Pretargeted Radioimmunotherapy Using Anti-CD45 Monoclonal Antibodies to Deliver Radiation to Murine Hematolymphoid Tissues and Human Myeloid Leukemia

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Abstract

Radioimmunotherapy (RIT) for treatment of hematologic malignancies frequently fails because of disease recurrence. We therefore conducted pretargeted (P)RIT studies to augment the efficacy in mice of therapy using a pretargeted anti-human (h)CD45 antibody (Ab)-streptavidin (SA) conjugate followed by a biotinylated clearing agent and radiolabeled 1,4,7,10-tetraazacyclododecane N,N',N',N'''-tetraacetic (DOTA)-biotin. Tumor-to-blood ratios at 24 hours were 20:1 using pretargeted anti-hCD45 RIT and <1:1 with conventional RIT. In vivo imaging studies confirmed that the PRIT approach provided high-contrast tumor images with minimal blood-pool activity, whereas directly labeled anti-hCD45 Ab produced distinct tumor images but the blood pool retained a large amount of labeled Ab for a prolonged time. Therapy experiments showed that 90Y-DOTA-biotin significantly prolonged survival of mice treated with pretargeted anti-hCD45 Ab-SA compared with mice treated with conventional RIT using 90Y-labeled anti-hCD45 Ab at 200 μCi. Because human CD45 antigens are confined to xenograft tumor cells in this model, and all murine tissues are devoid of hCD45 and will not bind anti-hCD45 Ab, we also compared one-step and PRIT using an anti-murine (m)CD45 Ab where the target antigen is present on normal hematopoietic tissues. After 24 h, 27.3% ± 2.8% of the injected dose of activity was delivered per gram (% ID/g) of lymph node using 131I-A20-Ab compared with 40.0 ± 5.4% ID/g for pretargeted 111In-DOTA-biotin. These data suggest that pretargeted methods for delivering RIT may be superior to conventional RIT when targeting CD45 for the treatment of leukemia and may allow for the intensification of therapy, while minimizing toxicities. [Cancer Res 2009;69(1):185–92]

Introduction

Antibodies (Ab) conjugated to radionuclides have been used for over a decade to deliver targeted radiation to bone marrow, spleen, and other sites of acute myeloid leukemia (AML) while sparing normal organs (1–4). At least 90% of myeloid leukemias express CD45 and the antigen is not found on tissues of nonhematopoietic origin, making CD45 an attractive target for treatment of leukemia (5–10). Our group has performed clinical studies treating acute leukemias using myeloablative doses of an anti-CD45 radiolabeled Ab in combination with hematopoietic cell transplantation to reconstitute hematopoiesis (11–13). These studies suggested that a greater radiation absorbed dose delivered to marrow may lead to lower posttransplant relapse rates for patients with high-risk AML (14). Although these higher doses of radioimmunotherapy (RIT) seem to be more efficacious, many patients treated with this approach still relapse. Because acute leukemias have a relatively steep dose-response curve with no apparent radiation threshold for cell injury, this therapy could be even more effective and less toxic if the tumor-to-normal organ dose ratios could be improved.

Although the use of RIT may lead to the accumulation of DNA damage in leukemia cells by delivering a prolonged, exponentially decreasing low dose-rate of radiation exposure, the suboptimal therapeutic index (risk-to-benefit ratio) currently achievable with conventional RIT methodologies is a general limitation of RIT. Pretargeted (PR)IT is a method that might improve the delivery of a cytotoxic radionuclide to target cells while reducing nonspecific radiation to normal organs and thus minimizing toxicity (15–23). PRIT first allows for maximal Ab targeting before delivery of the therapeutic radionuclide. The delivery of the radionuclide is mediated via attachment to a small molecule that allows for rapid tumor uptake and rapid excretion of nontumor bound radioactivity. Synthetic clearing agents (CA) have been introduced as an additional refinement to PRIT studies to remove nontargeting immunoconjugates lingering in the bloodstream before administration of the radioactive moiety (17–19).

To assess the merits of CD45 PRIT for leukemia, we here report comparative in vivo imaging, biodistribution, and therapy experiments using human leukemia xenografts implanted in athymic mice. In a series of fluorescent imaging studies, we have shown significantly superior localization to human erythroleukoblastic leukemia (HEL) tumor sites using PRIT compared with conventional RIT. In addition, a single treatment of pretargeted anti-human (h)CD45 Ab-streptavidin (SA) BC8 conjugate followed by a single dose of radio-biotin resulted in tumor-to-blood and tumor-to-normal organ radioactivity concentration ratios that improved by as much as 15-fold over those seen with a directly radiolabeled anti-hCD45 Ab, resulting in markedly enhanced therapeutic efficacy with the PRIT method. The murine tumor xenograft model has limitations, however, because only the human tumor cells bear the target antigen and the host immune system is defective. Furthermore, the HEL xenograft model consists of a single subcutaneous nodule, which is analogous to a chloroma but is dissimilar from the disease pattern in most leukemia.
patients who have blood- and marrow-based disease. To counterbalance these limitations, we also report experiments in a syngeneic murine system using an anti-murine (m)CD45 Ab A20 that targets normal hematopoietic CD45+ tissues, as is the case in human patients. Results from these syngeneic experiments showed marked improvement in the hematopoietic organ to nonhematopoietic organ radioactivity concentration and absorbed dose ratios using PRIT due primarily to elimination of the initial nonspecific radioactivity from circulating blood—when directly labeled Abs were used. Radiation dose calculations for the syngeneic model showed that at least twice as much radiation absorbed dose can be delivered to the marrow, and five times more to the spleen, using PRIT compared with doses delivered by a conventional radiolabeled Ab. These data suggest that anti-CD45 PRIT may be highly effective and may allow for intensification of the targeted radiotherapy, with diminished toxicity, to sites of leukemic involvement to decrease the risk of relapse.

Materials and Methods

Mice

Female BALB/c athymic mice, 6- to 8-wk-old, were purchased from Harlan Sprague-Dawley. Male B6 Ly5a mice were bred at the Fred Hutchinson Cancer Research Center (FHCRC) and housed in a pathogen-free environment with acidified water and autoclaved chow. The animals were housed under protocols approved by the FHCRC Institutional Animal Care and Use Committee. Results from all mouse studies are representative of at least two experiments.

Cell Lines, Abs, and Production of DOTA-Ab and Ab-SA Conjugates

All cells were maintained as described previously (19). The HEL cell line was obtained from American Type Culture Collection (ATCC). The BC8 hybridoma cell line expressing the anti-human IgG1 CD45 Ab was a gift from Claudio Anasetti (Fred Hutchinson Cancer Research Center, Seattle, WA). The hybridoma cell line secreting murine IgG2a A20 Ab, which recognizes the Ly5.1 epitope encoded by the Ly5a allotype of murine CD45, was a gift from Dr. Shoji Kimura of Memorial Sloan Kettering Cancer Center (New York, NY). The A20 Ab was produced from mouse ascitic fluid as previously described (19). Isotype-matched (IgG1) human anti-bovine herpesvirus-1 (BHV-1) Ab was produced from a hybridoma obtained from ATCC and used as nonspecific negative control Ab for human HEL xenograft experiments. Hybridoma culture supernatants from the BC8 and BHV-1 Abs were produced using hollow fiber bioreactor systems in the FHCRC. Rat polyclonal IgG (MS163) was obtained from Biomeda and used as nonspecific negative control for murine syngeneic studies. DOTA-Ab and Ab-SA conjugates were produced as described previously (19).

Radiolabeling

Abs were iodinated with Na131I or Na125I (PerkinElmer, Inc.) by the chloramine-T method as previously published (19). 111Indium (PerkinElmer, Inc.) radiolabeling of intact Ab-SA conjugates for PRIT was performed as described (19). Radiochemical purity was typically >99% as determined by high performance liquid chromatography for each construct, and labeling efficiencies were >90%. DOTA-biotin was synthesized as described (19).

Biotinylated CA

A synthetic biotinylated CA containing 16 N-acetyl-galactosamine residues per dendrimeric molecule was a gift from NeoRx Corporation and was used for PRIT experiments to eliminate excess Ab-SA molecules from the circulation before the administration of radiolabeled biotin. The N-acetyl galactosamine residues have a high affinity for hepatic asialoglycoprotein receptors and thus facilitate the rapid hepatic clearance of residual Ab-SA conjugates from the bloodstream and their endocytosis into liver cells (17).

In vivo Imaging Studies

Anti-CD45 BC8 whole Ab was used for conventional studies and was labeled with Xenluor 680 (Caliper Life Sciences) according to the manufacturer’s instructions. The fluoresceinated BC8 Ab (1.4 nmol) was delivered i.v. via tail vein to athymic mice (n = 3) bearing HEL tumor xenografts at time 0 h. The group of pretargeted mice (n = 3) received 1.4 nmol of unlabeled anti-CD45 BC8 Ab-SA i.v. at time ~27 h followed by 5.8 nmol of CA at time ~3 h. At time 0 h the pretargeted mice were injected i.v. with 100 μg of R-phycocerythrin biotin conjugate (Invitrogen). All mice were anesthetized with 2.5% isofluorane in O2. Each animal was imaged on a Xenogen IVIS 100 System (Caliper Life Sciences). Background fluorescence of each image was removed after calculation of a region of interest (ROI) located on negative control (untreated) mice, using the formula A - Bx2; with A indicating fluorescent excitation measured, B indicating fluorescent emission measured, and k being the constant being calculated by determining the ratio of the ROI from the excitation to the emission image. Fluorescence intensity and tumor-to-total body ratios were determined for each mouse using background-corrected fluorescent whole-body images and a standard background ROI for comparison to measurements obtained from a ROI on each tumor.

Biodistribution Studies

Mice were injected with 1 × 106 HEL cells s.c. in each flank to generate human leukemia xenograft tumors. Mice were selected for experimentation at a time when similar HEL tumor sizes (~ 100 mm3) were detectable (~7 to 10 d after the s.c. administration of the HEL cells). Mice were placed on a biotin-free diet (Harlan Teklad) for 5 d before receiving an Ab construct. Groups of 5 mice were injected i.v. via the tail vein with 1.4 nmol of 111In-labeled (50 μCi) anti-hCD45 (BC8) Ab or nonbinding control BHV-1 Ab (conventional RIT) or unlabeled Ab-SA or BHV-1 Ab-SA conjugate (PRIT). Mice in PRIT groups were subsequently administered 5.8 nmol (50 μg) of CA i.v. 22 h later followed by i.v. delivery of 1.2 nmol (1 μg) of 111In-DOTA biotin (50 μCi) 2 h after CA. At 24 or 48 h after injection, mice were bled from the retro-orbital venous plexus, euthanized, and tumors and normal organs were excised, weighed, and γ counted for 111In activity using a Packard 5000 γ counter (Packard Instrument Co). The percent administered activities per gram of tumor or organ (% ID/g) were determined (after correcting for radioactive decay using an aliquot of the injectate at each time point), as were the tumor-to-normal organ absorbed dose ratios.

To determine the biodistribution of anti-mCD45 (A20) DOTA-Ab and Ab-SA conjugates in syngeneic B6 Ly5a mice, animals were injected i.v. with either 0.67 nmol of A20 DOTA-Ab trace-labeled with 131I (50 μCi) or an equal molar amount of unlabeled A20 Ab-SA conjugate. Twenty hours after the injection of the Ab-SA conjugate, these mice were injected with 1.4 nmol of CA i.v. followed 4 h later by i.v. delivery of 1.2 nmol of 111In-DOTA biotin. Control groups were injected with 0.67 nmol of the nonbinding rat polyclonal IgG1 131I-DOTA-Ab or Ab-SA conjugate followed by CA and 111In-DOTA-biotin. Groups of 5 mice were sacrificed at multiple time points from 2 to 96 h after injection, and tissues were sampled, weighed, and counted as described above.

Radiation dosimetry. Time-activity curves were constructed for each organ from the syngeneic murine biodistribution data, and the infinite-time integrals were calculated for the areas under curve to estimate the total number of radioactive transformations for dosimetry, assuming that both A20 DOTA-Ab and A20 Ab-SA conjugates were labeled with 131I. Absorbed fractions of β-particle energy for 131I were calculated by applying electron transport theory to a dosimetric model for the laboratory mouse (24). This model accounts for the size, shape, position, and density of organs, as well as for the overlap and contact from surrounding organs and tissues. The absorbed fractions included contributions from same-organ irradiation as well as from cross-organ irradiation. Contributions to the absorbed dose from 131I penetrating γ radiation in mouse organs were negligible (<1% of the total dose) and were neglected. The femoral marrow was assumed to be a cylinder of 0.5 mm in radius; therefore, only a proportion (46%) of the radioiodine present in marrow was assumed to deposit its energy inside the marrow. The β-particle absorbed fraction for lymph nodes was estimated to be 73%. Results were expressed as organ absorbed dose (Gy).
RIT of Leukemia Xenografts

To compare the therapeutic efficacy of pretargeted and conventional radiolabeled Abs, groups of 10 HEL leukemia tumor-bearing mice were placed on biotin-free chow for 7 d and injected with 1.4 nmol (215 μg; 200–400 μCi) of directly labeled 90Y-anti-hCD45 BC8 Ab, control 90Y-BHV-1 Ab, or equimolar amounts (300 μg) of anti-hCD45 BC8 Ab-SA or BHV-1 Ab-SAs conjugates followed 22 h later by 5.8 nmol CA, and 2 h later by 1.2 nmol (1 μg) 90Y-DOTA-biotin labeled with 400, 800, or 1,600 μCi of 90Y. Mice were monitored every other day for general appearance, tumor volume measurements, and body weight. Mice were euthanized if xenografts exceeded 10% of total body weight, caused obvious discomfort, or impaired ambulation. Tumor volumes (mm³) were calculated as previously described using the following equation: 4/3 A × B × C, where A, B, and C are the three dimensional radii in mm (19). Time-to-tumor growth (taken as time to development of tumor volume >400 mm³) and time-to-death were treated as time-to-event end points, and Cox regression was used to test for a dose-response effect. For mice that received PRIT, levels of 1 to 5 were assigned to the groups that received no treatment, the control Ab, PRIT at the dose of 400 μCi, PRIT at the dose of 800 μCi, and PRIT at the dose of 1,600 μCi 90Y-DOTA-biotin, respectively. For mice that received conventional RIT, levels of 1 to 4 were assigned to the groups that received no treatment, the control Ab, RIT at 200 μCi, and RIT at 400 μCi 90Y-anti-hCD45 Ab, respectively. A factor treating these levels as a continuous linear variable was then included in a Cox regression model to test for a dose-response effect (i.e., to ask if the risk of failure was associated with an increase in dose).

Results

In vivo fluorescent imaging. In vivo fluorescent imaging was performed in athymic mice bearing HEL leukemia xenografts at multiple time points after injection of either 1.4 nmol fluorophore-labeled anti-hCD45 DOTA-Ab (conventional) or 100 μg R-Phycocerythrin-biotin after delivery of 1.4 nmol unlabeled anti-hCD45 Ab-SA and CA (pretargeted), as described in the methods section (Fig. 1). In the conventional RIT group, most of the fluorescence after 12 hours was found in the blood pool and minimal tumor localization was observed (Fig. 1A). Although tumors were visible in the conventional RIT group at 24 and 48 hours, a large amount of fluorophore remained in the blood pool. The ratio of fluorescence in tumor-to-total body was 1:8.5 (Fig. 1C). Rapid uptake of fluorophore was observed in tumors after 12 hours in the pretargeted group (Fig. 1B) as well as rapid urinary excretion of unbound PE-biotin, manifested by a large amount of fluorescence in the bladder. By 24 hours, the majority of fluorescent activity in the bladder had cleared in the pretargeted group, resulting in high-contrast tumor images with a relative ratio of fluorescence in tumor-to-total body of 1:3 (Fig. 1C).

Biodistributions of 111In in HEL leukemia tumor-bearing mice treated with directly labeled 111In-anti-hCD45 Ab. Groups of 5 mice bearing human HEL xenografts were injected i.v. with 1.4 nmol of 111In-anti-hCD45 Ab or negative control 111In-anti-BHV-1 Ab. Mice were sacrificed 24 and 48 hours 111In-Ab administration (Fig. 2A). The peak concentration levels of 111In obtained in HEL tumors after conventional RIT using 111In-anti-hCD45 Ab after 24 and 48 hours were 11.7% ± 1.0% ID/g and 17.4% ± 3.3% ID/g, respectively. The concentrations of radioactivity in normal organs at 24 and 48 hours using directly labeled 111In-anti-hCD45 Ab ranged from 2.5% ID/g in stomach to 14.1% ID/g in kidney. In addition, circulating blood radioactivities after 24 and 48 hours were relatively high in mice receiving 111In-anti-hCD45 Ab. For example, concentrations in blood of 111In-anti-hCD45 Ab were 19.1% ± 0.29% and 20.5% ± 1.5% ID/g at 24 and 48 hours, respectively. At each time point, control animals injected with the nonbinding 111In-BHV-1 Ab had low tumor uptake of radioactivity in HEL xenografts at 24 and 48 hours (5.6% ± 1.1% and 5.9% ± 1.3% ID/g, respectively), demonstrating the specificity of targeting.

Biodistributions of radioactivity after PRIT using anti-hCD45 Ab-SA conjugate. Pretargeted biodistribution studies were performed using HEL leukemia-bearing mice injected with 1.4 nmol of anti-hCD45 (BC8) Ab-SA, followed 22 hours later by 5.8 nmol of CA and, 2 hours after that, by 1.2 nmol 111In-DOTA-biotin. Groups of 5 mice each were sacrificed 24 and 48 hours after administration of the Ab-SAs conjugate (Fig. 2B). The uptake of radioactivity in tumors of mice treated with pretargeted anti-hCD45 Ab-SA conjugate reached a maximum of 6.7% ± 2.4% ID/g 24 hours after administration of 111In-DOTA-biotin, and was still 6.9% ± 2.5% ID/g after 48 hours. The specificity of tumor targeting by anti-hCD45 Ab-SA conjugate in these experiments was shown by comparison to control groups treated with BHV-1-Ab-SA followed by CA and 111In-DOTA-biotin. Control mice did not exhibit any appreciable radiobiotin uptake in tumors at any time point (e.g., 1.0% ± 0.23% ID/g at 24 hours).

At all time points assessed, the tumor-to-normal organ ratios were superior with the pretargeted anti-hCD45 Ab-SA conjugate compared with the ratios seen using the DOTA-Ab conjugate (Fig. 2C). At the 48-hour time point, the tumor-to-blood ratio was 10.1 using pretargeted anti-hCD45 Ab-SA but only 0.81 with directly labeled DOTA-Ab conjugate. Additional tumor-to-normal organ ratios using the pretargeted approach with the BC8 Ab-SA conjugate at 48 hours varied from 50:1 in stomach to >35:1 in kidney. At the same time point using conventional radiolabeled BC8 DOTA-Ab, the tumor-to-stomach ratio was 7.3:1 and the tumor-to-kidney ratio was ~1:1. These data suggest that PRIT produces markedly superior tumor-to-normal organ radioactivity concentration ratios compared with conventional radiolabeling, despite lower uptake of radioactivity into tumor xenografts.

RIT with either conventional or pretargeted anti-hCD45 Ab conjugates. In view of the promising findings of the comparative biodistribution experiments described above, we performed therapy experiments to assess whether the superior biodistributions obtained with the PRIT method would translate to enhanced efficacy compared with conventional one-step RIT. Experimental groups of 10 human leukemia-bearing mice were injected with 1.4 nmol anti-hCD45 (BC8) Ab-SA, followed 22 hours later by 5.8 nmol CA, and then 2 hours after CA by 1.2 nmol 90Y-DOTA-biotin labeled with 400, 800, or 1,600 μCi/mouse. Comparison groups were injected with 1.4 nmol directly radiolabeled 90Y-anti-hCD45 Ab (200 or 400 μCi/mouse). Control groups were either untreated or injected with 1.4 nmol of the nonbinding BHV-1 Ab labeled with 400 μCi of 90Y or BHV-1 Ab-SA control conjugate followed by 5.8 nmol CA and then 1,600 μCi 90Y-DOTA-biotin. All mice in the control groups experienced exponential growth of their leukemia xenografts requiring euthanasia before day 16 (Fig. 3). Mice treated with conventional one-step RIT using 200 μCi 90Y-anti-hCD45 Ab experienced transient remissions with complete regression of tumor seen in 30% of mice by day 11 after treatment (Fig. 3A). However, 90% of mice in this group exhibited huddling behavior, developed extensive petechiae, and died by day 17 (Fig. 3C). Mice treated with 400 μCi conventional one-step 90Y-anti-hCD45 Ab experienced more striking tumor regressions, with complete disappearance of xenografts in 40% of the animals by day 9 after therapy (Fig. 3A). However, all mice in this group experienced lethal toxicity, losing 26.7% ± 15.5% of their initial weight and dying, likely from marrow suppression and infection, by day 11 (Fig. 3C). Mice were not treated with doses of direct conjugates higher than 400 μCi as this was a uniformly lethal dose.
Experimental mice pretargeted with BC8 Ab-SA fared much better than other groups in terms of toxicity, tumor responses, and survival (Fig. 3B and D). Transient tumor responses were seen in mice receiving pretargeted anti-hCD45 Ab-SA plus 800 μCi ⁹⁰Y-DOTA-biotin with a maximal response seen ~6 days after treatment; however, tumors rapidly recurred in all mice, leading to death by day 41 (Fig. 3B and D). In contrast, all mice receiving pretargeted BC8 Ab-SA plus 1,600 μCi ⁹⁰Y-DOTA-biotin achieved complete remissions before day 15. Three mice in the 1,600 μCi ⁹⁰Y-DOTA-biotin group died by day 16 from toxicity without tumor, whereas the remaining 7 mice died from tumor relapse between day 42 and 55 (Fig. 3D). There was minimal toxicity in mice pretargeted with anti-hCD45 Ab-SA followed by 400 or 800 μCi ⁹⁰Y-DOTA-biotin, with only 4.7% ± 2.0% weight loss in the 800 μCi ⁹⁰Y-DOTA-biotin by day 4; all mice regained pretreatment weight by day 11 (data not shown).

Overall, the hazard of tumor growth to >400 mm³ decreased as radionuclide dose increased in each group as dose increased but was statistically significant only for the mice treated with PRIT (P < 0.0001 PRIT; P = 0.31 for conventional RIT). However, the hazard of death decreased as radionuclide dose increased both for mice who received conventional RIT and for mice who received PRIT (P < 0.0001 for each group).

Biodistributions of radioactivity after conventional and PRIT using a syngeneic murine model using anti-mCD45 DOTA-Ab and Ab-SA conjugates. We first assessed the efficacy of the CA using the PRIT strategy on blood clearance in a relevant syngeneic murine system using an anti-mCD45 Ab (A20) that targets normal hematopoietic CD45+ tissues, as is the case in human therapies directed toward the CD45 antigen. Groups of 5 syngeneic B6 Ly5a mice received 1.4 nmol of either ¹²⁵I-A20 DOTA-Ab or ¹²⁵I-A20 Ab-SA administered i.v., followed 22 hours later by 5.8 nmol of CA. The concentration of ¹²⁵I-A20 Ab-SA in the blood decreased from 31.3% ± 0.29% ID/g of blood to 3.3 ± 2.1% ID/g within 1 hour after CA administration. By 48 hours after CA injection, 2.6% ± 0.4% ID/g of ¹²⁵I-A20 Ab-SA conjugate was still circulating in blood.

The hematolymphoid biodistributions of radioactivity of directly labeled anti-mCD45 (A20) DOTA-Ab and pretargeted A20 Ab-SA conjugates were then compared in the syngeneic B6 Ly5a mouse model. These biodistribution studies were performed using mice injected with 1.4 nmol of either directly labeled ¹³¹I-A20 Ab alone or pretargeted A20 Ab-SA followed 20 hours later by 5.8 nmol of CA and 4 hours after that by 1.2 nmol ¹¹¹In-DOTA-biotin. Groups of 5 mice were sacrificed at time points between 2 and 96 hours after injection of radioactivity, and their organs were harvested, weighed, and assayed for ¹³¹I and ¹¹¹In activity. Specific localization of conventional ¹³¹I-A20 Ab or pretargeted ¹¹¹In-DOTA-biotin in hematolymphoid organs including blood, bone marrow, lymph node, and spleen is depicted in Fig. 4. At all time points, control animals injected with nonspecific ¹³¹I-labeled rat polyclonal anti-murine IgG Ab and ¹¹¹In-Ab-SA exhibited negligible uptake of the radiolabel in hematolymphoid organs demonstrating the specificity of targeting in these experiments (data not shown).

Specific uptake of directly labeled ¹³¹I-anti-mCD45 A20 Ab in target tissues reached a maximum value by 20 hours with 15% ±
2.8% ID/g delivered to marrow and 42% ± 2.7% ID/g to the spleen (Fig. 4A). The targeted concentrations of radioactivity remained at relatively high levels in the bone marrow and spleen using the directly-labeled anti-mCD45 Ab with >8% ID/g present in these target tissues 48 hours after administration of the radiolabeled Ab. The amount of absorbed radioactivity also remained relatively high in the blood and normal organs, however, with 11.2% ± 1.8% ID/g remaining in the blood and 6.2 ± 0.88% ID/g in the lung after 20 hours (Fig. 4A). Despite the relatively rapid clearance of the radiolabeled Ab from the blood clearance from the circulation may still be too slow for optimal RIT without a pretargeted approach. In particular, PRIT with a CA has the potential to eliminate the initial 20 hours of nonspecific radiation exposure from blood-borne directly labeled A20 Ab (represented by the shaded area-under-the-curve, Fig. 4A). Consequently, a series of biodistribution experiments using the PRIT strategy were performed in the syngeneic murine CD45 system using nontumor bearing B6 Ly5a mice to examine the effect of eliminating the nonspecific radiation exposure from blood-borne radiolabeled Ab. Pretargeted anti-mCD45 A20 Ab-SA conjugate followed by the CA resulted in favorable biodistributions of radioactivity at each time point assessed (Fig. 4B). After 24 hours, 27.3% ± 2.8% ID/g was delivered to lymph nodes using 131I-A20 Ab compared with 40.0% ± 5.4% ID/g for pretargeted 111In-DOTA-biotin after A20 Ab-SA administration. Superior hematopoietic organ-to-nonhematopoietic organ ratios of radioactivity were seen using the A20 Ab-SA pretargeted
conjugate compared with directly labeled $^{131}$I-A20 Ab. The lymph node-to-blood ratios after 24 hours were 40:1 with the pretargeted A20 SA conjugate compared with 13.5:1 for conventional $^{131}$I-A20 Ab. Table 1 summarizes the mean radiation absorbed doses imparted to each hematolymphoid organ using either conventional targeting of mCD45 or PRIT under conditions delivering 10 Gy to the liver. The calculated radiation absorbed doses to the marrow and lymph node were at least 2-fold greater using the PRIT strategy, whereas 5 times more was delivered to the spleen compared with doses delivered by a directly labeled anti-mCD45 DOTA-Ab. A liver dose of 10 Gy was selected because this dose produces dose-limiting hepatic toxicity in human clinical trials of $^{131}$I-BC8 combined with high-dose chemotherapy and TBI (11).

**Discussion**

The data presented in this study are the first to compare conventional and pretargeted anti-hCD45 RIT in an acute leukemia xenograft model. The results showed clearly that anti-CD45 PRIT provided rapid tumor localization and improved biodistributions of radioactivity with significant improvements in efficacy compared with conventional anti-CD45 RIT. Although the concentration of activity in leukemia xenografts decreased with the PRIT strategy compared with conventional RIT (6.7% ID/g versus 11.7% ID/g, respectively, at 24 hours), the concentration of $^{111}$In in the blood at 24 hours decreased by 93% and the level in kidneys by 89%. These findings are likely attributable to the use of the CA and its ability to not only remove excess circulating Ab-SA conjugate from the circulation but also conjugate localized to tumors. Indium-111 was used as a surrogate $\gamma$-emitting radiometal in biodistribution experiments because $^{90}$Y does not emit $\gamma$ photons (25, 26). Because the toxic effects on normal tissues must be balanced by the cytotoxic effects on tumor cells, the pretargeted approach was markedly superior to conventional RIT when the therapeutic index (target-to-nontarget ratio) of each targeting strategy was considered. Conventional radiolabeled anti-hCD45 Ab achieved low tumor-to-blood ratios of <1:1 and tumor-to-normal organ ratios of 7:1 or less. Conversely, the PRIT approach produced tumor-to-normal organ radioactivity concentration ratios that were far superior, with tumor-to-blood ratios of up to 10:1 and tumor-to-normal organ ratios of 50:1 or higher, albeit at the risk of removing a nonnegligible amount of pretargeted Ab-SA conjugate from the xenograft. In vivo images displayed excellent tumor imaging with rapid localization of fluorophore-DOTA-biotin into the tumors.

**Figure 3.** Regression of HEL leukemia xenografts and survival of mice after conventional and PRIT. Athymic BALB/c mice bearing HEL leukemia xenografts were injected i.v. with either 1.4 nmol (A and C) BC8 Ab directly labeled with 200 to 400 $\mu$Ci of $^{90}$Y, or (B and D) unlabeled BC8 Ab-SA conjugate followed 20 h later by 5.8 nmol of CA, and 2 h after that with 400 to 1,600 $\mu$Ci $^{90}$Y-DOTA-biotin. Control mice bearing xenograft tumors were either left untreated or treated in a similar manner with directly labeled BHV-1 Ab or pretargeted BHV-1-SA, respectively. Tumor volume curves (A, conventional RIT; B, PRIT) are truncated at the time of euthanasia of the first mouse in each group. These mice bearing HEL leukemia xenografts were also analyzed for survival as a function of time (C, conventional RIT; D, PRIT).
tumors of the pretargeted animals within 5 hours. The efficient clearance of excess circulating immunoconjugate from the vasculature by the CA would suggest that in the setting of radiolabeled-DOTA-biotin, rapid urinary excretion of any excess unbound radionuclide would occur, thus minimizing nonspecific irradiation to normal organs and contributing to the superiority of the tumor images with the pretargeted method. Conversely, tumor images generated using conventional RIT with fluoresceinated anti-hCD45 Ab exhibited a high residual level of activity persisting in the blood and other nontarget organs. The improved tumor localization of the anti-hCD45 Ab using PRIT was predicted to translate into improved xenograft response rates and survival rates. We thus performed comparative therapeutic RIT studies in mice treated with either directly labeled anti-hCD45 Ab or anti-hCD45 Ab-SA, followed by CA, and then increasing doses of 90Y-DOTA-biotin. Because the therapeutic index of an antineoplastic agent depends on the balance between toxic effects on normal tissues and cytotoxic effects on tumor cells, it is not surprising that the pretargeted approach may be superior to conventional RIT in terms of tumor regressions and improved overall survival.

Although these mouse leukemia xenograft experiments with anti-hCD45 PRIT were encouraging and established the ability of a pretargeted anti-hCD45 Ab-SA conjugate to effectively localize to CD45 expressing human tumor cells, this model differs significantly from the state of naturally occurring leukemias in patients. In the xenograft system, the human target CD45+ cells are confined to tumors, which are more similar to chloromas than to typical disseminated leukemia. In addition, all other tissues in the xenograft model are devoid of hCD45 and will not bind anti-hCD45 Ab. Leukemia patients, however, display the hCD45 antigen on almost all normal hematopoietic tissues (bone marrow, lymph node cells, Kupfer cells, etc.) as well as on malignant cells. Toxicity profiles, particularly hematopoietic toxicities, in the human may thus not be reliably mimicked in xenograft systems. To overcome these limitations, we tested anti-CD45 PRIT in a complementary murine syngeneic system in which the target antigen was present on normal myeloid, lymphoid, and reticuloendothelial tissues. We have shown in the murine syngeneic model that the CA efficiently reduced circulating anti-mCD45 Ab-SA conjugate from the blood and thus limited the amount of radionuclide delivered to nontarget normal organs, resulting in higher target organ to nontarget organ radioactivity ratios using PRIT compared with the ratios seen using standard directly labeled Ab. These data are supported by preliminary studies in a separate preclinical syngeneic leukemia model where PRIT appears to improve the therapeutic index over that currently achievable with conventional RIT methodologies (20).

In addition to the SA-biotin PRIT system, other methods of PRIT have been described including bispecific Abs that recognize a radiolabeled hapten and the targeted tumor antigen as well as biotinylated Abs capable of binding avidin or SA (27–29). Although these PRIT approaches form a base of noteworthy principles and encouraging results, further advances are required to achieve optimal targeting and elimination of leukemia cells by PRIT. One recent improvement in the anti-CD45 PRIT technology has been the enhanced uniformity of the Ab-SA targeting.

Figure 4. Biodistributions of radioactivity in blood, bone marrow, spleen, lymph node, and lung of nontumor bearing B6 Ly5a syngeneic mice injected with directly radiolabeled anti-mCD45 DOTA-Ab or 111In-DOTA-biotin after pretargeted anti-mCD45 Ab-SA conjugate. Nontumor bearing B6 Ly5a mice were injected with 1.4 nmol of (A) conventional trace-labeled 131I-anti-mCD45 (A20) DOTA-Ab or (B) pretargeted anti-mCD45 Ab-SA conjugate followed 20 h later by 5.8 nmol of CA and 2 h after that by 1.2 nmol 111In-DOTA-biotin. Groups of 5 mice were euthanized at either 2, 20, 48, 72, and 96 h after injection of the antimurine Ab conjugates. The radioactivity concentrations in blood, bone marrow, spleen, lymph node, and lung were quantified by γ counting and expressed as %ID/g. The shaded area under the curve in A represents the initial 20 h of nontarget specific radiation dose from blood-borne radiolabeled Ab that can potentially be eliminated with the use of PRIT.

Table 1. Estimated absorbed radiation doses (Gy) delivered to marrow, spleen, lymph nodes, and thymus using conventional RIT and PRIT when targeting 10 Gy to the liver

<table>
<thead>
<tr>
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<th>Conventional RIT (Gy)</th>
<th>PRIT (Gy)</th>
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<tbody>
<tr>
<td>Spleen</td>
<td>49</td>
<td>250</td>
</tr>
<tr>
<td>Marrow</td>
<td>27</td>
<td>49</td>
</tr>
<tr>
<td>lymph node</td>
<td>31</td>
<td>66</td>
</tr>
<tr>
<td>Thymus</td>
<td>12</td>
<td>12</td>
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molecule. Our group has developed a recombinant fusion protein consisting of an anti-CD45 single-chain Ab fused to SA that can be expressed in a soluble tetrameric form in the periplasm of \textit{Escherichia coli} (21). This fusion protein has been shown to maintain the full antigen and biotin binding capabilities of its parent molecules, allowing for large-scale cGMP production of the anti-CD45 fusion protein for future PRIT trials.

In conclusion, the data presented in this report suggest that anti-CD45 PRIT has the potential to significantly augment the efficacy of RIT and reduce the radiation exposure to normal organs compared with a conventional, directly labeled anti-CD45 radioimmunoconjugate in patients with AML. The results from this study have prompted large-scale cGMP production of the anti-CD45 fusion protein for PRIT trials to be conducted in patients with advanced myeloid leukemias. It remains to be determined if using an anti-CD45 Ab-SA conjugate in patients with disseminated leukemia will be more effective and less toxic than directly labeled monoclonal Abs as suggested by these murine experiments, however, the current studies pave the way for human clinical trials that we anticipate opening in 2009.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


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Pretargeted Radioimmunotherapy Using Anti-CD45 Monoclonal Antibodies to Deliver Radiation to Murine Hematolymphoid Tissues and Human Myeloid Leukemia

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