Therapeutic advantages of Auger electron- over β-emitting radiometals or radioiodine when conjugated to internalizing antibodies

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Abstract. Recent studies suggest a higher anti-tumour efficacy of internalizing monoclonal antibodies (MAbs) when labelled with Auger electron emitters, as compared with β-emitters. The aim of this study was to compare the anti-tumour efficacy and toxicity of the internalizing MAb, CO17-1A, labelled with Auger electron emitters (^{125}I, ^{111}In) versus conventional β$^-$-emitters (^{131}I, ^{90}Y) in a colon cancer model, and to assess whether the residualizing radiometals may have therapeutic advantages over the conventionally iodinated conjugates. Biodistribution studies of ^{125}I-, ^{111}In- or ^{88}Y-labelled CO17-1A were performed in nude mice bearing subcutaneous human colon cancer xenografts. For therapy, the mice were injected with either unlabelled or ^{125}I-, ^{131}I-, ^{111}In- or ^{90}Y-labelled CO17-1A IgG_{2a}, whereas control groups were left untreated or were given a radiolabelled isotype-matched irrelevant antibody. The influence of internalization was assessed by comparing the results with those obtained with an anti-carcinoembryonic antigen (CEA) antibody which does not internalize to a relevant extent. The maximum tolerated activities (MTA) and doses (MTD) of each agent were determined. Myelotoxicity and potential second-organ toxicities, as well as tumour growth, were monitored. Bone marrow transplantation (BMT) was performed in order to enable dose intensification. Radiometals showed significantly better tumour-to-blood ratios than the respective iodinated conjugates. The MTAs of ^{131}I- and ^{125}I-CO17-1A without artificial support were 11.1 MBq (300 μCi) and 111 MBq (3 mCi), respectively; the MTA of the metals was reached at 4 MBq (100 μCi) for ^{90}Y-, and at 85 MBq (2.3 mCi) for ^{111}In-CO17-1A. Myelotoxicity was dose limiting in all cases. BMT enabled an increase in the MTA to 15 MBq (400 μCi) of ^{131}I-labelled CO17-1A, to 4.4 MBq (120 μCi) of ^{90}Y-labelled CO17-1A, and to 118 MBq (3.2 mCi) of ^{111}In-labelled CO17-1A, while the MTA of ^{125}I-CO17-1A had not been reached at 185 MBq (5 mCi) with BMT. Whereas no significant therapeutic effects were seen with unlabelled CO17-1A, tumour growth was retarded significantly with its radiolabelled forms. The therapeutic results were significantly (P<0.01) better with both Auger electron emitters (^{125}I and ^{111}In) than with the β-emitters, and, in accordance with the biodistribution data, a trend towards better therapeutic results was found with radiometals (more complete remissions) as compared with radioiodine. In contrast, at equitoxic doses, no significant difference was observed in the therapeutic efficacy of ^{131}I- versus ^{125}I-labelled non-internalizing anti-CEA antibody, F023C5. These data suggest that, at equitoxic doses, the therapeutic efficacy of internalizing MAbs labelled with Auger electron emitters, such as ^{125}I or ^{111}In, is superior to that of internalizing MAbs labelled with conventional β-emitters. The lower toxicity of Auger electron emitters may be due to the short path length of their low-energy electrons, which can reach the nuclear DNA only if the antibody is internalized (as is the case in antigen-expressing tumour tissue, but not in the stem cells of the red marrow).

Key words: Radioimmunotherapy – Internalizing monoclonal antibody – Auger/conversion electron emitter – Radiometal – Indium-111 – Yttrium-90

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Introduction

Radioimmunotherapy is an attractive therapeutic concept, aiming to deliver tumoricidal radiation doses to tumours without causing significant radiation toxicity to normal tissues [1]. Indeed, in radiosensitive tumours, such as non-Hodgkin's lymphoma, radioimmunotherapy has led to long-term remissions or even cures in a high percentage of patients [2]. In solid tumours, however, success is still limited [3]. Frequently, myelotoxicity becomes dose limiting before sufficiently high tumour doses can be reached [4, 5], owing to the radiation exposure of haematopoietic stem cells to beta and gamma emissions of most radionuclides currently used [4, 5].

More recently, the use of low-energy Auger electron emitters, conjugated to antibodies that internalize selectively into the target cancer cells, has been proposed [6, 7]. Due to their short path length, the low-energy Auger electrons can reach the nuclear DNA only if the antibody is internalized, as is the case in the antigen-expressing tumour tissue but not in other non-malignant cells, e.g. the haematopoietic cells in the red marrow. Therefore, higher anti-tumour efficacy at lower toxicity may be anticipated [8]. Iodine-125 is the most commonly used Auger electron emitter in this setting. It decays by electron capture with a physical half-life of 60.1 days to stable tellurium-125, thereby releasing a shower of as many as 21 low-energy Auger and conversion electrons [9, 10, 11]. The path length of these electrons is, at 0.06–17 µm, too short to reach the DNA if the nuclear decay does not occur within the cell; however, ^{125}I disintegrating close to the DNA has a relative biological effectiveness which is close to high linear energy transfer (LET) radiation [8, 9].

Almost a decade ago, Woo and co-workers showed selective chromosomal damage and cytotoxic effects of the ^{125}I-labelled monoclonal antibody CO17-1A in human colon cancer cells in vitro [12]. This antibody (IgG_{2a} subytpe) reacts with a tumour-associated 41-kDa glycoprotein expressed on gastrointestinal adenocarcinomas and is internalized after antigen binding [12, 13, 14, 15]. More recently, two clinical studies using ^{125}I-labelled internalizing antibodies against gastrointestinal malignancies have been published: Meredith et al. [6] showed in 28 patients that high activities (up to 250 mCi) of the ^{125}I-labelled chimeric form of CO17-1A did not result in any significant bone marrow suppression or other toxicity [6]. Welt et al. [7] reported on 21 patients treated with the ^{125}I-labelled internalizing antibody A33. They observed some encouraging anti-tumour effects at injected activities of up to 728 mCi per patient, although dose-limiting toxicity was not reached.

Despite extensive literature on the biological effects of ^{125}I in vitro [16], only very few *systematic* analyses have been published that compare the toxicity and anti-tumour efficacy of an *internalizing monoclonal antibody* labelled with ^{125}I versus ^{131}I at *equitoxic* doses in vivo [17, 18, 19]. Therefore, the aim of this study was to determine the maximum tolerated doses and dose-limiting organ toxicities of the ^{125}I-labelled monoclonal antibody CO17-1A in comparison with its ^{131}I-labelled form, as well as to assess the therapeutic efficacy of both radiolabels in a human colon cancer xenograft model in nude mice. We additionally wanted to assess whether residualizing radiometals will lead to therapeutic advantages over non-residualizing radioiodinated conjugates. Preliminary parts of these findings, suggesting a therapeutic advantage of ^{125}I over ^{131}I, have been published recently [20], whereas here we present the complete study, extending to the therapeutic efficacy and toxicity data of Auger electron-emitting residualizing radiometals as compared with the non-residualizing iodine isotopes.

Materials and methods

Antibodies. The murine monoclonal antibody CO17-1A was obtained from GlaxoWellcome (Hamburg, Germany). Its generation and production have been described in detail previously [13, 14, 15]. It is an IgG_{2a} isotype directed against a 41-kDa glycoprotein which is found on human gastrointestinal malignancies, but to a lesser extent on normal epithelia as well [15]. Its affinity has been determined as 5×10^7 l/mol [21, 22]. The internalizing properties of CO17-1A have been described in detail previously [12].

Two control antibodies were used. The first, tumour-specific, is an anti-CEA antibody (clone F023C5) which specifically binds to CEA-expressing cells without being internalized to a significant extent. It belongs to the IgG_1 isotype and has an affinity similar to that of CO17-1A [23, 24]. The second, non-specific isotype-matched control was the anti-human-CD3 antibody, OKT3. OKT3 is a murine IgG_{2a} and was obtained from CILAG (Sulzbach/Taunus, Germany) [20].

Radionuclides and radiolabelling procedures. Iodine-125 and iodine-131 were purchased as sodium iodide in 0.1 *M* NaOH from DuPont (Bad Homburg, Germany). Radioiodination was performed using the iodogen method as described previously [24].

Isothiocyanate benzyl-diethylene tri-amine penta-acetate (SCN-Bz-DTPA) conjugates were prepared according to Brechbiel et al. [25]. For biodistribution studies, yttrium-88 was obtained as $^{88}YCl_3$ in 0.1 *M* HCl from Amersham Life Science (Braunschweig, Germany). Yttrium-90 was obtained from Pacific Northwest National Laboratory (Richland, Wash.) as $^{90}YCl_3$ in 0.05 *M* HCl. Indium-111 was obtained as $InCl_3$ from Mallinckrodt (Petten, The Netherlands) in 0.05 *M* HCl. Radiometal labelling followed procedures described previously [26]. Briefly, the antibody chelate was buffered in 0.5 *M* NaOAc (pH 5.5). The activity was added in the same buffer and incubated for 30 min at 37°C in a water bath. Unbound radiometals were removed by gel filtration chromatography on a PD-10 column (Sigma Chemie, Deisenhofen, Germany) which was eluted with phosphate-buffered saline. Excess of DTPA was added to each preparation to chelate any unbound indium or yttrium, in order to assure its rapid renal excretion. The quality of each preparation was tested by high-performance liquid chromatography as well as by measuring its immunoreactivity.

Animal model and biodistribution studies. Female nude mice, 19–23 g and 4–5 weeks old, were purchased from Charles River

(Sulzfeld, Germany). The human colon carcinoma cell line, GW-39 [27], was serially propagated as described previously [20, 26]. After approximately 10 days, subcutaneous tumours reached the size of approximately 100–200 mg [26], which was the size used in this study unless otherwise indicated.

Tumour-bearing animals were injected into the tail vein with the radiolabelled antibodies at the protein doses indicated. The mice were sacrificed at 1, 4, 24, 72, 168 and 336 h. They were bled by retro-orbital puncture. For determination of the whole-body retention, the mice were measured in a well counter. After cervical dislocation, the animals were dissected. The amount of activity in the tumours and various tissues was determined by gamma scintillation counting using an injection standard to account for physical decay, as described previously [26]. The number of animals used for each study was typically five animals per group at each time-point.

Radiation dosimetry. The biodistribution data were used to generate time-activity curves [26] which were fitted to the difference or sum of two exponents for curves with and without an uptake component, respectively. Cumulated activity in each organ was obtained by analytically integrating the resulting expressions over time.

Absorbed dose calculations for ^{125}I and ^{131}I were performed by assuming uniform deposition of all electron emissions within source volumes. For ^{125}I, a second estimate of the blood dose, which excludes energy deposition associated with electrons having energies <20 keV, is provided, since electrons with initial energies below 20 keV are not likely to reach the nucleus of non-antigen-expressing cells and, therefore, will not contribute to a biological effect. This blood dose may be seen as an index of potential haematological toxicity [4, 26, 28, 29].

The photon contribution to the dose estimates was obtained by adding the average photon dose contribution from a 25-g sphere, containing the whole-body cumulated activity obtained from the whole-body fits. The decays were assumed to be uniformly distributed throughout the 25-g volume in an infinite water medium [30]. Absorbed fractions for elliptical geometries were used to estimate the photon contribution to the dose to each organ [30].

Tumour absorbed dose estimates were performed using S factors published by Goddu et al. [31]. Additionally, the contribution of low-energy Auger electrons to the tumour doses was estimated. For this purpose, some fraction (for details, see Results) of the tumour-associated ^{125}I was assumed to be internalized and uniformly distributed within the cytosol of tumour cells. The cumulated activity associated with this cytosolic fraction was multiplied by "cytosol to nucleus" S factors [11, 32]. This increases the estimated tumour dose and better accounts for the effect of internalization and the short-ranged electron emissions of iodine-125.

For ^{111}In dosimetry, for all tissues except the bone, the assumption of uniform distribution of activity in a spherical mass was maintained, and doses were calculated, using S factors generated for ^{111}In in spheres of 0.1–1 g [26]. However, the γ contribution of the indium photons was neglected in this dose estimation. Since we have shown previously that accounting for cross-organ radiation is mandatory for ^{90}Y dosimetry in small animals, such as nude mice [33], the model recently published by Yoriyaz and Stabin was used [34].

Experimental radioimmunotherapy. Tumour sizes were determined by caliper measurement in three dimensions immediately before therapy and at weekly intervals thereafter [26]. Tumour volumes were estimated by multiplying the product of the three perpendicular diameters by 0.5, assuming an elliptical geometry. Tumours were either left untreated (controls) or injected with a single dose of radiolabelled antibody, with the activities indicated. Eight to 20 (mostly ten) animals were studied in each treatment group. Approximately 100- to 200-mg tumours were used for therapy experiments. As a non-specific, antibody isotype (IgG_{2a})-matched therapy control, the anti-CD3 antibody, OKT3, was used. As non-internalizing, but tumour-specific immunoglobulin, the anti-CEA antibody, clone F023C5, was studied, which has a similar affinity to CEA as CO17-1A has to its antigen. Body weight was recorded weekly, and survival was monitored. The maximum tolerated activities (MTAs) or doses (MTDs), respectively, were defined as the highest possible activities or doses under the respective conditions that did not result in any animal deaths, with the next higher dose level resulting in at least 10% of the animals dying [26].

Bone marrow transplantation for dose intensification; toxicity assessment. Bone marrow was harvested by sterile technique from untreated donor nude mice of the same strain. The marrow cavity of both mouse femurs was rinsed with 0.9% sterile saline, as described previously [35]. An inoculum of approximately 10^7 bone marrow cells was injected i.v. via the tail vein 120 h after radioantibody therapy injection.

Total and differential leucocyte and thrombocyte counts were determined on the day of therapy, and at weekly intervals thereafter [20]. Blood urea nitrogen (BUN) and creatinine, as well as glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase (AP), were determined on the day of radioimmunotherapy, and at weekly intervals thereafter [20].

Statistical analysis. Statistical analysis was performed as has been described previously [24]. Differences in the efficacy between the treatment modalities were statistically analysed by assuming an exponential tumour growth pattern. Non-linear regression analysis based on asymptotic approximation was used as described previously [36]. The Wilcoxon Rank Sum test was used to compare tumour and normal tissue uptake in biodistribution studies.

Results

Factors affecting the biodistribution of radioiodinated CO17-1A: the role of protein dose and tumour size

Figure 1 shows the biodistribution of radioiodinated CO17-1A at a 200-µg protein dose level in the major organs, as well as the whole-body and blood clearance. This protein amount has been shown previously to yield best tumour/non-tumour ratios [20]. Maximum tumour uptake occurred at 72 h p.i. (8.8%±1.9%ID/g in 100- to 200-mg tumours), the very time when tumour-to-blood ratios surpassed 1.0. The whole-body clearance followed a strictly mono-exponential pattern with a mean half-life of approximately 81 h (Fig. 1), whereas the blood clearance was bi-exponential ($t_{1/2}$-$\alpha \approx 6$ h, $t_{1/2}$-$\beta \approx 48$ h). The biodistribution of the anti-CD3 (i.e. irrelevant) IgG_{2a} isotype-matched antibody, OKT3, showed an almost identical blood and organ clearance pattern (data not shown), with exception of the tumour, where the uptake of the irrelevant antibody was, at 2.1%±0.4%ID/g at 72 h p.i.,

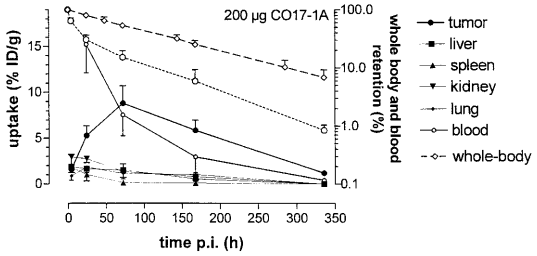


Fig. 1. Biodistribution of radioiodinated CO17-1A at a 200-µg protein dose level: whole-body and blood clearance (*dashed lines*, right y-axis) and biodistribution (uptake values in %ID/g) in the blood, major organs and the tumour (*solid lines*, left y-axis). The blood clearance is shown as retention of the injected activity in blood (*open circles, dashed line*, right y-axis) and in %ID/g (*open circles, solid line*, left y-axis)

significantly ($P<0.01$) lower than with the specific antibody (not shown).

Radioimmunotherapy with ^{125}I- versus ^{131}I-labelled CO17-1A: determination of MTDs, dose-limiting organ toxicities and anti-tumour efficacy as compared with unlabelled CO17-1A

In order to establish the MTA of ^{131}I- and ^{125}I-labelled CO17-1A, varying amounts of activity were injected, starting at 9 MBq (250 µCi) and 19 MBq (500 µCi), respectively, and proceeding in 10%–20% increments. At a 9-MBq level of ^{131}I-CO17-1A, moderate leukopenia and thrombocytopenia were observed, whereas no toxicity was noted in the 19-MBq ^{125}I group, or in animals given 200 µg of unlabelled CO17-1A. The MTA of ^{131}I-labelled CO17-1A was reached at 11 MBq (300 µCi), and the MTA of its ^{125}I-labelled form at 111 MBq (3 mCi). Severe myelosuppression occurred in both groups (Fig. 2a,b), and a further 10% increase in the administered activity resulted in 10%–30% deaths within 3–4 weeks p.i. in each group (Fig. 2c). No signs of second-organ toxicity were observed; BUN and creatinine as parameters of kidney function, as well as GOT and AP as indicators of liver function, did not show any significant changes.

Bone marrow transplantation given on day 5 after radioantibody injection allowed for an increase in the MTA by approximately 30% to 15 MBq (400 µCi) of ^{131}I-labelled CO17-1A (Fig. 2c). At this dose level, severe myelosuppression was again dose limiting. With ^{125}I-labelled CO17-1A and bone marrow transplantation, 185 MBq (5 mCi) was tolerated with only moderately severe myelotoxicity (nadirs at $1.36\pm0.34\times10^3$ white blood cells and $251\pm59\times10^3$ platelets per µl blood at 2 weeks post therapy), but the MTD had not yet been reached (Fig. 2c). Again, under bone marrow transplantation conditions, no signs of second-organ toxicity were noticed.

In untreated controls, the subcutaneous tumour xenografts grew rapidly, with a mean tumour volume doubling

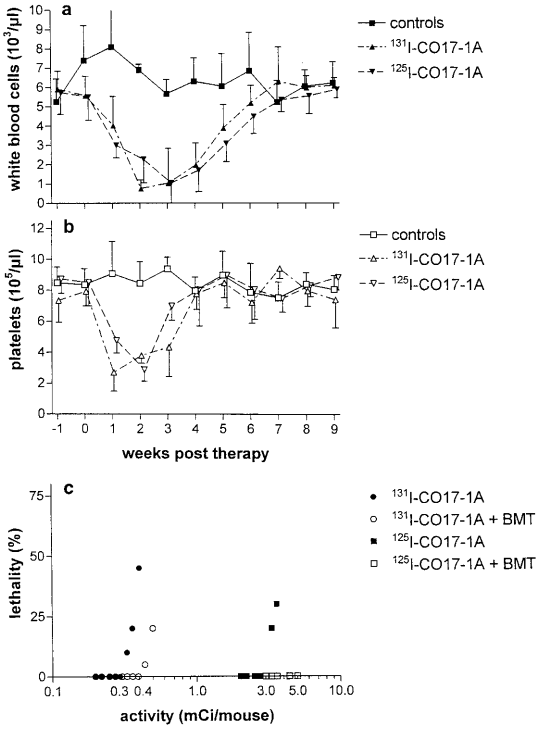


Fig. 2a–c. Myelotoxicity of ^{131}I- versus ^{125}I-labelled CO17-1A. **a** White blood cell and **b** platelet counts in the time course after therapy at the respective MTAs [11 MBq (300 µCi) ^{131}I-, 111 MBq (3 mCi) ^{125}I-CO17-1A]. **c** Lethality according to the administered activity of ^{131}I- and ^{125}I-labelled CO17-1A, defining the respective MTDs

time of less than 1 week (Fig.3a). At a 200-µg protein level, no significant anti-tumour effects were observed with unlabelled CO17-1A ($P=0.72$; Fig. 3a), whereas ^{131}I-labelled CO17-1A, at its MTD (11 MBq), led to a significant ($P=0.04$) growth retardation for approximately 7–8 weeks, at which time tumours again started to grow exponentially (Fig. 3a). At equitoxic dosing, ^{125}I-labelled CO17-1A (111 MBq) had significantly ($P=0.03$) better anti-tumour efficacy than its ^{131}I-labelled form (Fig. 3a). In contrast to the ^{131}I-label, ^{125}I was even able to induce "partial remissions" (i.e. a reduction in the tumour volume by more than 50%) in approximately half of the tumours. At the 111-MBq MTA of ^{125}I-CO17-1A, tumour growth was retarded by 10 weeks, and even afterwards, the slope of the growth curves was less steep than in ^{131}I-treated groups (cf. Fig. 3a).

Dose intensification by means of bone marrow transplantation led to a further improvement in the therapeutic efficacy of both ^{131}I- and ^{125}I-labelled CO17-1A antibody (Fig. 3b). However, whereas ^{131}I was not able to achieve remissions, even "complete remissions" (i.e. at least transient disappearance of the subcutaneous tumour nodules) were seen in 30% of animals treated with 185 MBq (5 mCi) ^{125}I-CO17-1A (partial remissions in 85%). In 15% of the animals so treated, no tumour regrowth was seen at 20 weeks and histological analysis of

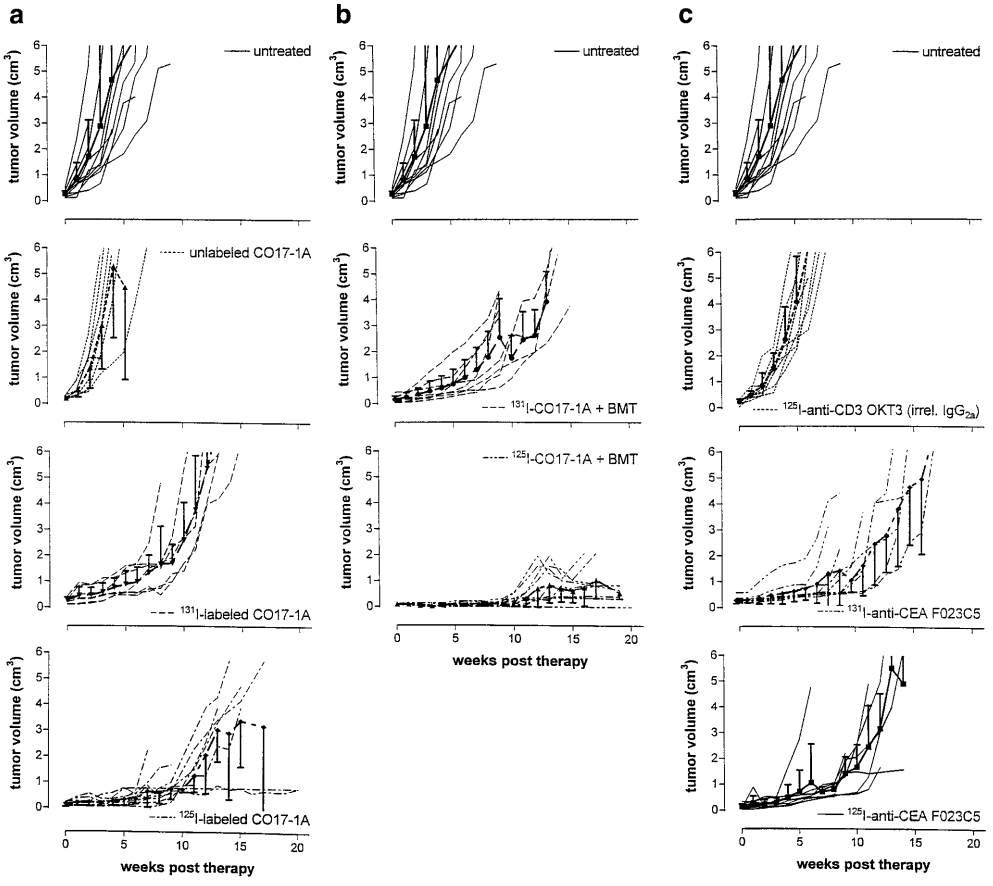


Fig. 3. a Therapeutic efficacy of ^{131}I-labelled and ^{125}I-labelled CO17-1A at their MTDs [11 MBq (300 µCi) and 111 MBq (3 mCi), respectively], as compared with untreated controls or animals given 200 µg unlabelled CO17-1A. **b** Therapeutic efficacy of ^{131}I-labelled CO17-1A at its MTD with bone marrow support [15 MBq (400 µCi)] and of 185 MBq (5 mCi) ^{125}I-CO17-1A with bone marrow support, as compared with untreated controls. **c** Efficacy of therapy with 111 MBq (3 mCi) ^{125}I-labelled irrelevant antibody (anti-CD3) or therapy with a non-internalizing anti-CEA antibody [11 MBq (300 µCi) ^{131}I-labelled vs 111 MBq (3 mCi) ^{125}I-labelled F023C5]. Again, results in untreated controls are also shown

the former tumour site did not show any viable tumour cells left. None of the tumours in animals treated with 185 MBq of ^{125}I-CO17-1A reached an exponential growth pattern again, whereas animals treated with ^{131}I-CO17-1A typically showed exponential tumour growth at 8–15 weeks post therapy (Fig. 3b).

Specificity of the observed effects: therapeutic efficacy of ^{131}I- versus ^{125}I-labelled non-internalizing or irrelevant antibodies

In order to evaluate whether the observed therapeutic superiority of ^{125}I-labelled CO17-1A is specifically due to its internalizing properties, control groups of animals were treated with 111 MBq (3 mCi) of the ^{125}I-labelled (irrelevant) anti-CD3 antibody, OKT3, or were given the ^{131}I- (11 MBq) or ^{125}I-labelled (111 MBq) non-internalizing anti-CEA antibody, F023C5. Although no formal MTD-finding studies were undertaken for these radiolabelled control immunoglobulins, the degree of the observed myelotoxicity was similar to that observed with the respective CO17-1A label, which renders the assumption of equitoxic dosing likely.

Whereas the irrelevant antibody, OKT3, was only able to achieve a slight growth retardation which was not significantly different (P=0.37) from untreated controls (Fig. 3c), the (specific) anti-CEA antibody, F023C5, had significantly (P=0.01) higher therapeutic efficacy. However, Fig. 3c shows that there was no significant difference in the therapeutic efficacy of this non-internalizing antibody, whether labelled with ^{125}I or ^{131}I (P=0.63). Furthermore, there was no significant difference in the therapeutic efficacy of $^{131/125}$I-F023C5 as compared with ^{131}I-CO17-1A.

Observed biological effects in relation to the radiation dosimetry

Table 1 shows the radiation dosimetry of ^{131}I- versus ^{125}I-labelled CO17-1A. This table differentiates into doses resulting exclusively from electrons and doses resulting from photons and electrons combined. Due to the almost tenfold lower energy liberated per nuclear transition, radiation doses per administered activity (i.e. in Gy/mCi) are considerably lower for ^{125}I than for ^{131}I label. This compares favourably with the empirically determined toxicities and MTAs, which differ by almost one order of magnitude between the two iodine isotopes. Whereas the photon contribution appears very low for ^{131}I, owing to the low probability of its high-energy photons being absorbed in the small volume of a mouse, it is much more prominent for ^{125}I, with its low-energy γ- and

Table 1. Radiation dosimetry of ^{131}I- versus ^{125}I-labelled CO17-1A in GW-39 bearing nude mice

Organ/ tissue	Electron contribution only				Photon contribution only[a]			
	^{131}I		^{125}I		^{131}I		^{125}I	
	Dose (Gy/mCi)	Dose at MTD (Gy/0.3 mCi)	Dose (Gy/mCi)	Dose at MTD (Gy/3 mCi)	Dose (Gy/mCi)	Dose at MTD (Gy/0.3 mCi)	Dose (Gy/mCi)	Dose at MTD (Gy/3 mCi)
Tumour	43.4	13.0	6.7	20.0	44.5	13.4	7.7	23.0
45% Internalization[b]	43.3	13.0	5.0	15.1	44.5	13.4	6.0	18.1
Liver	5.5	1.6	0.7	2.0	6.6	2.0	1.7	5.0
Spleen	4.2	1.3	0.5	1.5	5.4	1.6	1.5	4.5
Kidney	9.1	2.7	1.2	3.5	10.2	3.1	2.2	6.5
Lung	5.5	1.6	0.7	2.2	6.6	2.0	1.7	5.1
Intestine	7.1	2.1	0.8	2.4	n/d	n/d	n/d	n/d
Bone	3.7	1.1	0.6	1.8	n/d	n/d	n/d	n/d
Blood	56.0	16.8	7.1	21.2	57.2	17.2	8.1	24.2
No internalization[c]	56.0	16.8	3.2	9.7	57.2	17.2	4.2	12.7
Whole body	20.0	6.0	2.0	6.0	21.1	6.3	3.0	8.9

MTD, maximum tolerated dose; n/d, not determined

[a] The photon contribution assumes a 25-g sphere in infinite water medium [30]; thus, the given values most likely slightly overestimate the "true" absorbed fractions and doses

[b] Tumour doses in this row account for an internalized fraction of radioiodinated CO17-1A of 45% [20]; thus, it is assumed that only 45% of electrons with energies <20 keV will be able to reach the nuclear DNA

[c] Blood doses in this row exclude any contribution from electrons with an energy <20 keV (i.e. assume an internalized fraction of 0% in blood or bone marrow cells)

X-rays (cf. Table 1). Interestingly, at the respective MTDs [11 vs 111 MBq (0.3 vs 3.0 mCi) for ^{131}I and ^{125}I], calculated blood doses were approximately 25% higher for ^{125}I than for ^{131}I label (21 vs 17 Gy) when accounting for electrons only, regardless of their energy. When excluding any dose contribution from electrons with energies below 20 keV, ^{125}I blood doses drop by approximately half (Table 1). These low-energy electrons are not able to reach the nuclear DNA of bone marrow stem cells, if the decay occurs outside these non-antigen-expressing cells.

On the other hand, when assuming complete internalization of the radiolabel into the cytoplasm of tumour cells, tumour doses are almost twice as high with ^{125}I as compared with ^{131}I. However, as has been described in more detail elsewhere [20], the internalized fraction of radioiodinated CO17-1A is only approximately 45%±9% in vivo, which is well within the range reported for CO17-1A or similar antibodies in other tumour cell lines in vitro as well [11, 12]. When basing tumour dose calculations on this empirically determined internalized fraction, the differences in tumour doses at the respective MTDs are comparatively minor (e.g. 13 vs 15 Gy for ^{131}I- vs ^{125}I-CO17-1A, when accounting for the electron contribution only). Therefore, the dose estimates, as presented in Table 1, give the spectrum within which the actual radiation doses to tumours, bone marrow and organs will most likely range.

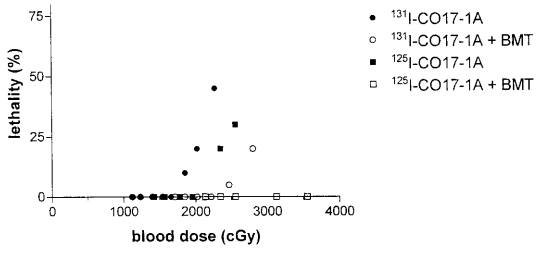


Fig. 4. Myelotoxicity-related lethality according to the radiation dose to the blood (cf. Fig. 2c). The dose estimates given in this figure account for all electron contributions regardless of their energy, but disregard any photon contribution

Figure 4 shows the myelotoxicity-related lethality in relation to the radiation doses to the blood, based on electron contributions only, i.e. excluding any photon influence. As per definition, no lethality occurred at the respective MTD, whereas an increase in administered activity by 10% led to at least 5%–10% deaths. Dose-limiting myelotoxicity was seen with ^{131}I-labelled CO17-1A at a blood dose of 17 Gy (cf. Fig. 4, Table1), and with its ^{125}I-labelled form at 21 Gy. This ^{125}I dose would drop to 10 Gy if the contribution from electrons with energies below 20 keV were to be excluded. On the other hand, mice would tolerate 21 Gy with ^{131}I label under BMT conditions, whereas 36 Gy administered by ^{125}I would not yet become dose-limiting with bone marrow support (corresponding to 16 Gy from electrons >20 keV).

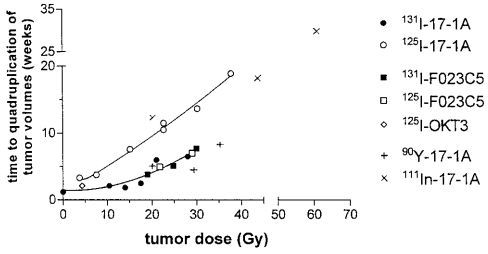


Fig. 5. Anti-tumour efficacy in relation to the tumour dosimetry for the Auger electron emitters, ^{125}I or ^{111}In, conjugated to an internalizing antibody (CO17-1A, here abbreviated as 17-1A) versus a tumour-specific but non-internalizing antibody (F023C5), an irrelevant antibody (OKT3), ^{90}Y-labelled CO17-1A and ^{131}I-labelled antibodies (internalizing as well as non-internalizing). The dose estimates given in this figure (x-axis) were calculated on the basis of the mean doses per activity administered (Tables 1 and 2), accounting for all electron contributions, but disregarding any photon contribution

Figure 5 shows the relation between calculated tumour doses (considering the electron contribution only, accounting for all energies, but disregarding the fact that, in reality, only a portion of the total activity will be internalized into the cytoplasm) and the resulting anti-tumour efficacy. As tumour growth retardation was the major observed effect in all treated groups, the correlation between mean tumour doses and the extent of induced growth retardation was analysed (Fig. 5). Since GW-39 is a very rapidly growing cell line with tumour volume doubling times of less than 1 week, and since only approximately half of the total radiation dose would be delivered to the tumour within this time frame, the time to volume duplication is not a good parameter for assessing anti-tumour efficacy. Therefore, Fig. 5 shows the correlation between tumour doses and the mean time needed for the *quadruplication* of tumour volume. No difference was seen in the anti-tumour efficacy of the ^{131}I-labelled antibodies according to whether they were internalizing (CO17-1A) or not (F023C5), and no difference was seen as compared with the ^{125}I-labelled non-internalizing antibody, F023C5. Below a tumour dose of approximately 10 Gy, no significant tumour growth delay was noticeable with these radiolabelled antibodies. Above this threshold, tumour growth was retarded in a dose-dependent fashion up to sixfold at approximately 20 Gy (the highest possible dose at the MTD with BMT). The resulting non-linear regression curve (regression coefficient: r=0.95) resembles a "shoulder curve" with its "shoulder" at approximately 10 Gy. In contrast, no threshold dose was seen with ^{125}I-CO17-1A; the time to tumour volume quadruplication was prolonged in an almost linearly increasing manner with increasing tumour dose (regression coefficient of the linear regression line: r=0.99). Comparable anti-tumour efficacy was seen with ^{125}I-CO17-1A at doses which were approximately half as high as those needed with the ^{131}I label or ^{125}I bound to non-internalizing antibodies.

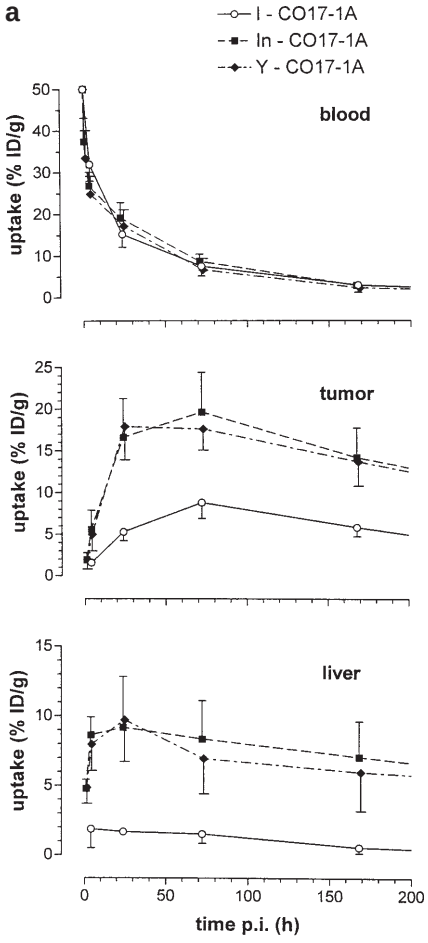
Biodistribution, toxicity and therapeutic efficacy of radiometal (^{111}In versus ^{90}Y)-chelated CO17-1A

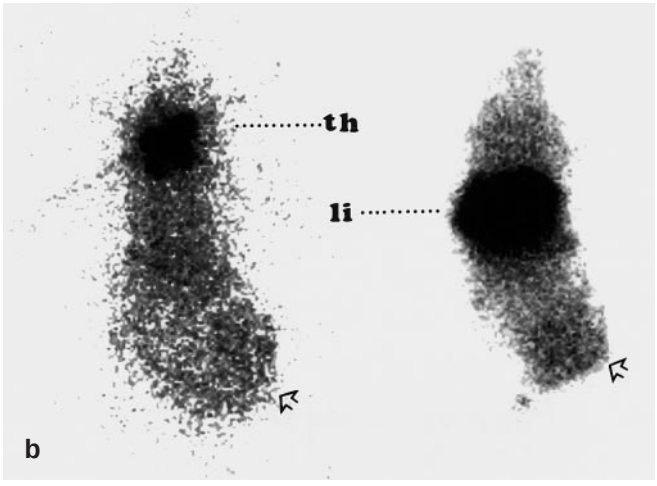
Figure 6 shows the comparative biodistribution of radioiodinated CO17-1A versus both radiometal (indium and yttrium) conjugates. Figure 6a shows the organ uptake in the blood (upper panel), the tumour (middle panel) and the liver (lower panel) for all three labels. Whereas no significant differences between the three labels were observed for the blood and typical "blood pool" organs (e.g. the lung or kidneys, data not shown), the uptake and retention of both metals was significantly higher in the tumour, liver and spleen (the latter not shown), which are those organs known to internalize the antibody either via antigen binding (i.e. in the tumour) or via IgG_{2a} receptor-mediated uptake (in the liver and spleen, see above). Accordingly, Fig.6b shows the comparative whole-body scans of the same animal at 48 h post ^{131}I-CO17-1A versus ^{111}In-CO17-1A injection (the view of the same animal at dissection is shown in Fig. 6c). Whereas the tumour is barely visible with radioiodine (left panel), it is clearly visualized with the radiometal (right panel). On the other hand, this figure also shows an intensive uptake of liberated radioiodine in the thyroid, which is in contrast to an intensive hepatic and splenic uptake with radiometal-labelled CO17-1A.

In order to establish the individual MTAs and dose-limiting toxicities, again, varying amounts of activity were injected, proceeding in 10%–20% increments. The MTAs of ^{90}Y- and ^{111}In-CO17-1A without artificial support were reached at 4 MBq (100 µCi) for ^{90}Y-, and at 85 MBq (2.3 mCi) for ^{111}In-CO17-1A. Again, myelotoxicity was dose limiting in both cases. In particular, no signs of (acute or chronic) hepatic toxicity were observed (no significant abnormalities in liver enzyme levels in blood, no significant changes in the liver histology). Accordingly, BMT enabled an increase in the MTA to 4.4 MBq (120 µCi) of the ^{90}Y- and to 118 MBq (3.2 mCi) of the ^{111}In-labelled CO17-1A.

The radiation dosimetry for the ^{111}In- versus ^{90}Y-labelled CO17-1A conjugates is shown in Table 2. The table shows that the blood dose at the MTD of ^{111}In (i.e. 24.8 Gy) compares very favourably with the blood dose at the MTD of ^{125}I-labelled CO17-1A (i.e. 24.2 Gy) (cf. Tables 1 and 2). Similar observations as for these Auger electron emitters hold true for both β-emitters as well (14.0 Gy vs 17.2 Gy for ^{90}Y- vs ^{131}I-CO17-1A). The observed lack of hepatic toxicity is in accordance with liver doses below 20 Gy with both ^{111}In- and ^{90}Y-labelled conjugates. The considerably lower tumour doses with ^{90}Y than with ^{111}In are due to differences in the absorbed fractions of the two radionuclides, where, in contrast to the Auger electrons of ^{111}In, the long path length of the electrons of ^{90}Y leads to a considerable loss of radiation energy outside the tumour [26].

Figure7 shows the therapeutic efficacy of ^{111}In- versus ^{90}Y-labelled CO17-1A in subcutaneous GW-39 xeno-





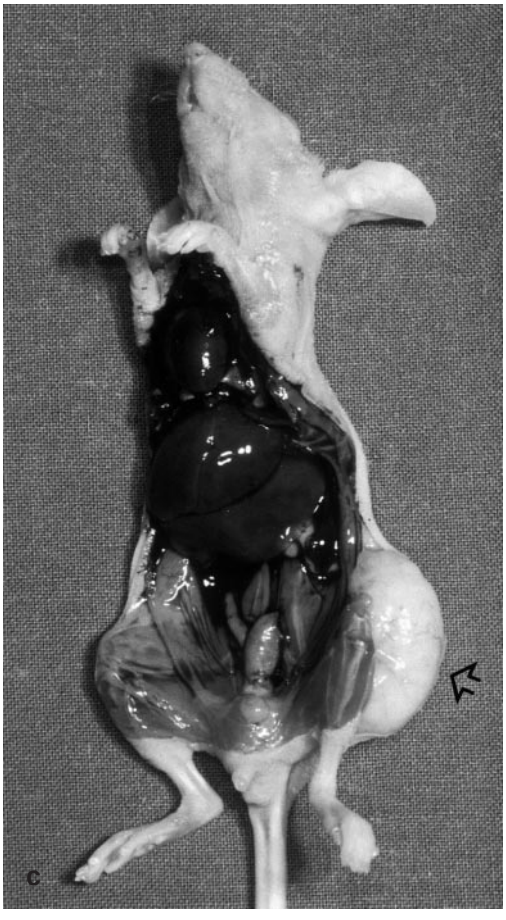


Fig. 6a–c. Biodistribution of iodinated versus radiometal-chelated CO17-1A. **a** The comparative biodistribution in the tumour (*middle panel*) and liver (*lower panel*) shows significantly higher uptake and retention of the radiometal chelates as compared with the radioiodinated antibodies, whereas no significant difference exists in the blood (*upper panel*) or blood pool organs (not shown). **b** External scintigraphy of the same animal, bearing a subcutaneous GW-39 xenograft, at 48 h after the injection of either ^{131}I- (*left*) or ^{111}In-labelled (*right*) CO17-1A. *Arrows*, tumour; *li*, liver; *th*, thyroid. The camera used was a Picker Prism 2000 gamma camera, equipped with high- or medium-energy parallel-hole collimators; 100 kcts were acquired in each case. **c** View of the same animal at dissection

graft-bearing nude mice. Again, a clear therapeutic advantage ($P<0.01$) of the Auger electron-emitting radionuclide ^{111}In over the conventional β-emitter ^{90}Y is demonstrated at equitoxic doses (i.e. the MTD). Interestingly, although no statistically significant difference in the mean tumour growth delay was observed between ^{111}In- and ^{125}I-CO17-1A at their respective MTDs (cf. Figs. 3a and 7), complete remissions at the conventional MTD without BMT were exclusively seen with ^{111}In, which is accordance with the higher tumour doses observed with this radiometal as compared with ^{125}I (cf. Tables 1 and 2). As had been observed for both radioiodinated immunoconjugates, dose intensification by means of BMT led to further improved therapeutic effects (data not shown).

In addition to the iodine isotopes, Fig. 5 shows the anti-tumour efficacy of the Auger electron-emitting radiometal, ^{111}In, conjugated to the internalizing antibody CO17-1A, versus its ^{90}Y-labelled form, in relation to the tumour dosimetry. Once more, the dose estimates given were calculated on the basis of the mean doses per activ-

Table 2. Radiation dose estimates of ^{90}Y- versus ^{111}In-labelled CO17-1A in GW-39 bearing nude mice (accounting for cross-organ irradiation according to the model of Yoriyaz and Stabin [34])

Organ/ tissue	^{90}Y		^{111}In	
	Dose (Gy/mCi)	Dose at MTD (Gy/100 µCi)	Dose (Gy/mCi)	Dose at MTD (Gy/2.3 mCi)
Tumour	293.8	29.4	19.0	43.7
Liver	116.8	11.7	7.5	17.3
Spleen	121.4	12.1	4.8	11.0
Kidney	61.9	6.2	3.6	8.3
Lung	47.6	4.8	4.3	9.9
Intestine	39.9	4.0	1.5	3.5
Blood	140.2	14.0	10.8	24.8

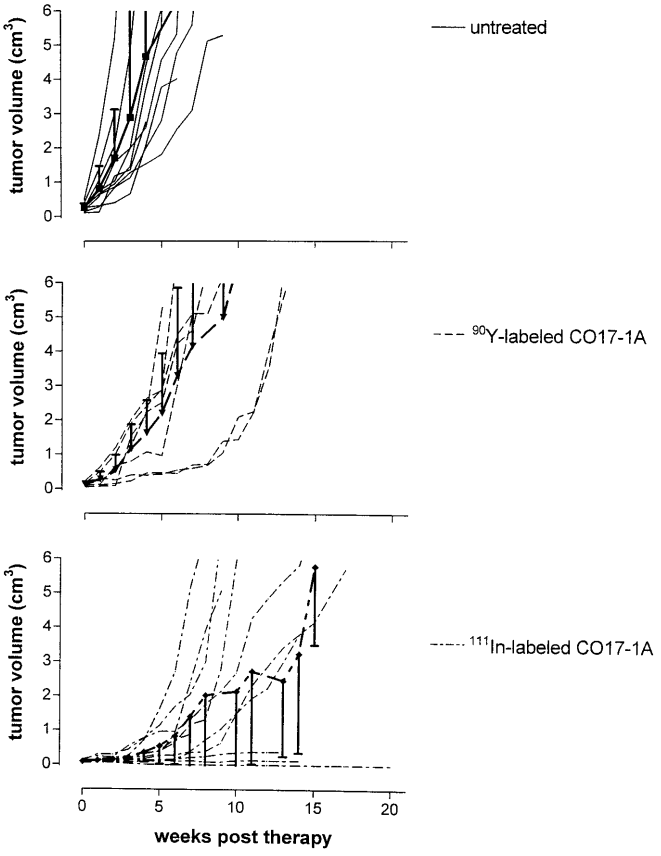


Fig. 7. Therapeutic efficacy of ^{90}Y-labelled versus ^{111}In-labelled CO17-1A at their MTDs [4 MBq (100 µCi) and 85 MBq (2.3 mCi), respectively], as compared with untreated controls

ity administered (Table 2), accounting for all electron contributions, including inter-organ cross-fire but disregarding any photon contribution from ^{111}In. Again, the data points of ^{111}In-CO17-1A very well match the results observed with the non-metallic Auger electron emitter ^{125}I, and, similarly, those of ^{90}Y-CO17-1A match well the results with the conventional β^--emitter ^{131}I.

Discussion

Although radioimmunotherapy is an attractive concept for a more target-oriented systemic cancer therapy than is usually achieved with other forms of systemic therapies [1, 2], it has a major drawback in solid tumours, namely the problem of achieving sufficiently high tumour doses [3, 4, 5, 6, 7]. Frequently, myelotoxicity is dose limiting before therapeutically effective tumour doses are reached [4]. Doses of less than 20 Gy may be sufficient to eliminate radiosensitive tumours, such as lymphoma, but are not high enough to control more radioresistant solid tumours [2, 3].

The majority of radionuclides used to date for radioimmunotherapy have been β-emitters, which have a path length of several millimetres in tissue. These radionuclides possess the advantage of being able to deliver cross-fire doses to neighbouring cells which may not express the respective antigen, but will cause damage to non-target tissue as well, e.g. to the stem cells in the bone marrow. A more selective irradiation of tumour tissue with less radiation exposure of normal organs seems desirable. Short-ranged Auger electron emitters appear an attractive alternative, if they can be selectively directed *into* the target cells: this is important since most of their low-energy electrons can reach the nuclear DNA only when the disintegration takes place intracellularly, given that the few micrometres of cytoplasm form sufficient shielding against electrons originating from outside these cells [6, 7, 8, 9, 11, 12].

Several decades ago, ^{125}I was compared with ^{131}I for therapy of hyperthyroidism [37, 38, 39]. Mostly, therapeutic results were disappointing and not superior to conventional Na^{131}I [37, 38, 39, 40]. More recently, ^{125}I-labelled meta-iodobenzylguanidine was compared with its ^{131}I form in in vitro as well as in vivo sytems [41, 42, 43, 44]. Also here, no clear advantage was seen for the Auger electron-emitting form, and this holds true for ^{125}I-labelled steroid hormone analogues as well [45], although they even bind to *intranuclear* receptors. However, encouraging preclinical as well as (in pilot studies) clinical observations have been reported for ^{125}I-iododeoxyuridine (^{125}I-IUdr) (reviewed, e.g. in [16]), which acts as a deoxythymidine analogue, being incorporated into the nuclear DNA. ^{125}I disintegrating within the nucleus has a relative biological effectiveness 7–8 times higher than conventional β- or γ-emissions [46]. It consequently fulfils the criteria of a typical high-LET radiation [8, 9, 47, 48], and single decays are able to cause DNA double strand breaks which are characterized by the absence of the shoulder typically seen in cell survival curves with low-LET particles [48, 49]. If the decay occurs within the cytoplasm, the relative biological effectiveness factor is still approximately 2, as compared with conventional low-LET radiation [48, 49]. IUdr, however, has the disadvantage of being taken up as a nucleotide analogue in accordance with the cell proliferation rate,

regardless of whether the tissue is benign (e.g. stem cells in the red marrow) or malignant (i.e. the actual target tissue). Thus, a systemic but more selective and, therefore, more tumour-specific intracellular delivery of ^{125}I to the malignant tissue seems desirable.

Our study clearly shows that with ^{125}I- as well as with ^{131}I-labelled immunoglobulins, myelotoxicity is the only dose-limiting toxicity. The same holds true for both radiometals as well. The observation that approximately tenfold activities of ^{125}I as compared with ^{131}I are needed to cause comparable effects on the bone marrow may well be explained by the fact that the energy liberated per disintegration of one ^{125}I atom is roughly one-tenth of the energy set free by decaying ^{131}I [10]. In accordance with their respective γ-energies, the photon contribution is negligible for ^{131}I but may be quite substantial for ^{125}I (cf. Table 1). The dose assumptions, as presented in Table 1, assume an infinite water medium around the body of the mice according to the model of Furhang et al. [30]. Since, of course, in reality the mice are in air, the photon backscatter will be substantially lower. Therefore, Table 1 merely tries to give the range within which the actual radiation doses lie.

In good accordance with earlier observations with ^{131}iodinated IgG (e.g. [1, 4, 24]), the blood-based maximum tolerated red marrow dose was found at approximately 17 Gy for ^{131}I-CO17-1A. Depending upon the fraction of ^{125}I being internalized into the bone marrow cells and the extent of the photon contribution, blood doses will range between 10 and 24 Gy for ^{125}I-CO17-1A, which would be well within the same range. Since the bone marrow cells do not express the CO17-1A antigen, ideally no internalization of the antibody is anticipated; thus the low-energy electrons will not be able to reach the nuclear DNA of these cells, and only higher-energy electrons, as well as x- and γ-rays, will be able to cause radiation damage to the cells. Their energy would sum up to approximately 13 Gy to the blood (cf. Table 1). Very similar blood doses were also observed with ^{90}Y-CO17-1A at its respective MTD (cf. Tables 1 and 2).

In contrast to this lower biological effectiveness of ^{125}I-CO17-1A in the bone marrow, significantly higher efficacy was observed at comparable doses in tumour tissue. Interestingly, plotting tumour volume multiplication times against tumour doses (cf. Fig. 5) yields a diagram resembling the sort of inverted cell survival curve ("shoulder curve") known from irradiating cells in cell culture. Conventional ^{131}I-labelled antibodies, whether internalizing (CO17-1A) or not (anti-CEA or irrelevant), as well as irrelevant or non-internalizing ^{125}I-labelled antibodies, do not show any significant anti-tumour effect at doses below 10 Gy, with increasing growth delay at increasing doses above this threshold. The resulting curve resembles the typical "shoulder" curve when plotting cell survival in cell culture against radiation doses. In contrast, ^{125}I or ^{111}In bound to the specific and internalizing antibody lacks a pronounced shoulder, and dis-

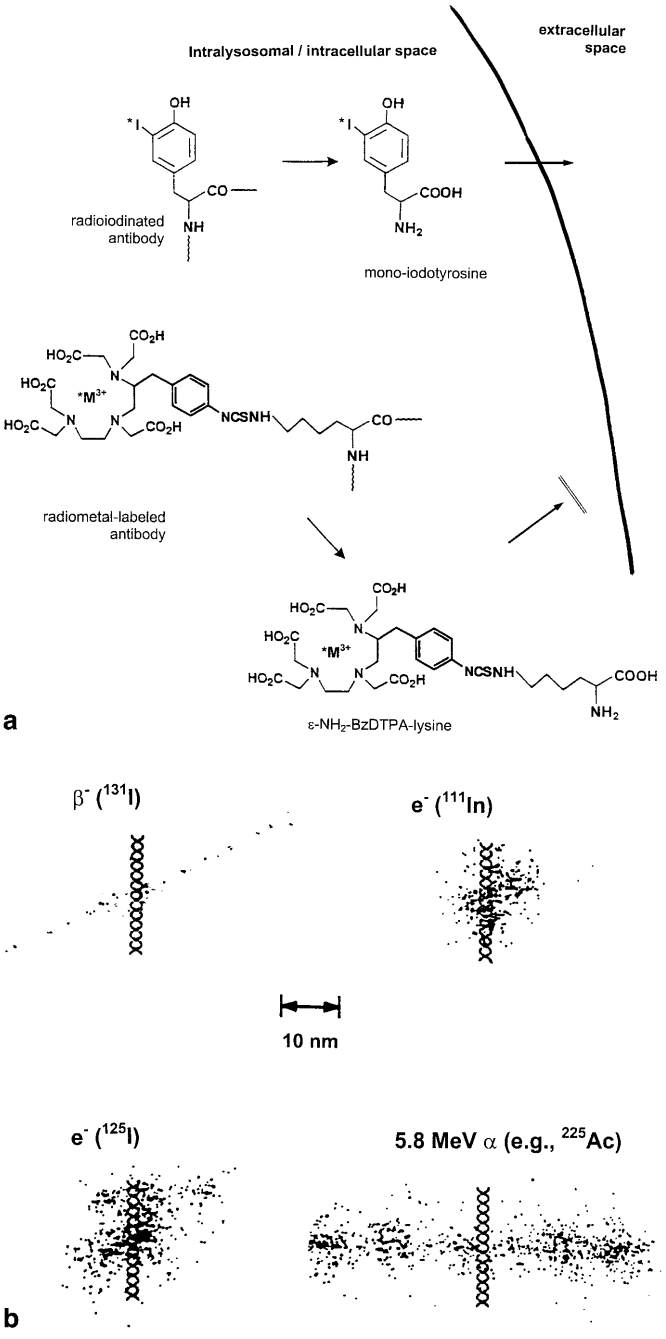


Fig. 8a,b. Advantage of residualizing radiometals over conventionally radioiodinated internalizing antibodies and high-LET characteristics of Auger/conversion electron emitters over conventional β⁻-emitters. **a** Schematic comparison of the metabolism of radioiodinated versus radiometal-labelled antibodies: whereas radioiodinated tyrosyl moieties, released from proteins after proteolytic cleavage within the lysosomes, are released from the cells, the metabolites of radiometal-labelled proteins (e.g. ε-amino-BzDTPA-lysine) cannot leave the cells and, thus, are called a form of "residualizing" label. **b** Comparison of the initial positions of the reactive chemical species (e.g. $OH^{\cdot}$, $H^{\cdot}$, e^{-}_{aq} etc.) for Auger electron (e.g. ^{125}I or ^{111}In) as compared with low-LET β⁻ (e.g. ^{131}I) or high-LET α-emitters (e.g. ^{225}Ac), each crossing the DNA (modified from ref. [51])

plays an almost linearly increasing efficacy with increasing dose. Interestingly, with ^{125}I comparable anti-tumour efficacy is achieved at approximately half the dose needed with ^{131}I, which matches surprisingly well the relative biological effectiveness factor known for ^{125}I decaying in the cytoplasm inside the cell [11, 49]. On the other hand, the therapeutic efficacy of ^{125}I bound to antibodies that do not internalize to a significant extent (e.g. anti-CEA antibodies) does not seem to be superior to the efficacy of conventional β-emitters.

Since conventionally radioiodinated proteins undergo lysosomal degradation to mono- or di-iodotyrosine that is rapidly released from the cells, Auger electron-emitting residualizing radiolabels (e.g. *radiometals*, such as ^{67}Ga or ^{111}In), which are retained intracellularly in lysosomes [50] (Fig. 8a), were investigated. We examined whether they may help to further improve the therapeutic results by increasing the tumour accretion, and thus the radiation dose to the tumour. Again, the therapeutic advantage of the Auger electron-emitting radiometal (^{111}In) over the conventional β-emitter (^{90}Y) was clearly demonstrated. The fact that significantly higher liver and spleen uptake, and thus higher doses to these organs, occurred with both metals than with both radioiodine isotopes is most likely due to the IgG_{2a} receptor-mediated uptake of CO17-1A in the liver and spleen; the consequence is that the radiometal-labelled metabolites are retained intracellularly not only in the tumour, but also in the liver and spleen, whereas the iodine label is easily released. However, in accordance with normal organ doses below 20 Gy, no signs of normal organ toxicity other than myelosuppression were observed.

Summarizing, internalizing antibodies seem to show encouraging therapeutic results when labelled with Auger electron-emitting radionuclides, such as ^{125}I or ^{111}In. They cause biological effects similar to those of typical high-LET radiation, such as α-emitters. Indeed, Auger electron emitters decaying in the neighbourhood of the DNA produce similar amounts of reactive chemical radical species (e.g. $OH^{\cdot}$, $H^{\cdot}$, e^{-}_{aq} etc.) as do α-emitters, which are regarded as the classical form of high-LET radiation (Fig. 8b). Further studies are ongoing in order to show whether internalizing antibodies of higher affinity, or approaches combining radioimmunotherapy with potentially radiosensitizing chemotherapeutic agents [24], may further enhance the therapeutic efficacy. Systematic clinical studies with internalizing antibodies labelled with Auger electron-emitting radionuclides seem to be warranted.

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