Biodistribution and Dosimetry of Pretargeted Monoclonal Antibody 2D12.5 and Y-Janus-DOTA in BALB/c Mice with KHJJ Mouse Adenocarcinoma

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Biodistribution and dosimetry of 88Y (and equimolar 90Y) Janus-dodecanetetraacetic acid (DOTA) were performed using a three-step pretargeting protocol in BALB/c mice bearing mouse mammary adenocarcinoma (KHJJ) implants. Pretargeting was performed with mouse monoclonal antibody (mAb) 2D12.5 specific for yttrium-DOTA, and the chase was Y-DOTA–human transferrin conjugate. In this article, we report extensive organ dosimetry and the theoretic limits of the radionuclide physical half-life (T_P) for pretargeting. Methods: Organ biodistribution data were obtained from bioassays on tissue taken from tumor mice killed at 3, 24, 48, 72, 96, and 120 h after intravenous injection of 88Y-Janus-DOTA. Uptake and retention of 88Y as a function of time were described by nonlinear least squares fits of the tissue data to multieponential functions. Radiation dose estimates for equivalent molar amounts of 90Y were subsequently derived from these time-integrated functions. Results: The results were as follows: rapid blood clearance of 88Y-Janus-DOTA; rapid uptake and slow clearance of 88Y-Janus-DOTA from the tumor over 5 d; rapid clearance from all organs and body; largest radiation absorbed dose (AD) per injected dose of 63.52 (cGy/MBq) to tumor; high therapeutic ratios (AD tumor/AD tissue), particularly for blood and bone; and optimal radionuclide T_P range from 30 min to 10 d. Conclusion: Although the absolute concentration of 90Y in the tumor is less using the hapten system than is achieved generally with the chelated radionuclide covalently attached to the mAb, the achievable tumor uptake of radioactivity, coupled with low radioactivity in bone, blood, and other organs, suggests that a three-step pretargeting protocol has considerable promise as a method for 90Y radioimmunotherapy.

Key Words: radioimmunoimaging; radioimmunotherapy; 90Y; 111In pretargeting; biodistribution; dosimetry; therapeutic ratio

Briefly, the antibody and radiolabel are administered separately. The first or pretargeting step consists of administration of mAb to BALB/c mice with KHJJ mouse tumors. Sufficient time (20 h) is allowed for the tumor to equilibrate with blood mAb. At equilibrium, the tumor has as high an mAb concentration as possible. The next step or chase step is the intravenous injection of a large-molecular-weight polyvalent hapten. This procedure leads to the rapid clearance from blood of excess circulating nonradioactive antibody (19). Finally, 1 h after administration of the chase, the radiolabeled hapten–chelate conjugate is given, and mouse organ and tumor assay or whole-body imaging is performed at various times after the addition of the radiolabel (20). Recognizing these principles, Axworthy et al. (21) used the avidin/biotin system to cure human carcinoma xenografts in mice by a single dose of pretargeted 90Y with negligible toxicity.

Substituting the γ-emitting radionuclides 88Y and 111In as tracers for 90Y-Janus-DOTA, we extend our previous analysis (17) to early blood radioactivity levels and a more complete description of the biodistribution and dosimetry of 90Y to tumor, blood, heart, lung, spleen, kidneys, muscle, bone, skin, and gut. 88Y has a physical half-life (T1/2) of 106.64 d and decays through both electron capture and positron emission. This decay produces both x-ray and γ-ray emissions, making it suitable for accurate organ assay in a scintillation well counter.

MATERIALS AND METHODS

Organ biodistribution data were obtained from bioassays performed on tissue taken from tumor mice killed at 3, 24, 48, 72, 96, and 120 h after 88Y-Janus-DOTA injection. Uptake and retention of 88Y as a function of time were described by nonlinear least squares fits of the tissue data to multiexponential functions. Radiation dose estimates for equivalent molar amounts of 90Y were derived subsequently from area-under-the-curve (AUC) estimations calculated from these integrated functions (17).

Mouse Tumor Model and Pretargeting Protocol

The KHJJ mouse breast adenocarcinoma was used in these experiments (22). Unlike the more commonly used nude mouse–human tumor system, this model is analogous to tumor imaging in an immunologically competent patient using human mAb. BALB/c mice were used 2 wk after trocar implantation in the flank, at which time the tumors were approximately 0.7 cm in diameter and weighed 558 ± 220 mg (mean ± SD; n = 18).

Pretargeting used three steps. First, 50–100 μg (0.33–0.67 nmol) 2D12.5 were given intravenously to BALB/c mice bearing KHJJ mouse tumors. Enough time (20 h) was allowed for the mAb to leak through the abnormally permeable tumor neovascularature and to equilibrate with the blood mAb to give the maximum tumor concentration. At this time, a multivalent Y-DOTA conjugate of human transferrin (35.4 μg HTr-2IT-BAD-Y, 0.442 nmol HTr, 3.09 nmol BAD-Y; 6:1 hapten/protein) was given intravenously to clear the blood quickly of the excess circulating nonradioactive antibody. One hour after the chase (21 h after mAb), the 88Y-labeled hapten–chelate was given intravenously (33.3 kBq 88Y, 0.744 pmol Y, 0.484 nmol Janus-DOTA).

The equilibrium association constant (Ka), measured by equilibrium dialysis, for the affinity of 2D12.5 and 88Y-DOTA is approximately 108 (17). A previous study in this laboratory (17) with 111In, 88Y, 67Ga, and mono- and divalent haptons using the same pretargeting mAb protocol suggested passive tumor uptake of the antibody through leaky capillaries with no organ uptake, because of the presence of cross-reacting antigen elsewhere in the tissues.

Organ Biodistribution

The tumor mice (n = 3 per group) were killed 3, 24, 48, 72, 96, and 120 h after 88Y-Janus-DOTA injection. The percentage injected dose per gram (%ID/g) of decay-corrected 88Y-labeled hapten was determined in blood, heart, lungs, liver, spleen, kidneys, tumor, muscle, bone and marrow (femur), skin, and gut. To obtain a better estimation of the early blood clearance of the metal–hapten chelate, a second experiment was performed as described previously, substituting 111In-Janus-DOTA for 88Y-Janus-DOTA and sampling at 0.08, 0.17, 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 h after injection.

Data Analysis

Uptake and retention of the 88Y- or 111In-Janus-DOTA as a function of time were described by iterative nonlinear least squares fits of the %ID/g tumor and organ data to multieponential functions using SAAM II (SAAM Institute, Inc., Seattle, WA), which is a simulation, analysis, and modeling software package.

If f(t) is defined as the %ID/g of metal-chelate as a function of time, then:

$$f(t) = \sum A_j \exp(-\lambda_j t), \quad \text{Eq. 1}$$

where $A_j$ (%ID/g) and $\lambda_j$ (biologic uptake or decay rate, time⁻¹) are the fitted parameters.

Absorbed Dose Estimates

Radiation absorbed dose (AD) estimates for equivalent molar amounts of 90Y were derived subsequently from AUC estimates. These were derived from the fitted exponential functions describing the biodistribution of the injected 88Y activity and the 90Y physical decay constant. If $f_0(t)$ is defined as 90Y MBq/kg as a function of time, then:

$$f_0(t) = c_1 f_1(t) \exp(-\lambda_0 t), \quad \text{Eq. 2}$$

where $c_1$ is a constant for the conversion of %ID/g to 90Y MBq/kg and $\lambda_0$ is the 90Y physical decay constant (time⁻¹). Substituting $\sum A_j \exp(-\lambda_j t)$ for $f_0(t)$ gives:

$$f_0(t) = c_1 \sum A_j \exp(-\lambda_j t) \exp(-\lambda_0 t), \quad \text{Eq. 3}$$

and

$$f_0(t) = c_1 \sum A_j \exp[-(\lambda_j + \lambda_0) t]. \quad \text{Eq. 4}$$

If the AUC from time zero to infinity is defined as the integral from zero to infinity, then:

$$\text{AUC}_{0→∞} = \int_0^\infty f_0(t) dt, \quad \text{Eq. 5}$$

or
\[
AUC_{0\rightarrow\infty} = c_1 \int_0^\infty \sum A_j \exp\left[ -\left( \lambda_j + \lambda_p \right) t \right] dt,
\]

Eq. 6

which, when integrated, becomes:

\[
AUC_{0\rightarrow\infty} = c_1 \sum A_j / \left( \lambda_j + \lambda_p \right).
\]

Eq. 7

Finally, if AD is defined as the product of the AUC from zero to infinity and the energy constant, then:

\[
AD = AUC_{0\rightarrow\infty} \times \Delta,
\]

Eq. 8

where \( \Delta \) is the \(^{90}\)Y energy constant (1.49 \times 10^{-5} \text{ cGy} \times \text{kg/MBq} \times \text{s}).

FIGURE 1. (A) Semilog plots of blood (■) clearance of both \(^{111}\)In- (0.08–2.5 h) and \(^{89}\)Y-Janus-DOTA, and tumor (●) uptake and clearance of \(^{89}\)Y-Janus-DOTA. Note predicted rapid uptake into tumor as blood levels decline rapidly as metal-Janus-DOTA is quickly distributed into interstitial volume. (B) Semilog plots of %ID/g for tumor (●), liver (■), and bone (▲) predicted uptake and clearance of \(^{89}\)Y-Janus-DOTA. Note much higher initial accumulation in tumor compared with bone and liver.
These calculations do not consider the reduction of the dose caused by the escape of energy from the small mouse organs or the effect of such escaped energy on the other organs. Because we are dealing primarily with pure β-emitters, this situation will have little implication for human dosimetry in treatment situations.

**Statistics**

The AD estimates for the tumor and organs were compared by ANOVA and the Dunnett multiple comparison test, which compared the AD of the tumor to the AD of blood and other tissue (23). In all cases, P < 0.01 was considered statistically significant.

**RESULTS**

Figure 1A is a plot of the %ID/g blood versus time after intravenous injection of 111In- and 88Y-Janus-DOTA 1 h after injection of the polyvalent hapten–protein conjugate. The data from 0 to 2.5 h are from 111In-Janus-DOTA, and those from 3 to 24 h are from 88Y-Janus-DOTA. The data were fitted to the following triexponential function:

\[ f_{\text{%ID/g blood}}(t) = A_1 \exp(-\lambda_1 t) + A_2 \exp(-\lambda_2 t) + A_3 \exp(-\lambda_3 t), \]  

Eq. 10

where \( A_j \) (%ID/g) and \( \lambda_j \) (h\(^{-1}\)) were the fitted parameters.

The uptake and clearance of 88Y-Janus-DOTA from the tumor and organs over 5 d is shown in Figure 1B. The tumor data were fitted to the following triexponential function:

\[ f_{\text{%ID/g tumor}}(t) = A_1 \exp(-\lambda_1 t) + A_2 \exp(-\lambda_2 t) + A_3 \exp(-\lambda_3 t), \]  

Eq. 11

where \( A_3 = -(A_1 + A_2) \) such that \( \Sigma A_j = 0 \) at \( t = 0 \), and the initial estimate for \( \lambda_1 \) (uptake phase) was obtained from the \( \lambda_1 \) (distribution phase) of the %ID/g blood versus time curve.

Clearance of the metal-DOTA from the tumor was slow compared with the rapid blood clearance. The metal-chelate was bound to the pretargeted antibody in the tumor with 4.44 and 1.93 %ID/g at 3 and 24 h, respectively. This result gave tumor-to-blood ratios of approximately 20:1 and 60:1 at 3 and 24 h, respectively.

The biodistribution data for the remaining organs were fitted to multieponential equations as above. Figure 1B shows the %ID/g 88Y-Janus-DOTA versus time for tumor, liver, and bone. Values of the %ID/g 88Y-Janus-DOTA at 3, 24, 48, 72, 96, and 120 h for the tumor and organs are listed in Table 1.

Table 2 summarizes the tumor and organ time-integrated activity and absorbed dose for 88Y-Janus-DOTA.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>%ID/g</th>
<th>SD</th>
<th>%ID/g</th>
<th>SD</th>
<th>%ID/g</th>
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<th>SD</th>
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<tr>
<td>3 h</td>
<td></td>
<td></td>
<td>24 h</td>
<td></td>
<td>48 h</td>
<td></td>
<td>72 h</td>
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<tr>
<td>Blood</td>
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<td>0.06</td>
<td>0.03</td>
<td>0.02</td>
<td>0.05</td>
<td>0.07</td>
<td>0.13</td>
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<tr>
<td>Bone</td>
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<td>0.22</td>
<td>0.04</td>
<td>0.10</td>
<td>0.04</td>
<td>0.01</td>
<td>0.02</td>
<td>0.06</td>
<td>0.10</td>
<td>0.03</td>
<td>0.08</td>
<td>0.06</td>
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<tr>
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<td>0.28</td>
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<td>0.20</td>
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<td>0.67</td>
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<td>Lung</td>
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<td>0.09</td>
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<td>0.04</td>
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<td>0.04</td>
<td>0.02</td>
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<tr>
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<td>0.08</td>
<td>0.15</td>
<td>0.10</td>
<td>0.12</td>
<td>0.01</td>
<td>0.10</td>
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<td>0.16</td>
<td>0.05</td>
<td>0.13</td>
<td>0.11</td>
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<tr>
<td>Tumor</td>
<td>4.44</td>
<td>1.63</td>
<td>1.93</td>
<td>0.54</td>
<td>1.04</td>
<td>0.20</td>
<td>0.78</td>
<td>0.77</td>
<td>0.78</td>
<td>0.11</td>
<td>0.47</td>
<td>0.40</td>
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</tbody>
</table>

Values are mean ± SD for three mice.

\[ AD = \exp \left[ \sum A_j / (\lambda_j + \lambda) \right] \times \Delta. \]  

Eq. 9

\[ f_{\text{%ID/g blood}}(t) = A_1 \exp(-\lambda_1 t) + A_2 \exp(-\lambda_2 t) + A_3 \exp(-\lambda_3 t), \]  

Eq. 10

\[ f_{\text{%ID/g tumor}}(t) = A_1 \exp(-\lambda_1 t) + A_2 \exp(-\lambda_2 t) + A_3 \exp(-\lambda_3 t), \]  

Eq. 11

Table 2 summarizes the tumor and organ time-integrated activity and absorbed dose for 88Y-Janus-DOTA.
radioactivity (\(90Y\) MBq/\(s/kg\)) and AD (cGy) for \(90Y\)-Janus-DOTA obtained from the fitted exponential functions describing the biodistribution of the injected dose. A summary of the AD (cGy) per megabecquerel injected \(90Y\) for tumor and organs is shown in Figure 2. Note that the tumor had a much greater AD per injected dose (cGy/MBq), which was highly significant compared with all other tissue (\(P < 0.01\), ANOVA, Dunnett). The therapeutic ratios (TRs) defined as AD tumor/AD tissue are shown in Figure 3. Note the large TRs for both blood and bone.

**DISCUSSION**

The mAb from clone 2D12.5 was used because it had the highest 24-h whole-body retention of \(88Y\)-DOTA caused by a high affinity for Y(III)-DOTA \((K_a = 10^8 \text{ mol/L})\). After intravenous injection in mice, uncomplexed \(88Y\)-, \(111\)In-, and \(67\)Ga-labeled DOTA were all excreted rapidly in the urine. The half-life for elimination was approximately 40 min; >93% of the injected dose was excreted by the kidneys in 24 h (17).

Our model showed a rapid uptake of the \(88Y\)-Janus-DOTA \((C_{max} \text{ at } t_{max} \text{ of } 0.4 \text{ h})\) into the leaky neovasculature of the tumor as the injected metal-DOTA was distributed rapidly into the interstitial water (Fig. 1B). Figure 1A is a plot of the %ID/g blood versus time after intravenous injection of \(111\)In- and \(88Y\)-Janus-DOTA 1 h after injection of the polyvalent hapten–protein conjugate. Rapid blood clearance was seen with both \(88Y\)- and \(111\)In-Janus-DOTA.

Previously, we had shown that rapid lowering (chase) of a long-circulating protein was possible by the intravenous injection of a specific antibody (19). In these experiments, the chase of 2D12.5 by injection of the polyvalent hapten–protein conjugate also rapidly lowered the circulating mAb concentration. This procedure had a striking effect on the whole-body 24-h retention of the metal-DOTA injected soon after. When the chase was administered to 20-h pre-targeted mice 1 h before \(88Y\)-DOTA injection, whole-body retention of the metal-chelate fell from >80% (no chase) to approximately 10% at 3 h and approximately 5% at 24 h after injection of the tracer.

As a matching isotope for \(90Y\), \(111\)In has been the nuclide of choice because of the similarities in its metabolic handling and coordination chemistry (24). Comparative studies with \(111\)In or \(90Y\) radiolabeled compounds have shown similar pharmacokinetic behavior of the two species (25). Moreover, nitrobenzyl-DOTA binds both yttrium and indium with high stability (26). On the basis of these reports showing that the physicochemical properties of the metal-DOTA are determined primarily by the chelate and not the metal ligand, the \(111\)In- and \(88Y\)-DOTA biodistribution data were pooled.
Pretargeting involves the administration of a long-circulating targeted macromolecule (mAb) having a high-affinity noncovalent binding site for a small, rapidly excreted effector molecule, which is given after the mAb has concentrated in the targeted tumor (20). Efficient removal of the hapten-binding macromolecule (mAb) from the circulation with a polyvalent chase macromolecule before giving the effector molecule greatly improves the target-to-blood ratio. Pretargeting without the chase step requires a long waiting period for the blood mAb concentration to fall to very low levels, because even small amounts of mAb remaining in the blood will immediately bind effector molecules on injection. To ensure free effector molecules for diffusion into the tumor and filtration and excretion by the kidneys, circulating mAb must be either saturated or removed. The aggregated mAb produced by cross-linking with the chase in the circulation is endocytosed rapidly by the reticuloendothelial cells, mostly in the liver (18). The intracellular location of the endocytosed mAb prevents the access and binding of a subsequently injected hydrophilic effector molecule. The chase quickly reduces the blood mAb to a very low level so that radioactivity can be administered within 1 h (27).

After injection of the effector molecule (radiolabeled hapten) 1 h after the chase molecule, maximum tumor concentration and tumor-to-normal-tissue ratios were achieved rapidly, and radioactivity was retained in the tumor for several days. The rapid elimination of unlocalized radioactivity greatly decreased radiation exposure to normal tissue, especially the bone marrow (Figs. 1B and 2). Thus, the pharmacokinetics of the effector molecules gave fast tumor uptake (minutes), slow tumor washout (days), and relatively fast washout from most organs (Figs. 1B and 2, Table 1). This result provided high tumor-to-background ratios and high TRs, which will improve both imaging and radioimmunotherapy (Figs. 2 and 3).

Rapid diffusion, exclusive renal excretion, and extracellular fluid distribution are among the chemical and physiologic properties of an effector molecule that are necessary for achieving the best pretargeting results (26). Our experimental determination of the apparent $V_D$ of 3.3 mL for the metal-DOTA was consistent with literature estimates of the extracellular fluid in the mouse (28). This result, combined with the net negative charge and hydrophilic nature of the metal-hapten, suggested that the metal-DOTA was confined essentially to the extracellular volume with very little intracellular uptake (29).

Rapid uptake of the metal-DOTA by both tumor and organs seen in our model (Figs. 1A and B) with a $C_{max}$ at approximately 0.4 h was consistent with rapid distribution of the metal-DOTA into the interstitial water. Clearance of the metal-DOTA from the tumor was slow compared with that of blood, because the metal-DOTA was bound to the pretargeted antibody (Fig. 1A).

**FIGURE 3.** Summary of TRs calculated as AD tumor/AD tissue. Note large TRs for blood and bone.

![Therapeutic Ratio (TR): Absorbed Dose Tumor/Absorbed Dose Tissue](image-url)

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**THREE-STEP PRETARGETING mAb PROTOCOL • Lubic et al.**
From the tumor and organ time-integrated radioactivity \((^{90}\text{Y MBq} \times \text{s/kg})\) and AD (cGy) for \(^{90}\text{Y-Janus-DOTA}\), the tumor had the largest AD compared with all other tissue (Fig. 2, Table 2). Moreover, TRs were >2.5/1 for all measured tissue (Fig. 3); TRs for both blood (21/1) and bone (18/1) were particularly dramatic. The tumor-to-blood TR of >20/1 was much greater than the 2–3/1 obtained with directly labeled mAb (1). This large gain in the tumor-to-blood TR is highly desirable for both radioimmunodetection and radioimmunotherapy.

Radiation dose is related to the effective retention of activity in tissue \((T_e)\), reflecting both radioactive decay and biologic clearance processes (30). Biologic retention and clearance values are best characterized by the AUC (%ID/g tissue) obtained from the integration of the equation describing the %ID/g tissue (obtained from a suitable tracer) as a function of time (Eq. 1) and is given by the following relationship:

\[
\text{AUC}_{0\rightarrow\infty} = \sum A_i / \lambda_i, \quad \text{Eq. 12}
\]

where \(A_i\) (%ID/g tissue) and \(\lambda_i\) (biologic decay and uptake rate constants, h\(^{-1}\)) are obtained from the fit of Equation 1. Cumulative activity in tissue is derived from the decay-corrected tissue count data and \(T_p\) of the radionuclide and is best characterized by the AUC (MBq \times s/kg tissue) obtained from the integration of Equation 4. Cumulated activity in tissue is given by the following relationship:

\[
\text{AUC}_{0\rightarrow\infty} = c_1 \sum A_i / (\lambda_i + \lambda_p), \quad \text{Eq. 13}
\]

where \(c_1\) is a constant for the conversion of %ID/g to MBq/kg and \(\lambda_p\) is the radionuclide decay constant (time\(^{-1}\)) (Eq. 7).

As a first approximation, the AD is related to the cumulative activity in a specific tissue (Eq. 8). For several reasons, the dose is not directly proportional to cumulative activity. Some of the energy emitted from radionuclides in an organ or tissue may be imparted to more distant organs or may completely escape the body. Moreover, accurate AD calculations also depend on the size and shape of the organ and the density and atomic composition of the tissue (30). In a mouse model, Hui et al. (31) and Beatty et al. (32) showed that the dosimetry calculation model, using the standard assumption for humans that all \(\beta\)-energy is deposited within the organs or tumor in which it is localized, is inadequate for determining the \(\beta\)-AD for mouse organs. Because the tumors in our mouse model weighed significantly more, we estimated the self-absorbed fraction to be approximately 0.5 for our tumors. Thus, the actual dose to our tumors is probably closer to half the value we calculated using standard assumptions. On the other hand, we did not consider

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**FIGURE 4.** Summary plot of several tumor-to-tissue TRs vs. \(T_p\) for select number of positron-emitting radionuclides.
the dose caused by β-particles entering from adjacent tissue or as circulating activity, which is lower with pretargeting than with conventional radioimmunoonotherapy. Although the mouse dosimetry is overestimated, the uncorrected values should apply to larger human organs (17).

It is likely, as shown for 111In, 88Y, and 90Y, that the biodistribution and biologic half-life (Tb) of many other metal-DOTA chelates will be very similar if not identical (33). Thus, the relative AD for each radiometal to the organs and the tumor should be directly proportional to the Tb for that radionuclide. Plotting several TRs as a function of Tb (Figs. 4 and 5) for various imaging and therapeutic radionuclides, we notice that the tumor-to-liver TR is <1 for radionuclides with half-lives >10 d. The reason for this result is suggested by a careful analysis of the biodistribution data for the liver. Although the absolute %ID/g is small for the liver, the radionuclide–DOTA–mAb complex is not cleared readily and thus has a very long biologic half-life compared with other tissue (Fig. 1B). Thus, for therapeutic radionuclides with Tb >10 d, the Tb of the complex in the liver is quite pronounced, with more energy imparted to the liver than to the tumor. Similarly, for radionuclides with Tb <1 h, the TR decreases rapidly, especially for the blood and gut.

CONCLUSION

The high tumor uptake of 90Y-DOTA and subsequent high radioactivity, coupled with low radioactivity in bone, blood, and other organs, suggests that a three-step pretargeting protocol has considerable promise as a method for 90Y-radioimmunoonotherapy.

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