo instability of the ^{188}Re-conjugate used. In our study, we observed both a significantly shortened biologic half-life of ^{188}Re-MAb, compared with the ^{99m}Tc-MAb (82 ± 65 and 140 ± 89 h, respectively), and a reduced bone marrow uptake (31% ± 13% and 52% ± 13%, respectively). The diminished bone marrow uptake might have been caused by a lower affinity of ^{188}Re-MAb than of ^{99m}Tc-MAb. The substantial difference between ^{99m}Tc- and ^{188}Re-MAbs suggests that the presumed in vivo instability of ^{188}Re-MAb manifested itself early. However, in vitro studies showed a completely intact immunoreactivity (*12*). The shorter biologic half-life can be explained by in vivo instability as assumed by Juweid et al. Quality control experiments confirmed that only minor amounts of free perrhenate were injected (<5%). However, in vitro quality control revealed 30% reoxidation of ^{188}Re-MAb within 24 h (Fig. 1), compared with nearly complete in vitro stability of ^{99m}Tc-MAb. These findings correspond to the 24-h urinary excretion of ^{99m}Tc- and ^{188}Re-MAb, with values of 15.4% ± 4.6% and 25.3% ± 3.3%, respectively. Furthermore, ^{188}Re was not readsorbed or bound at any protein but was still present as perrhenate. Thus, renal elimination by glomerular filtration is not hindered, and radiation protection can be performed by perchlorate (*24*). However, the differences in bone marrow and other organ uptake are not fully explained by the difference in urinary clearance of both MAbs, suggesting that some breakdown products (presumably low-molecular-weight peptides) of the ^{188}Re-MAb may have remained in the body (presumably in the kidneys) and resulted in an increased renal dose with ^{188}Re-MAb.

Differences between the biokinetic data of ^{99m}Tc- and ^{188}Re-MAb are not caused by the estimation of bone marrow size because the same value was assumed for both studies. Furthermore, the ROI size of the bone marrow of the spine—0.29 ± 0.03 of the total size—agrees well with the data of Cristy (*26*), who estimated that the spine contains 32.3% of the active bone marrow in healthy adults. The substantial difference between the attenuation for ^{99m}Tc (0.1153/cm) and ^{188}Re (0.0563/cm) is probably caused by the high-energy photons of ^{188}Re (478 and 633 keV, 1% each) and their scatter. Reduction of window width from 20% to 10% results in an increased attenuation for ^{188}Re (0.073/cm). However, biokinetic data are not substantially affected as long as the imaging technique is constant in a single patient. Different imaging intervals also did not affect the biokinetic data. Biokinetic data of ^{188}Re-MAb derived from 24- and 48-h measured intervals are not statistically different (mean differences for whole body and bone marrow were 0.8% ± 1.3% and 0.05% ± 1.9%, respectively).

Bone marrow accumulation of ^{99m}Tc-MAb was reported to be influenced by age and to depend on the type of hematologic disorder (*27,28*). The uptake depends on the number of binding sites; therefore, active bone marrow provides more cells with a larger surface area and more binding sites. The uptake of ^{188}Re-MAb was 12% in a patient with chronic myeloid leukemia and 46% in a patient with acute myeloid leukemia in relapse (Tables 1 and 3). Matthews et al. (*1*) observed that in cases of acute myeloid leukemia, the estimated radiation absorbed dose was 1.52-fold higher than in cases of acute lymphatic leukemia, and the dose was 2.2-fold higher in relapse than in remission. Therefore, the stimulation of bone marrow proliferation using growth-stimulating cytokines can potentially improve the attachment of radiolabeled MAb to bone marrow cells and reduce the radiation exposure of other organs (*1*).

The broad range of biokinetic data requires individual dosimetric measurements and calculations (*29*). Dosimetric data derived from high-quality images of ^{99m}Tc-MAb could not be transferred to ^{188}Re-MAb because no functional relationship existed between the 2 and interpatient differences were large. The mean ratio of radiation absorbed doses for bone marrow (^{188}Re/^{99m}Tc) was 0.60, but the range of values—0.16 to 0.81—is not acceptable for therapeutic use. Furthermore, different bone marrow activities rule out the use of mean values derived from ^{188}Re-MAb. In our study, the mean radiation absorbed dose of the bone marrow yielded 1.45 mGy/MBq, but individual values ranged from 0.27 to 2.78 mGy/MBq. At a minimum, exact dosimetric measurements are necessary to define the limits of toxicity. Dose escalation studies have the disadvantage of not consid-

ering the variation in number of bone marrow binding sites for different diseases (*30*).

One advantage of ^{188}Re-MAb over ^{131}I-MAb is that radiation absorbed doses are lower to the lung and similar to the liver. Using ^{188}Re-MAb, the mean ratio of radiation delivered to the marrow was 3.37 compared with the liver, 20.7 compared with the lung, and 2.04 compared with the kidney. Using ^{131}I-labeled anti-CD45 MAb, the mean ratio of radiation delivered to the marrow was 2.7 compared with the liver, 3.4 compared with the lung, and 11.3 compared with the kidney (*1*). Another advantage is the high (approximately 4-GBq/mg) specific radioactivity of ^{188}Re-MAb, with no pharmacologic effect or HAMA induction (*13,31*). The ^{131}I-MAb is given in a specific activity of 185–300 MBq/mg, requiring a protein dose of 50–74 mg (*1,4,32*). Therefore, a HAMA response was observed in 37%, with the earliest positive sera seen at 2 wk (*7*). A third advantage of ^{188}Re-MAb compared with ^{131}I-MAb concerns radiation protection. The γ energy is lower from ^{188}Re (155 keV compared with 364 keV) and is emitted with a probability of only 15% (compared with 81% in decay of ^{131}I). The low probability of γ emission, resulting in a dose of only 20 μSv/h 1 m from a patient who receives 4 GBq ^{188}Re-MAb, is advantageous in protecting the staff from radiation. Therefore, radioimmunotherapy might be possible even in outpatients (*11,33*).

CONCLUSION

Important differences found between the biokinetics of ^{188}Re- and ^{99m}Tc-MAb make individual dosimetry using ^{188}Re-MAb necessary. The differences were in the initial bone marrow uptake of labeled MAb and the speed of elimination. Marrow uptake of ^{188}Re-MAb was less, and elimination faster, compared with ^{99m}Tc-MAb. These differences were related to differences in the stability of the radiotracers, as shown by HPLC. A mean of 10 GBq ^{188}Re-MAb applied in 12 patients with leukemia yielded a mean of 14 Gy to the bone marrow and 7 Gy to the kidneys. This radioimmunotherapy was well tolerated in addition to standard conditioning therapy with whole-body irradiation and chemotherapy.

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