²¹¹At-Labeled Anti-CD45 Antibody as a Nonmyeloablative Conditioning for Canine DLA-Haploidentical Stem Cell Transplantation

Sofia H.L. Frost^{*1}, Johnnie J. Orozco^{*1,2}, Tom A. Bäck³, Brian W. Miller⁴, Erlinda B. Santos¹, Aimee Kenoyer¹, Sue E. Knoblaugh⁵, Donald K. Hamlin⁶, D. Scott Wilbur⁶, and Brenda M. Sandmaier^{1,2}

¹Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center, Seattle, Washington; ²Department of Medicine, University of Washington, Seattle, Washington; ³Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; ⁴Departments of Radiation Oncology and Medical Imaging, University of Arizona, Tucson, Arizona; ⁵Comparative Medicine Shared Resource, Fred Hutchinson Cancer Center, Seattle, Washington; and ⁶Department of Radiation Oncology, University of Washington, Seattle, Washington

The α -emitter ²¹¹At deposits a high amount of energy within a few cell diameters, resulting in irreparable DNA double-strand breaks while minimizing off-target toxicity. We investigated the use of the ²¹¹Atlabeled anti-CD45 monoclonal antibody (mAb) ²¹¹At-CD45-B10 as a nonmyeloablative conditioning regimen for dog-leukocyte-antigenhaploidentical hematopoietic cell transplantation. Methods: Seventeen healthy dogs were injected with either a 0.50 (n = 14) or 0.75 (n = 3) mg/kg dose of anti-CD45 mAb labeled with ²¹¹At (8.436-23.199 MBq [0.228-0.627 mCi/kg]) on day -3. Peripheral blood stem cells from dog-leukocyte-antigen-haploidentical donors were given on day 0. Peripheral blood chimerism was calculated by polymerase chain reaction assays, and blood clearance of the radioimmunoconjugate was studied using enzyme-linked immunosorbent assay and radioactivity measurements of serial blood samples. Results: All dogs achieved donor chimerism by day 28 (range, 27%-100%). The hematopoietic engraftment rate was 100%, though engraftment durability was variable. No difference in absorbed dose to blood was seen for the 2 mAb dosing levels studied. Neutropenia (0-29 cells/µL), lymphocytopenia (36–130 cells/ μ L), and thrombocytopenia (1.5–9 \times 10³/ μ L) with prompt recovery were observed. The main adverse nonhematologic event related to ²¹¹At-CD45-B10 was mild reversible transaminitis. Graft-versus-host disease was not seen. Twelve of the 17 dogs survived over 30 d, with donor chimerism ranging from 3% to 99%. Conclusion: The results suggest that nonmyeloablative conditioning with ²¹¹At-CD45-B10 could be used in haploidentical hematopoietic cell transplantation though with variable engraftment.

Key Words: radioimmunotherapy; ²¹¹At; haploidentical hematopoietic cell transplantation; canine

J Nucl Med 2024; 00:1–7 DOI: 10.2967/jnumed.124.267540

 Γ or a range of malignancies, α -radioimmunotherapy is a promising treatment modality merging high–linear-energy-transfer characteristics of α -particles with the discriminating targeting abilities of antigen-specific monoclonal antibodies (mAbs). The resulting radioimmunoconjugates enable efficient killing of target cells, with limited toxicity to surrounding healthy tissue. Our group has previously explored radioimmunotherapy preparative regimens for hematopoietic cell transplantation (HCT) in preclinical models, concluding that conditioning with anti-CD45 radioimmunoconjugates labeled with either α -emitter ²¹³Bi or ²¹¹At were efficacious, without significant nonhematopoietic toxicity (*I*–4). ²¹¹At was chosen over ²¹³Bi for subsequent radioimmunotherapy studies because of its superior myelosuppression, lower cost, greater availability, and clinically less challenging half-life and lower toxicity (*2,5*).

The safety and efficacy of ²¹¹At-CD45-B10 as a conditioning regimen have been demonstrated in a dog model of dog-leukocyteantigen (DLA)–identical allogeneic HCT (*1,2*). Our group has also explored optimal mAb dose, absorption, and distribution for ²¹¹At-CD45-B10 in autologous HCT. We demonstrated ²¹¹At activity distributed in T-cell–rich areas in lymphatic tissues, and anti-CD45 mAb at 0.75 mg/kg was efficiently targeted without severe toxicity (*6*).

Human-leukocyte-antigen (HLA)–haploidentical grafts have been applied as an alternative hematopoietic stem cell source for patients without suitable HLA-identical donors (7,8). We previously reported that ²¹³Bi-anti-CD45 mAb successfully allowed sustained engraftment in a DLA-haploidentical setting (3). Next, we speculated that radioimmunotherapy using ²¹¹At-CD45-B10 may have the ability to overcome graft rejection in haploidentical HCT. Here, we investigated whether nonmyeloablative conditioning with ²¹¹At-CD45-B10 without additional systemic chemotherapy would allow durable donor engraftment in a canine model of DLA-haploidentical HCT.

MATERIALS AND METHODS

Antibodies and Antibody Conjugates

The anticanine CD45 mAb CA12.10C12 and *closo*-decaborate(2-) (B10) were conjugated for subsequent ²¹¹At labeling according to a previously published protocol (9). Flow cytometry was used as previously described to analyze chimerism levels in peripheral blood lineage subsets, using mAbs against canine CD45, CD4, CD8, CD3, and TCR $\alpha\beta$ (*1*,2). Production and purification of anticanine antibodies were performed at the Biologics Production Facility at the Fred Hutchinson Cancer Center.

Radioactivity

All radioactive materials were handled according to approved protocols at the Fred Hutchinson Cancer Center and the University of

Received Jan. 31, 2024; revision accepted May 28, 2024.

For correspondence or reprints, contact Johnnie J. Orozco (jorozco@ fredhutch.org).

^{*}Contributed equally to this work.

Published online Jul. 18, 2024.

COPYRIGHT © 2024 by the Society of Nuclear Medicine and Molecular Imaging.

Washington. ²¹¹At was produced at the University of Washington, and isotope isolation was achieved through a wet chemistry method (9). Labeling of the CA12.10C12-B10 conjugate was performed as before (2).

Dogs

The experimental protocol was approved by the Fred Hutchinson Cancer Center Institutional Animal Care and Use Committee, and the study was executed according to principles outlined in the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research) (10). All dogs were healthy. The kennels were certified by the American Association for Accreditation of Laboratory Animal Care, International. DLA-haploidentical littermates were selected on the basis of family typing using highly polymorphic major histocompatibility complex class I and class II microsatellite markers and identity for DLA-DRB1 alleles as determined by direct sequencing (11,12).

Conditioning Regimen

All dogs were treated with 211 At-CD45-B10 on day -3 as a single conditioning agent without additional chemotherapy and then given peripheral blood stem cells intravenously from DLA-haploidentical littermate donors on day 0. Donor selection and peripheral blood stem cell harvest and infusion were performed as previously described (11,12). Mycophenolate mofetil (subcutaneous injection, 10 mg/kg twice daily on days 0-100, 5 mg/kg twice daily on days 101-130, 2.5 mg/kg twice daily on days 131-160, and then 2.5 mg/kg once daily on days 161-180) and cyclosporine (orally, 15 mg/kg twice daily on days -3 to 180) were administered for posttransplant immunosuppression. Ursodiol (orally, 7.5 mg/kg twice daily on days -10 to 180) was administered to minimize the risk of liver toxicity. In addition, 8 dogs (H751, H764, H765, H763, H766, H762, H834, and H856) received foscarnet (intravenously, 90 mg/kg once daily on days -13 to -7) to prevent issues related to canine herpesvirus (CHV). Standard supportive care after conditioning and HCT was provided (13,14).

Chimerism Analysis

Transplant engraftment was assessed weekly through donorrecipient cell chimerism, using a polymerase chain reaction-based assay of polymorphic (CA)n dinucleotide repeats after extraction of genomic DNA from cells of interest (15). Lineage-specific chimerism analysis was performed for mononuclear cells (MNCs), granulocytes, and CD3-positive (+) cells (T lymphocytes), via flow cytometry as before (1).

Antibody Dose, Blood Clearance, and Biodistribution

Dogs were infused ²¹¹At-CD45-B10 intravenously at a dose of 0.75 mg of mAb/kg (n = 3) or 0.5 mg of mAb/kg (n = 14). Blood was collected from 5 min before to 22 h after ²¹¹At-CD45-B10 infusion and used for pharmacokinetic assessment through an enzyme-linked immunosorbent assay (*I*). Biopsies of lymph nodes and bone marrow were taken at an early (2–4 h) or a late (19–22 h) time point after the ²¹¹At-CD45-B10 infusion. Harvested tissues were weighed and measured for radioactivity, and the results were expressed as percentage injected activity per gram after corrections for background and decay.

Dosimetry and α -Imaging

The absorbed dose to blood for each dog was calculated from the blood samples (δ). In short, the cumulated total number of ²¹¹At decays was derived (from exponential fits of individual time–activity curves of percentage of injected activity and multiplied by the mean energy per decay (α -particles only). Image-based small-scale 3-dimensional dosimetry was performed for lymph nodes from a representative dog, using voxel dose-point kernels and α -camera imaging of serial sections (δ).

Histopathology and Immunohistochemistry

Formalin-fixed, paraffin-embedded lymph node samples were stained with hematoxylin and eosin, and immunohistochemistry was performed on a BOND RX system (Leica) with antibodies against CD3 (3 mg/mL, MCA1477; Serotec), MAC 387 (0.2 mg/mL, M0747; Dako), and cleaved caspase-3 (0.3 mg/mL, CP229B; Biocare Medical) after ethylenediaminetetraacetic acid–based antigen retrieval. The CD3 antibody was followed by a rabbit antirat secondary, and all antibodies were then detected and visualized using PowerVision horseradish peroxidase polymers and Refine 3,3'-diaminobenzidine detection kit (Leica Biosystems).

Toxicity

Treatment-related toxicity was assessed through complete blood counts, blood urea nitrogen, creatinine, and liver enzymes. Samples were taken at baseline, then daily for the first 2 mo after treatment or until full hematopoietic recovery, and then weekly for the remainder of the study. Necropsies, gross examination, and tissue harvesting were performed at euthanasia, and samples were taken for pathologic analysis of microscopic abnormalities.

Statistical Methods

Mean (average) and median values of a dataset were defined per standard statistical approaches. Chimerism percentages (1) and dosimetry with α -imaging (6) were performed as described above.

RESULTS

Engraftment and Chimerism

Seventeen dogs were raised at the Fred Hutchinson Cancer Center and given standard immunizations. Their median age was 9.0 mo (range, 6-12 mo), and their median weight was 10.3 kg (range, 7.9-12.4 kg). All 17 dogs received ²¹¹At-CD45-B10 on day -3 without additional chemotherapy, with activity ranging from 8.436 to 23.199 MBq/kg (0.228-0.627 mCi/kg; Table 1; Fig. 1). On day 0, the dogs received peripheral blood stem cells (mean total mononuclear cells, 9.3×10^8 /kg; range, $2.9-19 \times 10^8$ /kg) intravenously from DLA-haploidentical littermate donors. All dogs achieved donor engraftment and maximum chimerism between 27% and 100% on median day +27 (range, days 5-221) after HCT. H695 received the lowest ²¹¹At activity (8.436 MBq/kg [0.288 mCi/kg]) and the lowest number of CD34+ cells $(0.7 \times 10^6 \text{ CD34+/kg})$ but achieved maximum chimerism of 55% on day 21 and final chimerism of 3% on day +194, when the animal was euthanized for posttransplant lymphoproliferative disorder (PTLD). Among all of the dogs, H856 showed the lowest maximum chimerism (27%) on day +13, which further decreased to 24% on day 28 and 6% by day 76, the day of death from hemorrhage.

Maximum granulocyte chimerism was rapid, with most dogs reaching peak donor chimerism in 2–3 wk (Fig. 1B). Unlike MNC chimerism, which showed variability across dogs, most dogs exhibited relatively stable granulocyte chimerism. H751, euthanized at the end of the study, exhibited nearly 100% donor granulocyte chimerism, whereas other dogs that lived longer but developed PTLD (H695 and H765) showed decreasing granulocyte chimerism before euthanasia.

Peripheral blood was also analyzed for CD3+ chimerism monthly, as this required blood lineages to be sorted. With CD3+ chimerism done at 1 mo after haploidentical HCT, H751 conditioned with 1.69 Gy had the highest CD3 chimerism of 91% and remained elevated (range, 83%–95%) throughout the study until day 285, when last assessed (Fig. 1C). A similarly conditioned dog H762 received 1.68 Gy for conditioning and achieved a CD3 chimerism of 80% 1 mo after HCT but continually decreased to 71%

 TABLE 1

 Chimerism and Outcomes in DLA-Haploidentical HCT with ²¹¹At-Anti-CD45 mAb

	²¹¹ At				% donor MNC engraftment				
Dog no.	MBq/kg	mAb/kg	MNC/kg	CD34+/kg	Max	Day 28	Final	Survival (d)	Cause of death
H707	14.06 (0.380)	0.75	12.5	2.9	81	76	65	45	CHV
H719	13.653 (0.369)	0.75	19.0	1.0	100	N.D.	82	23	Intussusception, CHV
H700	23.199 (0.627)	0.75	10.9	3.9	87	N.D.	87	5	Intussusception
H695	8.436 (0.228)	0.5	2.9	0.7	55	49	3	194	PTLD
H834	10.767 (0.291)	0.5	6.2	1.7	77	77	72	83	CHV
H856	10.767 (0.291)	0.5	4.0	1.1	27	24	6	76	Hemorrhage
H762	10.841 (0.293)	0.5	14.5	3.3	94	92	68	126	PTLD
H765	11.359 (0.307)	0.5	9.3	2.3	86	67	45	182	PTLD
H751	12.876 (0.348)	0.5	10.3	2.7	96	90	95	307	End of study
H699	13.949 (0.377)	0.5	13.0	2.5	94	87	91	63	CHV
H741	13.986 (0.378)	0.5	8.3	1.0	45	N.D.	45	10	Intussusception/CHV
H763	14.282 (0.386)	0.5	4.9	2.4	73	N.D.	73	10	Hemorrhage
H714	14.504 (0.392)	0.5	7.3	2.9	71	N.D.	71	23	Intussusception
H764	14.615 (0.395)	0.5	4.8	1.2	65	65	42	117	Bronchiolitis
H766	14.8 (0.400)	0.5	8.2	1.2	99	96	99	32	Intussusception
H502	22.644 (0.612)	0.5	15.9	6.3	72	68	22	132	PTLD
H505	22.792 (0.616)	0.5	13.5	1.9	88	83	88	32	Intussusception

At = astatine; max = maximum.

Data in parentheses are mCi/kg. Total of 17 dogs received ²¹¹At-anti-CD45 mAb. Initial donor engraftment was demonstrated in all subjects, with maximum chimerism ranging between 27% and 100%.

N.D. = not done.

at nearly 4 mo. The lowest CD3 chimerism was seen in H695, which received 1.17 Gy but achieved CD3 chimerism of only 23% at 1 mo and only 1% at months 2 and 3.

CD45 mAb Dose

Three dogs received a 0.75 mg/kg dose of anti-CD45 mAb with the 211 At activity ranging from 13.653 to 23.199 MBq (0.369–0.627 mCi/kg; Table 1). For these 3 dogs, the maximum MNC chimerism ranged from 81% to 100%, and the final MNC chimerism was 65%–87%. The remaining 14 dogs received a 0.5 mg/kg dose of anti-CD45 mAb with 211 At activity ranging from 8.436 to 22.792 MBq/kg (0.228–0.616 mCi/kg). Their maximum MNC chimerism ranged from 27% to 99%, with a final MNC chimerism of 3%–99%.

Recovery of Myelosuppression

All 17 transplanted dogs experienced transient pancytopenia. The median nadir levels for granulocytes, lymphocytes, and platelets were 9 cells/ μ L (range, 0–29 cells/ μ L), 64 cells/ μ L (range, 36–130 cells/ μ L), and 1,500 platelets/ μ L (range, 1,500–9,000 platelets/ μ L), respectively, with a median time to nadir of 4 d (range, 3–7 d), 0 d (range, -3–7 d), and 8 d (range, 5–14 d), respectively (Fig. 2). All dogs engrafted rapidly, with median neutrophil and platelet engraftment occurring at 12 d (range, 11–16 d) and 11.5 d (range, 9–24 d) after transplantation, respectively.

Toxicity

All dogs tolerated transplantation well, with no immediate adverse events related to ²¹¹At-CD45-B10 and no subsequent graft-versus-host disease. No renal toxicity was observed by renal

function laboratory testing. Aminotransferase (aspartate transaminase or alanine transaminase) levels were transiently elevated in 3 of 9 dogs not given prophylactic foscarnet and in 6 of 8 dogs given prophylactic foscarnet within 10 d of ²¹¹At-CD45-B10, though transaminases normalized within a few days if there was no CHV infection. Dogs H707, H719, and H699 showed grade 4 liver toxicity from CHV according to the Common Terminology Criteria for Adverse Events. Histopathologic examination of liver samples revealed centrilobular collapse with increased pigmentation and atypical, enlarged degenerating cells, attributed to CHV infections. All dogs except H751 were euthanized for a variety of etiologies of failure to thrive at a median of 54 d after transplantation (range, 5-194 d). Review of these necropsy samples showed that 6 dogs died of intussusception, 4 of PTLD, 2 of hemorrhage in the context of severe thrombocytopenia (platelet count, 1,500-3,000 on date of death), and one of bronchiolitis (Table 1).

Blood Clearance and Biodistribution

Blood samples were obtained serially (5 min through 22 h after ²¹¹At-CD45-B10 injection) to measure the clearance of circulating ²¹¹At and the absorbed dose to blood (Table 2; Fig. 3A). The absorbed dose to blood (range, 0.006–0.026 Gy/MBq) corresponded to total absorbed doses of 0.74–4.3 Gy for the injected ²¹¹At activity (range, 105–301 MBq [2.838–8.135 mCi]). The mean radiation dose per injected activity did not significantly differ between ²¹¹At dosed with 0.75 mg (0.019 Gy/MBq) and 0.5 mg (0.011 Gy/MBq) of anti-CD45 mAb per kilogram.

 α -camera imaging was performed to assess the distribution of ²¹¹At within lymph nodes (Fig. 4). Radioactivity distribution was



FIGURE 1. Percentage of donor chimerism in granulocytes, MNCs, and CD3+ cells in dogs treated with ²¹¹At-anti-CD45 mAb conditioning in DLA-haploidentical HCT. Peripheral blood was taken from recipient dogs at serial time points and assessed for donor origin by polymerase chain reaction assay of polymorphic (CA)n dinucleotide repeats after extraction of genomic DNA from cells of interest, to calculate percentage donor chimerism in MNCs (A), granulocytes (B), or CD3+ T cells (C) over time. N.D. = not done.

heterogeneous, with predominant ²¹¹At localization to T-cell–rich regions in the cortical follicles, given consecutive hematoxylinand eosin-stained cryosections. Immunohistochemical staining of formalin-fixed, paraffin-embedded lymph nodes allowed more detailed analysis (Fig. 5). Immunostaining indicated widespread apoptosis and macrophages, with multifocal apoptotic cellular debris in medullary cords, interfollicular zones, and occasional germinal centers.

Blood-Absorbed Radiation Dose and Chimerism

The relationship between levels of MNC chimerism and the blood's absorbed radiation dose from ²¹¹At-CD45-B10 was explored after dosimetry studies (Fig. 3B). Higher chimerism levels were seen in dogs that received higher absorbed radiation doses to blood. Dog H856, which received 0.7 Gy, achieved a mononuclear chimerism of 24% at month 1 after HCT, decreasing to 6% at the end of the study on day 76. However, dog H699, which received 1.9 Gy, achieved a mononuclear chimerism of 64% 1 wk after HCT, remaining high (89%) at week 6 after HCT.



FIGURE 2. Blood count recovery after ²¹¹At-CD45-B10 conditioning in DLA-haploidentical HCT. Peripheral blood from recipient dogs was drawn from baseline through HCT and followed up at serial time points to measure granulocytes (A), lymphocytes (B), and platelets (C). N.D. = not done.

DISCUSSION

The study assessed ²¹¹At-CD45-B10 as conditioning in DLAhaploidentical HCT. Previously, DLA-haploidentical HCT using ²¹³Bi-anti-CD45 mAb (*3*) resulted in all 6 dogs engrafting, and 3 of 6 dogs had sustained engraftment more than 10 wk after HCT. Because the ultrashort half-life of ²¹³Bi could limit wider application, we then used ²¹¹At-CD45-B10 to promote engraftment of DLA-identical HCT. Seven dogs had long-term donor MNC chimerism (19%–58%), whereas 1 dog treated with the lowest ²¹¹At activity had low donor MNC chimerism (5%) (*2*). Conditioning with ²¹¹At-CD45-B10 was thus sufficiently immunosuppressive to allow stable long-term engraftment.

Here, ²¹¹At-CD45-B10 is also capable of facilitating hematopoietic engraftment from haploidentical donors, though engraftment durability was variable. All 17 dogs achieved initial donor engraftment (maximum chimerism rate, 27%–100%), with only 2 dogs showing maximum chimerism below 50%. Dog H741 showed 45% maximum chimerism but died on day 10 because of intussusception. H856 showed 27% maximum chimerism, with 6% donor chimerism on death on day 76 from hemorrhage from severe thrombocytopenia. Pancytopenia after high-dose chemotherapy or

 TABLE 2

 Mean Absorbed Dose to Blood After Injection of ²¹¹At-Anti-CD45 mAb

Dog no.	mAb dose (mAb/kg)	²¹¹ At activity MBq (mCi)	Absorbed dose to blood (Gy)	Radiation dose/injected activity (Gy/MBq)
H700	0.75	301 (8.135)	4.30	0.014
H719	0.75	108 (2.919)	2.81	0.026
H707	0.75	136 (3.676)	2.28	0.017
H695	0.5	105 (2.838)	1.17	0.011
H834	0.5	123 (3.324)	1.09	0.009
H856	0.5	107 (2.892)	0.74	0.007
H762	0.5	133 (3.595)	1.68	0.013
H765	0.5	109 (2.946)	1.05	0.010
H751	0.5	122 (3.297)	1.69	0.014
H699	0.5	121 (3.27)	1.89	0.016
H741	0.5	172 (4.649)	1.04	0.006
H763	0.5	156 (4.216)	2.27	0.015
H714	0.5	147 (3.973)	1.58	0.011
H764	0.5	159 (4.297)	1.09	0.007
H766	0.5	184 (4.973)	2.22	0.012
H502*	0.5	226 (6.108)	N.D.	N.D.
H505*	0.5	227 (6.135)	N.D.	N.D.

*Tissues for α -camera imaging and dosimetry were not collected from H502 and H505; thus, absorbed radiation doses were not determined.

Data in parentheses are mCi.

total-body irradiation from conditioning regimens for allogeneic HCT is common and not unique to radioimmunotherapy approaches. Patients have access to robust blood product transfusion support, but the fact that canines do not could potentially alter hemorrhage outcomes. H695 had a maximum chimerism of 55% and final chimerism of 3% near its death on day 194 from PTLD. Rejection may have been from suboptimal CD34+ stem cells (0.7×10^6 CD34+/kg). Previous data suggested that more than 2.0×10^6 CD34+ cells/kg overcome the engraftment threshold in the haploidentical canine model (16), potentially limiting H695's chimerism. However, H766 received 1.2×10^6 CD34+ cells/kg and achieved maximum chimerism of 99%. Similarly, H719 received 1×10^6 CD34+ cells/kg but achieved maximum donor chimerism of 100%. These data suggest that a cellular threshold for engraftment may be a function of cell dose, radioactivity amount, or specific activity, as the mAb amount may influence radioimmunoconjugate biodistribution. Ten dogs that survived over 30 d had more than 50% donor chimerism on day 28 (H707, H699, H505, H751, H764, H765, H766, H762, H502, and H834). For improved durability of engraftment, pretransplant chemotherapy could be considered, given contemporary haploidentical HCT approaches that dose fludarabine/cyclophosphamide. Other haploidentical HCT murine studies showed that adding pretransplant chemotherapy before infusion of haploidentical stem cells improves chimerism levels and durability (17,18). A current clinical trial using ²¹¹At-anti-CD45 mAb before allogeneic HCT using haploidentical donors (NCT03670966) applies standard nonmyeloablative conditioning, which includes low-dose (2-3 Gy) total-body irradiation, fludarabine, and cyclophosphamide. To date, patients have durable full donor chimerism, and no graft failures have been observed.

²¹¹At-CD45-B10 was well tolerated and effective at the antibody doses (0.75 and 0.5 mg/kg) and ²¹¹At activities (8.436-22.792 MBg; 0.228-0.616 mCi/kg) studied here, with no distinguishable differences in hematologic, hepatic, or renal toxicity. The lower antibody dose (0.5 mg/kg) was sufficient to achieve engraftment, without serious toxicity in any dog, although radiation dose may have a larger effect on maximum engraftment. Dogs that received a higher absorbed radiation dose to the blood did indeed show higher mononuclear chimerism that was sustained up to 6 mo, compared with those that received lower absorbed radiation doses (Fig. 3B). As maximum engraftment levels were equivalent between the 2 antibody doses, the lower mAb dose (0.5 mg/kg) may be preferred, and current clinical trials using ²¹¹At-CD45-B10 before allogeneic HCT using HLA-matched related or unrelated donors (NCT03128034) (19) or HLA-haploidentical related donors (NCT03670966), and a different study using ¹³¹I-CD45 before haploidentical HCT (20), also use anti-CD45 mAb at 0.5 mg/kg.

Four dogs developed PTLD and reduction of final chimerism. Clinically, about 70% of PTLD is associated with Epstein–Barr virus infection, from either posttransplantation viral reactivation or primary infection. Risk factors for PTLD are HLA-haploidentical conditioning regimens and immune suppression from antithymoglobulin or T-cell–depleted grafts (21,22). No data correlate PTLD with engraftment, although there are reports that reduced immunosuppression for PTLD treatment is associated with acute allograft rejection, up to 40% in early reports (23-26). Although immunosuppression

N.D. = not done.



FIGURE 3. Blood clearance and weekly chimerism by blood's absorbed dose. (A) Blood samples were obtained at serial time points, and radioactivity measurements were made to estimate absorbed radiation dose (Gy) to blood for injected ²¹¹At activity. Animals receiving 0.75 mg/kg dose of mAb are indicated by dashed line, and animals receiving 0.50 mg/kg are indicated by solid line connecting solid circles. (B) Donor MNC chimerism by total absorbed radiation dose delivered to peripheral blood from weeks 1 to 6 after HCT. %ID = percentage injected dose.

was not withdrawn in the current study, the chimerism of PTLD dogs decreased.

PTLD is common after DLA-haploidentical HCT, as another study evaluating methotrexate to facilitate engraftment in canine



FIGURE 4. ²¹¹At distribution in lymph nodes taken from dog H700 at 2 different time points after ²¹¹At-CD45-B10 injection, as determined by α -camera imaging and hematoxylin and eosin staining of consecutive cryosections. Radioactivity was heterogeneously localized, predominantly to T-cell–rich regions in cortical follicles.



FIGURE 5. Histopathology and immunohistochemistry staining on lymph nodes from dog H700, biopsied 2 and 19 h after ²¹¹At-CD45-B10 injection, showing that apoptosis progressed with time. Representative lymph node sections were stained with hematoxylin and eosin (H&E) and immunostained for T cells (CD3), apoptosis (cleaved caspase-3 [CC3]), and macrophages (MAC 387). Scale bar represents 100 μm.

HLA-haploidentical HCT instead of cyclophosphamide reported PTLD in 1 of the 8 control dogs and in 5 of the 21 dogs transplanted with methotrexate (27). Although canine HCT does not have the same supportive care for infectious or PTLD complications as does clinical HCT, the precise mechanism of PTLD is unknown. First, antithymocyte globulin is increasingly used as conditioning in HLA-haploidentical HCT that may profoundly T-cell deplete. Antithymocyte globulin was not used in our study, but ²¹¹At-CD45-B10 conditioning might be as intensive as antithymocyte globulin. Second, T-cell reconstitution might be delayed in HLA-haploidentical HCT. These conditions might induce an immunocompromised state and lead to PTLD. Five dogs developed CHV, and 4 did not receive foscarnet, so 8 dogs received foscarnet to prevent infection. Nonetheless, 1 dog developed CHV despite prophylactic foscarnet, suggesting a sufficiently immunocompromised host.

 α -camera imaging revealed substantial variations in ²¹¹At absorbed dose rate within the lymph nodes. Comparison with hematoxylinand eosin-stained sections revealed colocalization of high-activity areas within lymphocyte-rich regions of the lymph nodes, facilitated by the targeting of CD45 expressed by T cells and B cells in nodal follicles. Immunohistochemical staining showed widespread cell death temporally. CD3+ T cells were scarce at 19 h after administration of ²¹¹At-CD45-B10. From the α -camera images, it appeared that most T cells were destroyed by ²¹¹At-CD45-B10, deepening immunosuppression of the host.

Thus, ²¹¹At-CD45-B10 conditioning was safe and successful in establishing DLA-haploidentical grafts in 17 of 17 dogs, albeit with variable durability.

CONCLUSION

The results suggest that nonmyeloablative conditioning with ²¹¹At-CD45-B10 could be used in haploidentical HCT, though with variable engraftment.

DISCLOSURE

This work was supported by NIH R0 CA172582, P01 CA078902, and R37CA252070. No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank the canine veterinarians and technicians at the Fred Hutchinson Cancer Center. Histology and immunohistochemistry were performed by Experimental Histopathology Shared Resources at the Fred Hutchinson Cancer Center. We thank Dr. George Sale for histopathology review of necropsy samples (Table 1) and Helen Crawford for manuscript preparation assistance.

KEY POINTS

QUESTION: Does single-modality ²¹¹At-CD45 have potential to facilitate hematopoietic engraftment from haploidentical donors in canine models?

PERTINENT FINDINGS:²¹¹At-CD45-B10 without additional chemotherapy resulted in engraftment from haploidentical donor dogs, achieving variable maximum chimerism levels (27%–100%) within 28 d after peripheral blood stem cell transplantation, though some chimerism levels did decrease with tapering of immunosuppression.

IMPLICATIONS FOR PATIENT CARE: Because better-tolerated and more targeted approaches are needed to make HCT less toxic, ²¹¹At-CD45-B10 may provide an optimizable option for patients needing HCT.

REFERENCES

- Sandmaier BM, Bethge WA, Wilbur DS, et al. Bismuth 213–labeled anti-CD45 radioimmunoconjugate to condition dogs for nonmyeloablative allogeneic marrow grafts. *Blood.* 2002;100:318–326.
- Chen Y, Kornblit B, Hamlin DK, et al. Durable donor engraftment after radioimmunotherapy using alpha-emitter astatine-211-labeled anti-CD45 antibody for conditioning in allogeneic hematopoietic cell transplantation. *Blood*. 2012;119:1130–1138.
- Nakamae H, Kerbauy FR, Wilbur DS, et al. Pilot study of a ²¹³bismuth-labeled anti-CD45 mAb as a novel nonmyeloablative conditioning for DLA-haploidentical littermate hematopoietic transplantation. *Transplantation*. 2010;89:1336–1340.
- Bethge WA, Wilbur DS, Storb R, et al. Radioimmunotherapy with bismuth-213 as conditioning for nonmyeloablative allogeneic hematopoietic cell transplantation in dogs: a dose deescalation study. *Transplantation*. 2004;78:352–359.
- Nakamae H, Wilbur DS, Hamlin DK, et al. Biodistributions, myelosuppression, and toxicities in mice treated with an anti-CD45 antibody labeled with the alpha-emitting radionuclides bismuth-213 or astatine-211. *Cancer Res.* 2009;69:2408–2415.
- Frost SH, Miller BW, Back TA, et al. α-imaging confirmed efficient targeting of CD45-positive cells after ²¹¹At-rdioimmunotherapy for hematopoietic cell transplantation. J Nucl Med. 2015;56:1766–1773.
- Guinan EC, Boussiotis VA, Neuberg D, et al. Transplantation of anergic histoincompatible bone marrow allografts. N Engl J Med. 1999;340:1704–1714.

- Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med. 2014;371:339–348.
- Wilbur DS, Chyan MK, Nakamae H, et al. Reagents for astatination of biomolecules. 6. An intact antibody conjugated with a maleimido-*closo*-decaborate(2-) reagent via sulfhydryl groups had considerably higher kidney concentrations than the same antibody conjugated with an isothiocyanato-*closo*-decaborate(2-) reagent via lysine amines. *Bioconjug Chem.* 2012;23:409–420.
- Guide for the Care and Use of Laboratory Animals. 8th ed. National Academy Press; 2011.
- Wagner JL, Burnett RC, DeRose SA, Francisco LV, Storb R, Ostrander EA. Histocompatibility testing of dog families with highly polymorphic microsatellite markers. *Transplantation*. 1996;62:876–877.
- Wagner JL, Works JD, Storb R. DLA-DRB1 and DLA-DQB1 histocompatibility typing by PCR-SSCP and sequencing. *Tissue Antigens*. 1998;52:397–401.
- Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLAidentical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood*, 1997;89:3048–3054.
- Ladiges WC, Storb R, Thomas ED. Canine models of bone marrow transplantation. Lab Anim Sci. 1990;40:11–15.
- Yu C, Ostrander E, Bryant E, Burnett R, Storb R. Use of (CA)n polymorphisms to determine the origin of blood cells after allogeneic canine marrow grafting. *Transplantation*. 1994;58:701–706.
- Fukuda T, Kerbauy FR, Gooley T, Santos EB, Storb R, Sandmaier BM. Dog leukocyte antigen-haploidentical stem cell allografts after anti-CD44 therapy and nonmyeloablative conditioning in a preclinical canine model. *Transplantation*. 2006; 82:332–339.
- Orozco JJ, Kenoyer A, Balkin ER, et al. Anti-CD45 radioimmunotherapy without TBI before transplantation facilitates persistent haploidentical donor engraftment. *Blood.* 2016;127:352–359.
- Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. *Blood.* 2001;98:3456–3464.
- Sandmaier BM, Wilbur DS, Hamlin DK, et al. A phase I trial of first-in-human alpha-emitter astatine-211-labeled anti-CD45 antibody (²¹¹At-BC8-B10) in combination with fludarabine and TBI as conditioning for allogeneic hematopoietic cell transplantation (HCT) for patients with refractory/relapsed leukemia or high-risk myelodysplastic syndrome (MDS): preliminary results of dose escalation [abstract]. *Transplant Cell Ther.* 2021;27:S54.
- Orozco JJ, Vo PT, Gooley TA, et al. Targeted radiation delivery before haploidentical HCT for high-risk leukemia or MDS patients yields long-term survivors. *Clin Cancer Res.* 2024;30:274–282.
- 21. Heslop HE. How I treat EBV lymphoproliferation. Blood. 2009;114:4002-4008.
- Cohen JM, Cooper N, Chakrabarti S, et al. EBV-related disease following haematopoietic stem cell transplantation with reduced intensity conditioning. *Leuk Lymphoma.* 2007;48:256–269.
- Pan K, Franke AJ, Skelton WP VI, et al. Reduction of immunosuppression for post-transplant lymphoproliferative disorder (PTLD): a single-center experience of allograft survival outcomes. *Leuk Lymphoma*. 2021;62:1123–1128.
- Jagadeesh D, Woda BA, Draper J, Evens AM. Post transplant lymphoproliferative disorders: risk, classification, and therapeutic recommendations. *Curr Treat Options Oncol.* 2012;13:122–136.
- Tsai DE, Hardy CL, Tomaszewski JE, et al. Reduction in immunosuppression as initial therapy for posttransplant lymphoproliferative disorder: analysis of prognostic variables and long-term follow-up of 42 adult patients. *Transplantation*. 2001; 71:1076–1088.
- Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol.* 2012;8:348–357.
- Thakar MS, Santos EB, Gooley TA, et al. Evaluation of posttransplant methotrexate to facilitate engraftment in the canine major histocompatibility complex-haploidentical nonmyeloablative transplant model. *Transplantation*. 2010;90:14–22.