

Advances in Brief

Therapy of Disseminated B-Cell Lymphoma Xenografts in Severe Combined Immunodeficient Mice with an Anti-CD74 Antibody Conjugated with ¹¹¹Indium, ⁶⁷Gallium, or ⁹⁰Yttrium¹

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Abstract

A radiolabeled antibody (Ab) to CD74 (the MHC class II invariant chain, Ii) was shown previously to effectively kill human B-lymphoma cells *in vitro*. Conjugates with both Auger electron and β -particle emitters were able to kill cells, but the former displayed less nonspecific toxicity in the *in vitro* assay used. In this report, we have extended the studies to an *in vivo* model of tumor growth. The human B-cell lymphoma Raji was injected i.v. into severe combined immunodeficient mice, and radiolabeled Abs were injected at various times after tumor inoculation. The maximum tolerated dose (MTD), as well as lower doses, was tested. Tumor growth was monitored by hind-leg paralysis. With a 3–5-day interval before Ab injection, anti-CD74 conjugated to either ¹¹¹In or ⁶⁷Ga, at a dose of 240–350 μ Ci/mouse, produced a strong therapeutic effect, with greatly delayed tumor growth, and many of the treated mice were tumor free for >6 months. Control mice became paralyzed in 16–24 days, uniformly. Treatment at later time points (9-day interval) had little therapeutic effect. The MTD was required for optimal therapy. With the β -particle emitter ⁹⁰Y, the MTD was much less, 25 μ Ci/mouse, and at this dose there was only a weak therapeutic effect. In conclusion, the data suggest that low-energy electrons are more effective than β -particles in this model system. These results may be applicable to humans, particularly in the case of micrometastatic disease. This approach may also be effective with other Abs that accrete in large amounts.

Introduction

Ab³ LL1 reacts with CD74, the invariant chain associated with the immature MHC class II antigen, which has normal

tissue expression primarily on B lymphocytes and macrophage-lineage cells (1). On B-cell lymphomas, this antigen is expressed at a relatively low level on the cell surface but is rapidly internalized and replaced by newly synthesized molecules so that $\sim 10^7$ Ab molecules are taken up per cell per day (2, 3). The internalized Abs are rapidly delivered to lysosomes and catabolized, but if “residualizing” radiolabels are used (which are trapped inside the cell, usually within lysosomes, after catabolism of the Ab; Refs. 4 and 5), a large amount of radioactivity accumulates intracellularly. We demonstrated recently that B-lymphoma cells *in vitro* were efficiently and specifically killed by LL1 conjugated to various radiolabels, including ¹¹¹In, ¹²⁵I, ^{99m}Tc, ⁶⁷Ga, ¹³¹I, and ⁹⁰Y (6, 7). Total killing was obtained (greater than ~ 6 logs) under conditions in which a nonreactive Ab labeled in the same way produced no significant toxicity. Many of these radionuclides (¹¹¹In, ^{99m}Tc, and ⁶⁷Ga) are considered “imaging” radionuclides, but these, as well as ¹²⁵I, emit low-energy electrons, including both Auger and conversion electrons, which are believed to be the toxic agents. Dosimetry calculations indicated that the amount of radiation delivered by the low-energy electrons was sufficient to produce the toxicity observed.

The β -particle emitters ¹³¹I and ⁹⁰Y also effectively killed target cells *in vitro* when linked to anti-CD74 (7). Such β -particle emitters, which are most commonly used for RAIT, differ markedly from Auger electron emitters in their depth of tissue penetration. Greater tissue penetration is both an advantage and a disadvantage; it allows nontargeted cells (such as antigen-negative tumor cells) to be killed, but it also increases nonspecific toxicity. Circulating β -particle emitters will deliver a substantial radiation dose to the bone marrow, whereas Auger electron-emitting radionuclides are much less toxic to nontargeted cells. Accordingly, much higher μ Ci doses of Auger electron emitters can be administered to mice (8). Our *in vitro* experiments similarly demonstrated that β -particle emitters had much greater nonspecific toxicity than Auger electron emitters (7). Thus, it seems plausible that with Auger electron emitters, tumor therapy might be achieved at doses at which little toxicity occurs. The present study was undertaken to determine whether specific therapeutic effects could be demonstrated *in vivo*, in a mouse xenograft model, with LL1 conjugates of ¹¹¹In, ⁶⁷Ga, and ⁹⁰Y.

Materials and Methods

Tumor Cell Lines, Antibodies, and Radiolabeling.

Raji B-cell lymphoma cells were obtained from the American Type Culture Collection (Rockville, MD). The culture medium was RPMI 1640 with 12.5% FCS, supplemented with penicillin, streptomycin, glutamine, and pyruvate (Life Technologies, Inc., Grand Island, NY). Cells were tested routinely for *Mycoplasma* contamination with the Mycotect Assay (Life Technologies,

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³ The abbreviations used are: Ab, antibody; RAIT, radioimmunotherapy; SCID, severe combined immunodeficient; MTD, maximum tolerated dose.

Inc.) and were negative. Ab LL1 was described previously (3) and was provided by Immunomedics, Inc. (Morris Plains, NJ). A control Ab, MN-14 (anti-carcinoembryonic antigen), is a mouse IgG1, as is LL1, and was also provided by Immunomedics, Inc. Labeling with ^{111}In -labeled benzyl-diethylenetriaminepentaacetic acid (benzyl-DTPA) was described previously (3). The conjugation ratio of diethylenetriaminepentaacetic acid:Ab was approximately 1.5:1, and the ^{111}In -labeled Ab had a specific activity of 10–80 mCi/mg, depending on the experiment. Labeling with ^{67}Ga used the chelator 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), as described previously (7), and resulted in specific activities of ~ 10 mCi/mg. ^{90}Y conjugation used the chelator 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA), as described (9), with a specific activity of ~ 5 mCi/mg. Labeled Abs were analyzed by either gel filtration high-performance liquid chromatography on a Bio-Sil SEC-250 column (Bio-Rad, Hercules, CA) or by instant TLC on silica gel strips (Gelman Sciences, Ann Arbor, MI), or both, and $>90\%$ of the counts were associated with Ab (usually $>95\%$). Immunoreactivity was determined on representative radiolabeled LL1 preparations, by binding to a large excess of Raji cells, and was 53–65%, with no evident differences between the radiolabels used.

Mice and Immunotherapy. Female SCID/NCr mice were obtained from the Animal Production Program of the National Cancer Institute (Frederick, MD). Their care was in accord with institutional guidelines. At 4–6 weeks of age, mice were injected with 2.5×10^6 Raji cells i.v. into the tail vein, following the model discovered and characterized by Ghetie *et al.* (10). Although these authors used the Daudi cell line, Raji cells grow similarly (11). The mice were monitored daily for hind-leg paralysis, which occurred in 16–24 days in control mice, and were sacrificed when paralysis was observed. For therapy, groups of 9–10 mice were injected at various times with radiolabeled Abs, i.v. into the tail vein. Although virtually all of the untreated control mice became paralyzed, some of the Ab-treated mice were found dead, without having paralysis detected. This tended to occur after 2–4 months of tumor growth, *i.e.*, 1–3 months after all control mice were found paralyzed. Such deaths can probably be attributed to tumor growth at sites other than the spinal canal, as described previously (10, 12), but in this study we did not attempt to determine the cause of death in such mice. Experiments were terminated after 6 months, and all remaining mice at this time were normal and apparently tumor free. Statistical comparisons between treatment groups used the log-rank test.

Results

The MTD of ^{111}In -labeled, ^{67}Ga -labeled, and ^{90}Y -labeled LL1 in SCID Mice. Non-tumor-bearing SCID mice, 6–8 weeks of age, were injected with increasing doses of ^{111}In -labeled, ^{67}Ga -labeled, or ^{90}Y -labeled LL1. To monitor toxicity, the body weight was determined weekly, in addition to visual observation of the mice. Table 1 shows the results in terms of survival. Many of the surviving mice treated with near-lethal doses had a substantial loss of body weight 2–4 weeks after injection. We define the MTD as the highest dose that does not kill any of the mice. The MTD for all radionuclides

Table 1 MTD determination for radiolabeled Ab LL1
SCID mice, 6–8 weeks of age, were injected i.v. with the dose indicated, in μCi .

^{111}In		^{67}Ga		^{90}Y	
Dose	Survivors	Dose	Survivors	Dose	Survivors
600	0/6	400	1/5	75	0/5
500	2/5	300	4/5	50	1/5
400	4/5	225	5/5	30	3/5
300	5/5				

was relatively low, much lower than would be expected for normal mice; this is primarily because of the SCID mutation, as discussed below. The MTD for ^{111}In -labeled LL1 was between 300–400 μCi ; we used 350- μCi doses in the experiments described below, with no radiation-induced deaths. With ^{67}Ga , the MTD was 225–300 μCi , apparently slightly lower than with ^{111}In (although the difference was not statistically significant). In a preliminary therapy experiment, 250 μCi of ^{67}Ga were more effective than 300 μCi , and approximately half of the mice treated with 300 μCi died without paralysis or other indications of tumor growth at days 25–30. It seems reasonable to assume that these deaths were attributable to radiation toxicity, perhaps combined with effects of tumor growth. The dose of 250 μCi also appeared to kill mice occasionally, despite its therapeutic effectiveness; therefore, we used maximum doses of 240 μCi in the experiments described below. ^{90}Y was much more toxic than either of the other two radionuclides, with 30 μCi still slightly above the MTD. We used doses of 25 μCi in the experiments described below, with no sign of toxicity.

Therapy of Disseminated B-cell Lymphoma in SCID Mice with ^{111}In -labeled Anti-CD74. Fig. 1A demonstrates the effective and specific Ab therapy of Raji B-lymphoma cells that were injected i.v. into SCID mice. In this experiment, the ^{111}In -labeled Ab was injected i.v. 5 days after injection of the tumor cells. The dose injected was 350 μCi /mouse (the MTD), and the specific activity was very similar for both the specific (LL1) and nonspecific (MN-14) Ab. As shown, LL1 greatly reduced the tumor growth rate in all of the mice and produced a complete cure (6 months without evidence of tumor growth) in 40% of the mice. Some mice succumbed to the tumor after 2–3 months, which was three to four times longer than the mean survival time of untreated mice (19.2 ± 3.1 days). The nonspecific Ab had a very slight, nonsignificant effect relative to the group of untreated control mice.

Fig. 1B shows a dose-response experiment in which varying doses of ^{111}In -labeled LL1 were injected. The maximum dose injected in this experiment was 275 μCi , slightly less than in the preceding experiment, but having a similar protective effect. With the 275- μCi dose, all of the mice showed delayed paralysis, and 70% were still alive at 10 weeks after tumor injection. However, some of these mice later developed tumors, and there were only 40% long-term survivors. The 92- μCi dose also produced a significant therapeutic effect, but only 20% of the mice survived for >50 days; these 2 mice remained tumor free for >6 months. The 31- μCi dose had only a small therapeutic effect, which however was still statistically highly significant ($P < 0.005$). A dose of 10 μCi was also tested in the

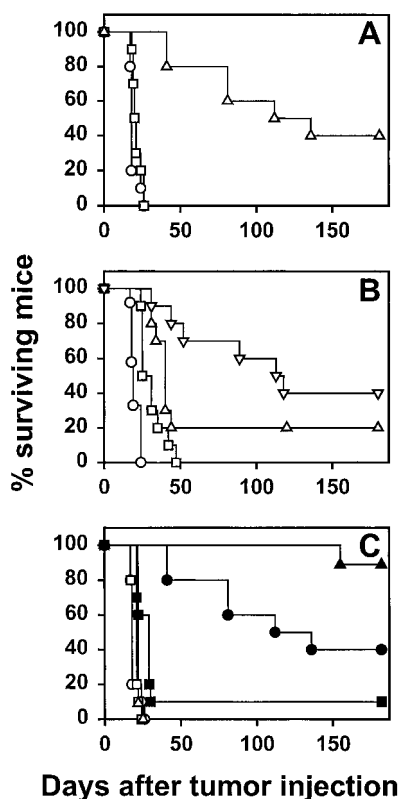


Fig. 1 Therapy of disseminated Raji xenografts in SCID mice with ^{111}In -labeled LL1. Mice were monitored daily for hind-leg paralysis, and paralyzed mice were sacrificed. The mice remaining at 180 days, when the experiments were terminated, appeared healthy. In A, 5 days after i.v. injection of tumor cells, groups of 10 mice were injected with 350 μCi of either ^{111}In -labeled LL1 (Δ) or ^{111}In -labeled MN-14, a nonreactive control antibody (\square). A control group of 10 mice had no antibody injected (\circ). The effect of LL1 was statistically significant, with $P < 0.001$ compared with either of the other two groups. In B, groups of 10 mice were treated on day 5 with 275 (∇), 92 (Δ), or 31 (\square) μCi of ^{111}In -labeled LL1. Results with 12 control untreated mice are also shown (\circ). The results with doses of 275, 92, and 31 μCi are significantly different from the control by the log-rank test (all $P < 0.005$). C, the time of antibody injection was day 3 (triangles), day 5 (circles), or day 9 (squares) after tumor cell injection, with a dose of 350 μCi of ^{111}In -labeled LL1. Results with untreated control mice (groups of 10–11 mice, open symbols) and antibody-treated mice (groups of 10 mice, filled symbols) are shown. The control mice were paralyzed at very similar times in all three experiments, as shown (which were therefore combined for statistical analysis). The day 5 results are the same as those presented in A and are included here to facilitate comparison. The results with the interval of 3 or 5 days were significantly different from the control by the log-rank test, with $P < 0.001$, and even the results with the 9-day interval were significantly different from the control, with $P < 0.025$, although the effect was relatively small.

same experiment, although it is omitted from Fig. 1B for the purpose of clarity; the therapeutic effect of this dose was slightly less than that of the 31 μCi dose but was still statistically significant ($P < 0.05$). Thus, a dose of 275–350 μCi appears to be necessary to maximize the therapeutic effect.

To demonstrate that the therapeutic effect was attributable to the radioactivity and not to the Ab itself, unlabeled LL1 was included as a control in two experiments, used at the same

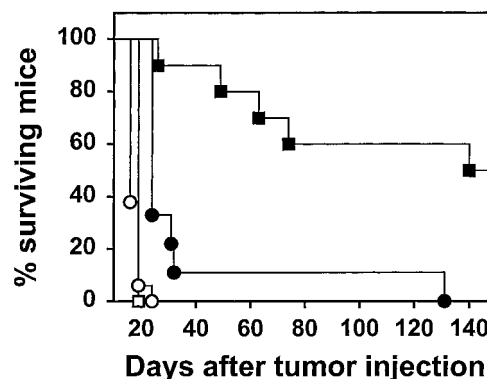


Fig. 2 Therapy of disseminated Raji xenografts with ^{67}Ga -labeled LL1, with the experimental system described in Fig. 1. On day 5 after i.v. injection of tumor cells, groups of 10 mice were injected with 240 μCi of either ^{67}Ga -labeled LL1 (\blacksquare) or ^{67}Ga -labeled MN-14, a non-reactive control antibody (\square). A control group of 16 mice had no antibody injected (\circ), and another group of 9 mice was injected with a lower dose, 75 μCi , of ^{67}Ga -labeled LL1 (\bullet). There were no additional mice paralyzed through 180 days, when the experiment was terminated, and the remaining mice appeared healthy. The dose of 240 μCi was significantly different from the control by the log-rank test, with $P < 0.001$, and even the dose of 75 μCi was significantly different from the control, with $P < 0.005$, although the effect was relatively small.

concentration as in the radiolabeled preparation. The protein dose injected ranged from 6 to 14 $\mu\text{g}/\text{mouse}$ in the separate experiments. This treatment had no effect relative to the control untreated group (data not shown).

All experiments described above used a 5-day interval between tumor injection and Ab administration. Experiments in which this interval was varied are shown in Fig. 1C. With an interval of 9 days, there was only a slight therapeutic effect, which however was still statistically significant ($P < 0.025$). With an interval of 3 days, tumor growth was totally prevented in essentially all of the mice. The only mouse not surviving for 6 months was found dead at day 155, without paralysis, and may have died from causes other than tumor growth.

The nonspecific toxicity of this therapeutic treatment was monitored by weekly determination of body weight for 6 weeks after Ab injection, as well as by visual inspection of the animals. This was included in the experiment shown in Fig. 1C, with therapy at day 3 after tumor inoculation, in which all of the mice survived for >154 days. Mice cured of tumor growth, by 350 μCi of ^{111}In -labeled LL1, displayed very little toxicity, with only a small loss in body weight of $5.1 \pm 2.5\%$ (mean \pm SD, $n = 9$), which was not a statistically significant weight loss. The nadir was at day 12 after Ab injection.

Therapy with ^{67}Ga -labeled and ^{90}Y -labeled LL1.

Therapy with ^{67}Ga -labeled LL1, at a dose of 240 $\mu\text{Ci}/\text{mouse}$ (the MTD), is shown in Fig. 2, together with the effect of a control Ab conjugated similarly. The delay between tumor inoculation and therapy was again 5 days. A strong therapeutic effect was observed, similar to the results with ^{111}In conjugates. Also shown are results with a lower dose of 75 μCi of ^{67}Ga ; this dose had a relatively small effect that was still statistically significant ($P < 0.005$). Fig. 3 shows results with ^{90}Y -labeled LL1, also used at the MTD, 25 μCi . This dose had only a slight

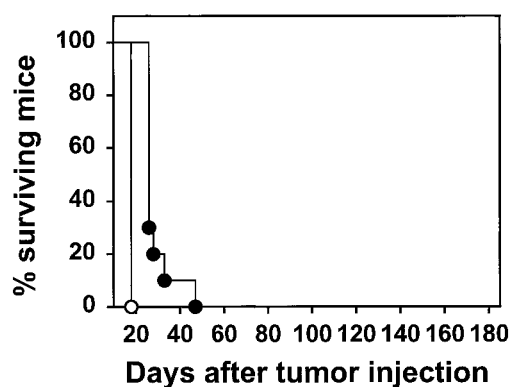


Fig. 3 Therapy of disseminated Raji xenografts with ^{90}Y -labeled LL1, with the experimental system described in Fig. 1. On day 5 after i.v. injection of tumor cells, a group of 10 mice was injected with 25 μCi of ^{90}Y -labeled LL1 (●), the MTD. A control group of 5 mice had no antibody injected (○). The X-axis scale is the same as used in the previous figures to facilitate comparison. The therapeutic effect was statistically significant ($P < 0.01$) but much weaker than that obtained with the other radionuclides tested.

therapeutic effect; all of the treated mice became paralyzed, and the mean time to paralysis was 29 days, compared with 18 days for the control mice. Although this effect was statistically significant ($P < 0.01$), it was much less effective than the other radionuclides tested. Therefore, we have not analyzed its specificity by testing a nonreactive Ab.

Discussion

The data presented demonstrate that ^{111}In -labeled and ^{67}Ga -labeled LL1 are potent and specific therapeutic agents for disseminated B-cell lymphoma in a mouse xenograft model. The results are consistent with our previous data showing *in vitro* toxicity with these conjugates (6, 7). Those studies demonstrated that the concentration required for near-100% kill *in vitro* was $\sim 30 \mu\text{Ci/ml}$ for ^{111}In conjugates and ~ 3 -fold lower for ^{67}Ga conjugates. With an injected dose of $\sim 300 \mu\text{Ci/mouse}$, the expected blood concentration after equilibration with interstitial fluid is $60 \mu\text{Ci/ml}$ (13), assuming a blood volume of 2.0 ml, and the expected concentration in interstitial fluid is $30 \mu\text{Ci/ml}$ (14). Thus, the dose-responses *in vivo* and *in vitro* are consistent. The very high cellular uptake of LL1 is unusual, but it seems possible that a similar approach may be effective with other Abs that are not internalized at such a high level. We showed recently that Abs binding in large amounts to the cell surface, without a high rate of internalization, were also able to deliver toxic amounts of ^{111}In or ^{125}I to cells *in vitro* (15).

^{111}In and ^{67}Ga are primarily used for imaging and are infrequently used for therapy. However, they emit Auger and conversion electrons that will kill cells at a sufficiently high dose (16). Although this is the first description of therapy with an ^{111}In -labeled Ab, therapy with ^{111}In -labeled octreotide was reported previously (17). However, it is very unlikely that cellular uptake of octreotide is at a level comparable with that of LL1. It has been suggested that the therapeutic effect of ^{111}In -labeled octreotide is attributable to nuclear localization (18). Ab conjugates of ^{125}I , also an Auger electron emitter, have been

used previously for tumor therapy in animal models (8, 19) and in clinical studies (20, 21). These conjugates displayed some cell killing *in vitro* (19, 21), although much less than that obtained with LL1. The conventional iodine label that was used in these studies is not useful with LL1, as we have shown previously *in vitro* (6), because the radioactive catabolic products are rapidly released from the target cell (5).

The comparison of Auger electron emitters with β -particle emitters is an important issue. *In vitro*, β -particle emitters (^{131}I and ^{90}Y) killed B-lymphoma cells efficiently and specifically when conjugated to Ab LL1 (7). Such toxicity is predicted by the intracellular S-values of the radionuclides used (16). But β -particle emitters produced greater nonspecific toxicity *in vitro* than Auger electron emitters (7), and our *in vivo* studies with ^{90}Y demonstrated this same difference. The MTD of the Ab conjugate was only 25 μCi for ^{90}Y , ~ 10 -fold lower than for ^{111}In or ^{67}Ga . At the MTD, ^{90}Y conjugates produced a very weak therapeutic effect. Again, these *in vivo* results were consistent with the *in vitro* dose-response. That is, the 25- μCi dose is expected to produce a peak concentration in interstitial fluid of $\sim 2.5 \mu\text{Ci/ml}$; this concentration, *in vitro*, produced a small but significant level of cell kill, $\sim 90\%$. However, the conclusions for ^{90}Y may or may not be applicable to lower-energy β -particle emitters such as ^{131}I . Lower energy β -particles will have greater potency in single-cell kill and possibly less nonspecific toxicity than ^{90}Y . Thus, ^{131}I appeared preferable to ^{90}Y in our *in vitro* studies. Unfortunately, the various residualizing forms of ^{131}I that we have tested *in vitro* are not suitable for *in vivo* experiments because of low specific activity and/or high accumulation in the kidney or liver (22). Improved residualizing iodine labels are currently under development. However, on the basis of our *in vitro* data, it seems unlikely that any of the β -particle emitters will display the therapeutic specificity seen with the Auger electron emitters. Even so, β -particle emitters may have an advantage in treating solid tumor masses because of the ability of β -particles to kill cells at some distance away from the site of emission. This factor may constitute a major advantage in cases in which full penetration of the tumor by the Ab is not readily achieved or where antigen expression on cells is heterogeneous. An attractive approach is to combine two radionuclides, on different or the same Abs, to adequately attack both microscopic disease and large tumor masses.

The apparent limitation of this method to treatment of a low disease burden should be noted. Although effective at day 5 after tumor inoculation, it had little effect at day 9. It is possible that better control of larger tumors may be achieved by administering multiple doses, or including β -particle emitters, as noted above. On the other hand, the rapid growth of this tumor xenograft (control mice normally become paralyzed at days 16–24) makes it a challenging model for therapy. In any case, this strategy seems most directly applicable to patients with suspected microscopic tumors.

A comparison of ^{125}I (Auger electron emitter) with ^{131}I (β -particle emitter) for RAIT was reported recently by Behr *et al.* (8). The MTD was $\sim 3.0 \text{ mCi/nude mouse}$ for ^{125}I and ~ 10 -fold lower for ^{131}I , and at the MTD ^{125}I was more potent than ^{131}I in tumor therapy using a nude mouse model with a s.c. carcinoma xenograft. These results, together with ours, suggest the possibility that Auger electron emitters may be generally

preferable to β -particle emitters for RAIT. We are not aware of any case in which the inverse was found, with both radionuclides used at their MTDs. But much additional work is required to determine whether this speculation is correct. Behr *et al.* (8) also demonstrated that the dose-limiting toxicity for ^{125}I -labeled Abs was bone marrow toxicity, as it is for Ab conjugates with β -particle emitters. Work with various radiometals, such as ^{90}Y or ^{67}Cu , which are residualizing labels, demonstrate that the bone marrow is also the dose-limiting organ with these labels (23, 24), at least if stable chelators are used. Although we have not examined toxicity to other organs, the bone marrow is likely to be the dose-limiting organ for radioconjugates of LL1.

In vitro, ^{67}Ga appeared to be better than ^{111}In as a toxic conjugate because of its greater level of specific killing (7). This difference was not confirmed in these therapy experiments, in which ^{111}In was equivalent to ^{67}Ga or better. Although the reason for this discrepancy is not known, the most likely explanation is the difference in specific activity, which was up to 8-fold higher for the ^{111}In conjugates. It may therefore be useful to develop methods of conjugating ^{67}Ga to Abs with higher specific activity.

Effective RAIT in animal models, with human tumor xenografts, has been reported many times, using tumors of various histological types (25). However, we are not aware of previous reports of effective RAIT of disseminated B-cell lymphoma using the SCID mouse model, as used herein. Using nude mouse s.c. xenograft models of B-cell lymphoma, effective RAIT was described with ^{131}I -labeled anti-CD37 (26) and ^{67}Cu -labeled Lym-1 (anti-MHC class II; Ref. 24). Recently, Wei *et al.* (12) described effective RAIT of s.c. lymphomas in immunodeficient mice, with ^{131}I -labeled anti-CD22 or anti-CD19. The therapy was much more effective in nude mice than in SCID mice because of the greater radiation sensitivity of the latter. These authors also attempted to treat disseminated tumors; RAIT alone was ineffective, but combined treatment with RAIT and an immunotoxin was very potent. We are currently evaluating the ability of ^{111}In -labeled LL1 to provide therapy for s.c. lymphoma xenografts. The ability of LL1 to specifically localize to s.c. xenografts was demonstrated recently (9).

The SCID mouse model used herein can be considered to be a particularly challenging model for RAIT, because SCID mice are known to be unusually sensitive to radiation (27, 28). Cells from SCID mice are approximately 2–3-fold as sensitive to ionizing radiation as cells from normal mice. This was reflected by the low MTDs obtained with all of the radionuclides tested, which were 2–3-fold lower than the MTDs in normal or nude mice.⁴ Similarly, Wei *et al.* (12) found that the MTD of ^{131}I -labeled Abs was 3–5-fold lower in SCID mice than in nude mice. Accordingly, the ratio of the MTD to the effective therapeutic dose, although quite low in this SCID mouse model, would be 2–3-fold greater in normal animals.

The key difference between the mouse model and the human situation is, of course, the lack of antigen expression on normal tissues of the mouse. CD74 is present on normal B cells and macrophage-lineage cells, including Kupffer cells in the

liver. It is uncertain how such normal tissue expression would impact on RAIT with this Ab. Because most bone marrow stem cells, including the most primitive population, are negative for the MHC class II antigen (29) and presumably for CD74, they will not be targeted. The lysis of normal B cells, as a side effect of cancer therapy, appears to be acceptable (30, 31), and the lysis of differentiated macrophage-lineage cells may also be acceptable. RAIT results with Ab Lym-1 (32) bear directly on this issue; this Ab reacts with the mature MHC class II antigen and thus has essentially the same normal tissue distribution as CD74. Although some liver uptake has been observed with Lym-1, it has been reduced by preinjection of unlabeled Ab and has not prevented the treatment of patients with high mCi doses of β -particle emitters linked to Lym-1. Moreover, the toxicity of LL1 linked to Auger emitters should be less than that of Lym-1 linked to β -particle emitters, because the toxicity of the Auger emitter is largely limited to cells which directly bind and internalize the Ab, as noted above. Thus, the delivery of ^{111}In -labeled LL1 to Kupffer cells would be expected to have little effect on adjacent hepatocytes, although this is not the case for β -particle emitters.

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References

- Dorke, B., Möller, P., Pezzutto, A., Schwartz-Albiez, R., and Moldenhauer, G. B-cell antigens: CD74. *In*: W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, and A. E. G. K. von dem Borne (eds.), *Leukocyte Typing*, Vol. 4, pp. 106–108. Oxford, United Kingdom: Oxford University Press, 1989.
- Roche, P. A., Teletski, C. L., Stang, E., Bakke, O., and Long, E. O. Cell surface HLA-DR-invariant chain complexes are targeted to endosomes by rapid internalization. *Proc. Natl. Acad. Sci. USA*, 90: 8581–8585, 1993.
- Hansen, H. J., Ong, G. L., Diril, H., Roche, P. A., Griffiths, G. L., Goldenberg, D. M., and Mattes, M. J. Internalization and catabolism of radiolabeled antibodies to the MHC class II invariant chain by B-cell lymphomas. *Biochem. J.*, 320: 293–300, 1996.
- Henderson, L. A., Baynes, J. W., and Thorpe, S. R. Identification of the sites of IgG catabolism in the rat. *Arch. Biochem. Biophys.*, 215: 1–11, 1982.
- Shih, L. B., Thorpe, S. R., Griffiths, G. L., Diril, H., Ong, G. L., Hansen, H. J., Goldenberg, D. M., and Mattes, M. J. The processing and fate of antibodies and their radiolabels bound to the surface of tumor cells *in vitro*: a comparison of nine radiolabels. *J. Nucl. Med.*, 35: 899–908, 1994.
- Griffiths, G. L., Govindan, S. V., Sgouros, G., Ong, G. L., Goldenberg, D. M., and Mattes, M. J. Cytotoxicity with Auger electron-emitting radionuclides delivered by antibodies. *Int. J. Cancer*, 81: 985–992, 1999.
- Govindan, S. V., Goldenberg, D. M., Elsamra, S. E., Griffiths, G. L., Ong, G. L., Brechbiel, M. W., Burton, J., Sgouros, G., and Mattes, M. J. Radionuclides linked to a CD74 antibody (LL1) as therapeutic agents for B-cell lymphoma: comparison of Auger electron emitters with β -particle emitters. *J. Nucl. Med.*, 41: 2089–2097, 2000.
- Behr, T. M., Sgouros, G., Vougioukas, V., Mentsoudis, S., Gratz, S., Schmidberger, H., Blumenthal, R. D., Goldenberg, D. M., and Becker, W. Therapeutic efficacy and dose-limiting toxicity of Auger-electron vs.

⁴ R. Stein, unpublished data.

- β emitters in radioimmunotherapy with internalizing antibodies: evaluation of ^{125}I - vs. ^{131}I -labeled CO17-1A in a human colorectal cancer model. *Int. J. Cancer*, 76: 738–748, 1998.
9. Shih, L., Ong, G. L., Burton, J., Mishina, D., Goldenberg, D. M., and Mattes, M. J. Localization of an antibody to CD74 (MHC class II invariant chain) to human B cell lymphoma xenografts in nude mice. *Cancer Immunol. Immunother.*, 49: 208–218, 2000.
 10. Ghetie, M.-A., Richardson, J., Tucker, T., Jones, D., Uhr, J., and Vitetta, E. S. Disseminated or localized growth of a human B-cell tumor (Daudi) in SCID mice. *Int. J. Cancer*, 45: 481–485, 1990.
 11. Van Horsen, P. J., Preijers, F. W. M. B., Van Oosterhout, Y. V. J. M., and De Witte, T. Highly potent CD22-recombinant ricin A results in complete cure of disseminated malignant B-cell xenografts in SCID mice but fails to cure solid xenografts in nude mice. *Int. J. Cancer*, 68: 378–383, 1996.
 12. Wei, B.-R., Ghetie, M.-A., and Vitetta, E. S. The combined use of an immunotoxin and a radioimmunoconjugate to treat disseminated human B-cell lymphoma in immunodeficient mice. *Clin. Cancer Res.*, 6: 631–642, 2000.
 13. Dewey, W. C. Vascular-extravascular exchange of ^{131}I -plasma proteins in the rat. *Am. J. Physiol.*, 197: 423–431, 1959.
 14. Jain, R. K. Transport of molecules in the tumor interstitium: a review. *Cancer Res.*, 47: 3039–3051, 1987.
 15. Ong, G. L., Elsamra, S. E., Goldenberg, D. M., and Mattes, M. J. Single-cell cytotoxicity with radiolabeled antibodies. *Clin. Cancer Res.*, 7: 192–201, 2001.
 16. Goddu, S. M., Howell, R. W., Bouchet, L. G., Bolch, W. E., and Rao, D. V. *MIRD Cellular S Values*. Reston, VA: Society of Nuclear Medicine, 1997.
 17. McCarthy, K. E., Woltering, E. A., Espenan, G. D., and Cronin, M. *In situ* radiotherapy with ^{111}In -pentetreotide: initial observations and future directions. *Cancer J. Sci. Am.*, 4: 94–102, 1998.
 18. Janson, E. T., Westlin, J.-E., Öhrvall, U., Öberg, K., and Lukinius, A. Nuclear localization of ^{111}In after intravenous injection of [^{111}In -DTPA-D-Phe 1]-octreotide in patients with neuroendocrine tumors. *J. Nucl. Med.*, 41: 1514–1518, 2000.
 19. Bender, H., Takahashi, H., Adachi, K., Belser, P., Liang, S., Prewett, M., Schrappe, M., Sutter, A., Rodeck, U., and Herlyn, D. Immunotherapy of human glioma xenografts with unlabeled, ^{131}I - or ^{125}I -labeled monoclonal antibody 425 to epidermal growth factor receptor. *Cancer Res.*, 52: 121–126, 1992.
 20. Brady, L., Miyamoto, C., Woo, D. V., Rackover, M., Emrich, J., Bender, H., Dadparvar, S., Steplewski, Z., Koprowski, H., Black, P., *et al.* Malignant astrocytomas treated with iodine-125 labeled monoclonal antibody 425 against epidermal growth factor receptor: a Phase II trial. *Int. J. Radiat. Oncol. Biol. Phys.*, 22: 225–230, 1992.
 21. Welt, S., Scott, A. M., Divgi, C. R., Kemeny, N. E., Finn, R. D., Daghighian, F., St. Germain, J., Richards, E. C., Larson, S. M., and Old, L. J. Phase I/II study of iodine 125-labeled monoclonal antibody A33 in patients with advanced colon cancer. *J. Clin. Oncol.*, 14: 1787–1797, 1996.
 22. Govindan, S. V., Mattes, M. J., Stein, R., McBride, B. J., Karacay, H., Goldenberg, D. M., Hansen, H. J., and Griffiths, G. L. Labeling of monoclonal antibodies with DTPA-appended radioiodinated peptides containing D-amino acids. *Bioconjug. Chem.*, 10: 231–240, 1999.
 23. Sharkey, R. M., Kaltovich, F. A., Shih, L. B., Fand, I., Govelitz, G., and Goldenberg, D. M. Radioimmunotherapy of human colonic cancer xenografts with ^{90}Y -labeled monoclonal antibodies to carcinoembryonic antigen. *Cancer Res.*, 48: 3270–3275, 1988.
 24. DeNardo, G. L., Kukis, D. L., Shen, S., Srivastava, S. C., Miers, L. A., and DeNardo, S. J. Efficacy and toxicity of ^{67}Cu -2IT-BAT-Lym-1 radioimmunoconjugate in mice implanted with human Burkitt's lymphoma (Raji). *Clin. Cancer Res.*, 3: 71–79, 1997.
 25. Goldenberg, D. M. *Cancer therapy with radiolabeled antibodies*. Boca Raton, FL: CRC Press, Inc., 1995.
 26. Buchsbaum, D. J., Wahl, R. L., Normolle, D. P., and Kaminski, M. S. Therapy with unlabeled and ^{131}I -labeled pan-B-cell monoclonal antibodies in nude mice bearing Raji Burkitt's lymphoma xenografts. *Cancer Res.*, 52: 6476–6481, 1992.
 27. Biedermann, K. A., Sun, J., Giaccia, A. J., Tosto, L. M., and Brown, J. M. *scid* mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. *Proc. Natl. Acad. Sci. USA*, 88: 1394–1397, 1991.
 28. Bosma, M. J., and Carroll, A. M. The SCID mouse mutant: definition, characterization, and potential uses. *Annu. Rev. Immunol.*, 9: 323–350, 1991.
 29. Knapp, W., Strobl, H., Scheinecker, C., Bello-Fernandez, C., and Majdic, O. Molecular characterization of CD34+ human hematopoietic progenitor cells. *Ann. Hematol.*, 70: 281–296, 1995.
 30. Jurcic, J. G., and Scheinberg, D. A. Radioimmunotherapy of hematological cancer: problems and progress. *Clin. Cancer Res.*, 1: 1439–1446, 1995.
 31. Wahl, R. L., Zasadny, K. R., MacFarlane, D., Francis, I. R., Ross, C. W., Estes, J., Fisher, S., Regan, D., Kroll, S., and Kaminski, M. S. Iodine-131 anti-B1 antibody for B-cell lymphoma: an update on the Michigan Phase I experience. *J. Nucl. Med.*, 39 (Suppl.): 21S–27S, 1998.
 32. DeNardo, G. L., DeNardo, S. J., Macey, D. J., Shen, S., and Kroger, L. A. Overview of radiation myelotoxicity secondary to radioimmunotherapy using ^{131}I -Lym-1 as a model. *Cancer (Phila.)*, 73: 1038–1048, 1994.