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Autologous Peripheral Blood Hematopoietic Cell Transplantation in Dogs with T-Cell Lymphoma

E.E. Warry, J.L. Willcox, and S.E. Suter

Background: Peripheral blood hematopoietic cell transplantation (PBHCT) is a feasible treatment option for dogs with B-cell lymphoma.

Objective: To examine apheresis and PBHCT outcomes in dogs diagnosed with T-cell lymphoma (TCL).

Animals: Fifteen client-owned dogs diagnosed with high-grade TCL.

Methods: After high-dose cyclophosphamide and rhG-colony-stimulating (rhG-CSF) factor treatment, peripheral blood mononuclear cells were collected using cell separators. The harvested cells then were infused after varying doses of total body irradiation (TBI). Postirradiation adverse effects were managed symptomatically and dogs were discharged upon evidence of hematopoietic engraftment.

Results: More than 2×10^6 CD34+ cells/kg were harvested from 15/15 dogs. Thirteen of 15 (87%) dogs engrafted appropriately, whereas 2 (13%) of the dogs died in the hospital. One dog developed cutaneous B-cell lymphoma 120 days post-PBHCT. The median disease-free interval and overall survival (OS) of the 13 dogs transplanted in first remission from the time of PBHCT were 184 and 240 days, respectively. Stage and substage of disease at diagnosis had no effect on OS. Two of 13 (15%) dogs were alive 741 and 772 days post-PBHCT.

Conclusions and Clinical Importance: PBHCT may be considered as a treatment option for dogs with TCL.

Key words: Apheresis; Bone marrow transplantation; CD34+; Engraftment; Total body irradiation.

T-cell lymphoma (TCL) accounts for approximately 10–38% of canine non-Hodgkin's lymphoma (LSA).^{1,2} The disease is considered to have a more aggressive clinical course and is associated with a poorer prognosis (6–9 months median survival) when compared to B-cell lymphoma (BCL; 12–14 months median survival).³

Treatment of all LSA phenotypes historically has involved the administration of multiagent CHOP-based (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy protocols of varying length.^{4,5} In addition, the use of cranial and caudal half-body irradiation in conjunction with chemotherapy has been described for the treatment of both B- and T-cell LSA.^{6,7} In a recent study, 31% of veterinarians treated dogs with TCL using alternative front-line protocols by including lomustine, procarbazine, and mechlorethamine while discontinuing the use of doxorubicin and cyclophosphamide.⁸ However, despite these different treatment regimens, complete remission rates and overall survival have remained relatively unchanged over the last decade.⁹

Autologous peripheral blood hematopoietic cell transplantation (PBHCT) after high-dose cyclophos-

Abbreviations:

BCL	B-cell lymphoma
CBC	complete blood count
CHOP	cyclophosphamide, doxorubicin, vincristine, prednisone
DFI	disease-free interval
G-CSF	granulocyte colony-stimulating factor
HCT	hematocrit
HDC	high-dose cyclophosphamide
LRS	lactated Ringer's solution
LSA	lymphoma
PARR	polymerase chain reaction for antigen receptor rearrangements
PBHCT	peripheral blood hematopoietic cell transplantation
PBMC	peripheral blood mononuclear cells
SHC	sterile hemorrhagic cystitis
TBI	total body irradiation
TCL	T-cell lymphoma
WBC	white blood cells

phamide and lethal total body irradiation (TBI) for the treatment of canine B-cell LSA (BCL) has been reported.¹⁰ The median overall survival time for dogs transplanted before they relapsed was 531 days, with 33% of the dogs still alive >2 years after PBHCT. In addition, a TerumoBCT (formally CaridianBCT)^a cell separator was able to collect a higher number of both total CD34+ cells and CD34+ cells/kg when compared to a Baxter-Fenwal CS-3000 Plus^b cell separator. Therefore, we reasoned that apheresis using a TerumoBCT cell separator and autologous PBHCT represent a feasible treatment alternative for dogs with TCL.

In this study, we report the apheresis product analysis, toxicities, and treatment outcome of dogs diagnosed with TCL treated with an aggressive myeloablative protocol including high-dose cyclophosphamide (HDC), mononuclear cell apheresis, and total

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body irradiation (TBI) followed by autologous PBHCT.

Materials and Methods

Study Design

Between November 2008 and March 2012, 15 dogs definitively diagnosed with high-grade TCL were enrolled. Dogs in a first and second clinical remission (CR), and 1 dog not in CR, were included. All clients signed a written informed consent form approved by the North Carolina State University (NCSU) Veterinary Health Complex Hospital Board.

Donor Preparation for PBMC Apheresis

Donor preparation, including administration of HDC^c and rhG-CSF,^d was carried out as previously described.¹⁰ All dogs received IV HDC and mesna^e at 500 (5 dogs), 600 (1 dog), 650 (7 dogs), 700 (1 dog), or 750 (1 dog) mg/m². In some dogs, glutamine^f (30 mg/kg PO q12h) also was administered.

PBMC Apheresis

PBMC aphereses were performed as previously described,¹⁰ using either a Baxter-Fenwal CS-3000 *Plus* (1 dog) or TerumoBCT (formally CaridianBCT) (14 dogs) cell separator machine. In 4 dogs weighing <15 kg, the TerumoBCT cell separator was primed to ensure hemodynamic stability during the initial period of apheresis. Three of these dogs have been previously described.¹¹ The priming solution used in the remaining dog (6.7 kg) was 100 mL autologous whole blood and 100 mL 0.9% NaCl. In addition, the rinse back products (purged machine contents after completion of apheresis) in these cases were collected into an empty bag rather than being returned to the dogs. This blood (approximately 400 mL) was subsequently infused into the dogs at approximately 50 mL/h beginning immediately after the aphereses were completed.

Apheresis Product Analysis

Midprocedure and final harvest products were evaluated as previously described.¹⁰ Polymerase chain reaction for antigen receptor rearrangements (PARR)¹² analysis was used to detect tumor cell contamination in the 14 dogs with PARR-positive tumors at diagnosis. When the apheresis product contained $>1 \times 10^7$ CD34+ cells/kg, one-half of the product was centrifuged to remove the plasma, and the cells were diluted 1:1 in freezing media containing 20% autologous plasma, 20% Normosol, and 10% dimethyl sulfoxide,^g placed in a freezing press,^h and frozen to -80°C in a controlled rate freezing chamber.¹³ The remaining apheresis product was refrigerated at 4°C until transplantation.

Mononuclear Cell Product Yield and Collection Efficiency Calculations

Mononuclear cell product yield, CD34+ cells/kg, and mononuclear cells collection efficiencies (MCCE) were calculated as previously described.¹⁰

Total Body Irradiation

The day after apheresis, 14 dogs received TBI at a dose rate of 7 cGy/min administered with 6 MV photons from a Varian

Clinac 1800,ⁱ as previously described.¹⁰ In these dogs, TBI consisting of either two 5 Gy fractions at least 3 hours apart (10 Gy, 3 dogs), two 4 Gy fractions at least 3 hours apart on day 1, and a single 3 Gy fraction on day 2 (11 Gy, 9 dogs), or two 3 Gy fractions at least 3 hours apart on 2 consecutive days (12 Gy, 1 dog). In 1 dog, two 4 Gy fractions at least 3 hours apart were given on day 1 and the machine faulted on day 2 after the dog received approximately 38% of a final 3 Gy fraction (target total dose of 11 Gy). Therefore, because of the 1-day delay, another 4 Gy dose was administered the next day after the machine was repaired, for a total of 12 Gy. One dog received TBI at a dose rate of 10 cGy/min administered with 6 MV photons from a Varian Clinac 21EX^j with the dose prescribed to the 100% isodose line as previously described,¹⁰ although a dose miscalculation resulted in this dog receiving only 8 Gy (of a prescribed 11 Gy dose) over 3 days. This dog received another 6 Gy (two 3 Gy fractions at least 3 hours apart) approximately 93 days after the initial PBHCT. TBI was considered day 0. Immediately after TBI, the apheresis product was warmed to room temperature and administered IV over a period of 1 hour.

Post-PBHCT Care

Post-PBHCT care was essentially as previously described.¹⁰ Toxicities are described using the Veterinary Co-operative Oncology Group v1.1 common terminology criteria for adverse events.¹⁴ In the event of relapse after PBHCT, the dogs received a variety of chemotherapy protocols at the discretion of the local veterinarian.

Statistical Analysis

All apheresis data were normally distributed as determined by a D'Agostino and Pearson omnibus K2 normality test and Kolmogorov-Smirnov normality test. A Welch's unpaired *t*-test was used to calculate significant differences between the dogs in this study (Group 1) and 16 dogs from a previous study diagnosed with BCL that underwent apheresis using a TerumoBCT cell separator and PBHCT (Group 2).¹⁰ The relationship between preapheresis parameters, collected CD34+ cells, and MCCE in both groups was estimated by linear regression and correlation analysis. One dog that underwent apheresis using a Baxter-Fenwal CS-3000 *Plus* cell separator was not included in the analyses for Tables 2 and 3. Disease-free interval (DFI), defined as the time from PBHCT until the time of relapse, and overall survival (OS), defined as the time from PBHCT until death, were calculated using the Kaplan-Meier method. For comparison to historical data, OS also was calculated from the time of diagnosis until death. DFI and OS were compared between the groups using the log rank test. Two dogs that died from treatment-related causes were censored from the DFI analysis but included in the overall survival analysis.¹⁰ Because only 2 dogs had not maintained their first remission before PBHCT, remission status before PBHCT was not considered in this analysis. All statistics were calculated using GraphPad.^j A *P*-value $\leq .05$ was considered significant.

Results

Patient Characteristics

Fifteen dogs, representing 12 breeds, met the inclusion criteria (Table 1). T-cell phenotype was confirmed using immunohistochemistry (3 dogs), flow cytometry (1 dog), or PARR (11 dogs), and the preponderance of large, immature lymphoblasts with a moderate to high mitotic rate was used to classify the malignancies as

Table 1. T-cell lymphoma case details before PBHCT.

Signalment	
Number of dogs	15
Breeds	12 (2 Box, 2 GR, 2 LR)
Age (months)	Mean, 63.6 (range, 36–108)
Spay/neuter	9NM, 1IM, 4SF, 1IF
Weight (kg)	Mean, 28.4 (range, 6.7–54.4)
T-cell Lymphoma (15)	14 LN FNA/biopsy, 1 liver/spleen FNA
Diagnosis	
Stage III	4
Stage IV	3
Stage V	8
Substage a	9
Substage b	6
Relapse before PBHCT	1
Remission before PBHCT	14
Days from dx to PBHCT	Mean 121 (range, 61–217)
PARR + tumors	14

Box, Boxer; GR, Golden Retriever; LR, Labrador Retriever; NM, neutered male; IM, intact male; SF, spayed female; IF, intact female; LN FNA, lymph node fine needle aspirate; PBHCT, peripheral blood hematopoietic cell transplantation; PARR, PCR for antigen receptor rearrangements.

high-grade. Four of these dogs were hypercalcemic at diagnosis, and 3 dogs had a mediastinal mass (2 dogs had both a mediastinal mass and hypercalcemia). Eight dogs had stage 5 disease, 3 dogs had stage 4 disease, and 4 dogs had stage 3 disease. Nine dogs were substage a, whereas 6 dogs were substage b (5/6 substage b dogs had stage 5 disease). One dog was in second remission before PBHCT, whereas 1 dog was not in CR upon arrival at NCSU.

Toxicity

Acute HDC Toxicity. Seven of 15 (47%) dogs (1 at 500 mg/m², 1 at 600 mg/m², 3 at 650 mg/m², 1 at 700 mg/m², and 1 at 750 mg/m²) were reported to have experienced an adverse event. One dog experienced grade-2 lethargy and inappetence only, whereas 6 dogs developed grade-4 neutropenia. Of the 6 neutropenic dogs, 3 also developed grade-3 thrombocytopenia, 3 became febrile, and 3 exhibited grade-2 gastrointestinal (GI) signs. Four of these dogs were hospitalized and given supportive care, including fluid administration, antibiotics, and antiemetics. Within 24 hours of hospitalization, all 4 dogs were discharged and cytopenias resolved within 7 days. Only 1 dog (650 mg/m² HDC) exhibited clinical signs consistent with sterile hemorrhagic cystitis (SHC) and this dog eventually required surgery to repair a ruptured urinary bladder, presumably secondary to severe SHC (moderate to marked transmural necrotizing cystitis). Regardless of the severity of HDC toxicities, all dogs recovered and were able to proceed with rhG-CSF treatment and PBMC apheresis.

Acute TBI Hematologic Toxicity. All dogs developed grade-4 neutropenia and thrombocytopenia as previ-

ously described.¹⁰ Grade-4 neutropenia (<500/ μ L) lasted from 3 to 11 days (median, 6 days) whereas grade-4 thrombocytopenia (<25 \times 10³/ μ L) persisted for 0–21 days (median, 8 days), with 8 (53%) dogs having grade-4 thrombocytopenia at discharge. No statistically significant difference in either duration or grade of neutropenia and thrombocytopenia was observed between dogs that received 10 Gy or 11 Gy of irradiation ($P = .583$). Nine of 15 (60%) dogs experienced grade-2 petechiation, ecchymosis, or both, 1 (7%) dog developed hematuria, and 1 (7%) dog developed epistaxis. Fifteen (100%) dogs received irradiated¹⁵ (20 Gy) blood products which included DEA 1.1-negative fresh whole blood, donor-matched fresh whole blood, frozen platelet concentrate, donor-matched packed red blood cells, and fresh platelet concentrate. There was no statistically significant difference in the median duration of hospitalization for dogs receiving 10 Gy of TBI (median, 18 days; range, 14–23 days) when compared to dogs receiving 11 Gy of TBI (median, 22.5 days; range, 17–34 days; $P = .1874$).

Acute TBI Gastrointestinal Toxicity. Fourteen of 15 (93%) dogs experienced GI adverse events, including vomiting, diarrhea, inappetence, or some combination of these. Six (40%) dogs vomited during their hospitalization (3 grade-2, 3 grade-3). Eleven (73%) dogs also developed diarrhea (3 grade-1, 4 grade-2, 4 grade-3). Inappetence developed in 10 (67%) dogs for a variable amount of time (3 grade-1, 2 grade-2, 3 grade-3, 2 grade-4). Three (20%) dogs also received nutritional support including partial parental nutrition (1), total parental nutrition (1), or a combination of partial parental nutrition and amino acid supplementation (1).

Chronic TBI Toxicity. One (7%) dog developed multiple SC nodules 120 days post-PBHCT that were diagnosed as cutaneous B-cell LSA by flow cytometry. This dog continued to develop additional SC nodules despite receiving a variety of chemotherapeutic agents and local irradiation, and was euthanized because of progressive B-cell LSA 605 days post-PBHCT.

TBI-Related Mortality. Two (13%) dogs died shortly after PBHCT. One dog that received 11 Gy TBI (two 4 Gy fractions at least 3 hours apart on day 1, and a single 3 Gy fraction on day 2) died of clinical signs consistent with sepsis 13 days post-PBHCT. One dog, that was not in remission upon arrival at NSCU, received doxorubicin^k (40 mg/m² IV) and melphalan^l (30 mg/m² PO) followed by rhG-CSF (5 μ g/kg SC) and plerixafor^m (240 μ g/kg SC) 1 week later. After apheresis and 12 Gy TBI (two 3 Gy fractions at least 3 hours apart on 2 consecutive days), the dog died of clinical signs consistent with severe GI toxicity 4 days post-PBHCT.

Apheresis

Effects of rhG-CSF Treatment. As reported previously,¹⁰ no signs of adverse effects were seen in any dogs receiving rhG-CSF before apheresis. In all dogs, preapheresis WBC (mean, 45,340 \pm 15,963 cells/ μ L)

and mononuclear cell counts (mean, $3,181 \pm 1,872$ cells/ μ L) were much higher than our hospital's normal reference range (WBC reference interval, 4,390–11,610 cells/ μ L; mean, 8,000 cells/ μ L; monocyte reference interval, 75–850 cells/ μ L; mean, 462 cells/ μ L; Table 2). There was no statistically significant difference when comparing these dogs to a previous report of 16 dogs with BCL harvested using a TerumoBCT cell separator¹⁰ with respect to preapheresis WBC count ($P = .1761$) and monocyte count ($P = .0623$). All rhG-CSF-treated dogs underwent apheresis.

Apheresis Procedure. All 15 dogs completed the apheresis. There was no statistically significant difference when comparing these dogs to a previous report of 16 dogs with BCL harvested using a TerumoBCT cell separator¹⁰ with respect to collection duration ($P = .7365$), total blood volume processed ($P = .7563$), postapheresis monocyte count ($P = .0636$), and Hct ($P = .1954$; Table 2). There was a statistically significant difference when comparing postapheresis WBC counts ($P = .0442$) and the amount of ACD-A infused during the procedure ($P = .0262$).

Effects of Apheresis. Fourteen of 15 dogs tolerated the procedure. One dog developed clinically relevant bleeding from the jugular catheter site during the last 15 minutes of the procedure. The dog's platelet count (preapheresis, 177×10^3 cells/ μ L; postapheresis, 30×10^3 cells/ μ L) and hematocrit (preapheresis, 45%; postapheresis 20%) decreased substantially during the procedure. A coagulation panel immediately postapheresis was abnormal (PT, no clot after 40 s; PTT, no clot formation after 120 s; d-dimer, >2,000 ng/mL). A coagulation panel the next day was normal (PT, 8.1 s; PTT 13 s, d-dimer <250 ng/mL) except for grade-4 thrombocytopenia (26×10^3 cells/ μ L), although all signs of bleeding slowly resolved during the day without medical management. One dog experienced mild generalized urticaria immediately after the procedure that responded appropriately to diphenhydramine administration. For the 4 dogs weighing <15 kg, machine priming with a variety of fluids was sufficient to maintain cardiovascular stability and the aphereses were completed uneventfully.

Apheresis Cell Yield. Table 3 compares the apheresis products of these dogs with a previous report of 16 dogs with BCL harvested using a TerumoBCT cell separator.¹⁰ There were no statistically significant differences when comparing the mean WBC ($P = .6836$), red blood cells ($P = .4089$), or mononuclear cells ($P = .8369$) harvested. In addition, we found no statistically significant difference in the number of CD34+ cells/kg harvested ($P = .3690$) and the cell separator MCCE ($P = .7643$) between the 2 groups. There was a statistically significant difference when comparing the mean number of platelets ($P = .0422$) harvested. PARR analysis was negative in 13/14 harvests for which the original tumor was PARR-positive. The one dog with a PARR-positive harvest also had PARR-positive blood at presentation to NCSU. Two other dogs with PARR-positive blood at presentation to NCSU had PARR-negative harvests.

Table 2. Apheresis characteristics of dogs with T- and B-cell lymphoma collected with a CaridianBCT cell separator.

	Weight (kg)	Coll Length (m)	TBV	ACD-A (mL)	Pre Aph WBC/ μ L	PreAph Mono/ μ L	Pre Aph Hct	Post Aph WBC/ μ L	Post Aph Mono/ μ L	Post Aph Hct
TCL ^a (n = 14)										
Range	6.7–54.4	181–395	2.6–4.8	322–1,115	20,210–75,550	606–6,800	29.7–51.5	21,200–77,020	432–3,569	24.5–50
Mean \pm SD	28.5 \pm 13.0	308 \pm 56	3.3 \pm 0.7	752 \pm 306	45,340 \pm 15,963	3,181 \pm 1,872	40.2 \pm 5.7	46,365 \pm 16,698	1,655 \pm 853	37.3 \pm 8.3
BCL ¹⁰ (n = 16)										
Range	11.3–55.6	240–420	2.3–4.2	299–1,625	36,600–85,060	1,935–10,618	31.5–48.2	31,820–89,180	1,273–8,000	17.9–55.3
Mean \pm SD	33.0 \pm 8.6	323 \pm 59	3.2 \pm 0.6	1,071 \pm 351	54,951 \pm 16,723	4,708 \pm 2,453	41.2 \pm 5.0	59,140 \pm 16,531	3,245 \pm 1,775	40.7 \pm 10.3
TCL versus BCL aphereses										
P-value	.2721	.7365	.7563	.0262	.1761	.0623	.7491	.0442	.1994	.1954

TCL, T-cell lymphoma; BCL, B-cell lymphoma; TBV, total blood volume; ACD-A, acid-dextrose formula; Pre Aph, preapheresis; Post Aph, postapheresis; Mono, mononuclear cells; WBC, white blood cells; Hct, hematocrit.

^aDoes not include 1 dog who underwent apheresis using a Baxter-Fenwal CS-3000 Plus cell separator.

Table 3. Apheresis product counts of dogs with T- and B-cell lymphoma using a CaridianBCT cell separator.

	WBCs (Cells/ μ L)	Plts (Cells/ μ L)	Grans (%)	Hct (%)	Mono (Cells/ μ L)	CD34+ (%)	CD34+ (Cells/kg)	MCCE (%)
TCL ⁹ (n = 14)								
Range	13,770–357,300	92 x 10 ³ –1,373 x 10 ³	0–45	2.1–4.5	10,643–110,763	1.3–6.7	2 x 10 ⁶ –42 x 10 ⁶	3.6–68.8
Mean \pm SD	92,217 \pm 87,051	725 x 10 ³ \pm 403 x 10 ³	18.0 \pm 16.2	5.7 \pm 0.7	43,846 \pm 34,105	2.4 \pm 1.4	1.4 x 10 ⁷ \pm 1.2 x 10 ⁷	36.2 \pm 18
BCL ¹⁰ (n = 16)								
Range	18,310–313,560	85 x 10 ³ –2,833 x 10 ³	3.0–4.7	1.4–6.2	4,944–131,695	0.2–4.9	3.5 x 10 ⁶ –54.5 x 10 ⁶	8.6–76
Mean \pm SD	105,646 \pm 91,341	1,195 x 10 ³ \pm 750 x 10 ³	14.8 \pm 13.2	3.3 \pm 1.2	59,639 \pm 42,036	2.4 \pm 1.4	1.8 x 10 ⁷ \pm 1.4 x 10 ⁷	37.6 \pm 19.3
TCL versus BCL aphereses	.6836	.0422	.6243	.4089	.8369	.9878	.3690	.7643
<i>P</i> -value								

TCL, T-cell lymphoma; BCL, B-cell lymphoma; WBC, white blood cells; Plts, platelets; Grans, granulocytes; Hct, hematocrit; Mono, mononuclear cells; MCCE, mononuclear cell collection efficiency.

^aDoes not include 1 dog who underwent apheresis using a Baxter-Fenwal CS-3000 Plus cell separator.

Factors Affecting Apheresis Yield. We examined the effect of preapheresis Hct on the machine MCCE because we reported previously that preapheresis Hct was moderately correlated ($R^2 = 0.3371$, $P = .0184$) with increased MCCE in dogs with BCL.¹⁰ In the TCL dogs, this correlation was not evident ($R^2 = 0.1280$, $P = .2091$; Fig 1A). When combining these data from both groups of dogs (n = 30), however, preapheresis Hct was correlated ($R^2 = 0.2021$, $P = .0127$) with machine MCCE (Fig 1B). Concordant with previous results,¹⁰ preapheresis leukocyte counts did not affect the machine MCCE ($R^2 = 0.01627$, $P = .6639$) or the number of CD34+ cells/kg ($R^2 = 0.05644$, $P = .4135$; data not shown). Finally, concordant with previous results,¹⁰ preapheresis monocyte count was not significantly correlated with the number of CD34+ cells/kg harvested ($R^2 = 0.00385$, $P = .9516$; Fig 1C), although when combining the 2 groups of dogs there was a significant correlation ($R^2 = 0.2119$, $P = .0105$; Fig 1D).

PBHCT Outcome

Platelet Recovery. We examined the effects of the number of transplanted CD34+ cells/kg on platelet recovery at day +14 after PBHCT. Contrary to previous findings,¹⁰ there was no statistically significant difference ($P = .1983$) in platelet counts at discharge in dogs receiving $<6 \times 10^6$ CD34+ cells/kg (mean, 43,000 \pm 57,000/ μ L) compared with dogs receiving $\geq 6 \times 10^6$ CD34+ cells/kg (mean, 119,000 \pm 123,000/ μ L), although only 3/4 dogs that received $<6 \times 10^6$ CD34+ cells/kg were included in this analysis (1 dog died in the hospital). When we combined these data with the previously reported BCL dogs, the difference in day +14 platelet counts became significant ($P = .0234$). There was no statistically significant difference in day +14 platelet counts when examining TCL dogs that received $<1 \times 10^7$ CD34+ cells/kg versus $>1 \times 10^7$ cells/kg alone or when combining these data with the previously reported BCL dogs.

Remission Duration. The median DFI for the 13 dogs that did not relapse before PBHCT and were in CR at the time of PBHCT from the time of PBHCT to relapse was 184 days (range, 28–738 days; Fig 2A). Three (23%) of these 13 dogs relapsed within 4 months of PBHCT, 3 (23%) dogs relapsed between 4 and 8 months after PBHCT, 5 (38%) dogs relapsed after 8 months of PBHCT (at 8.3, 10, 12.9, 18.3, and 24.6 months), and 1 (8%) dog had not relapsed after 24.7 months. There was no statistically significant difference ($P = .7665$) in DFI between dogs that received 10 or 11 Gy TBI. When we compared the median DFI between the dogs with TCL in this study and the previously reported BCL dogs,¹⁰ there was no statistically significant difference ($P = .0704$) between the 2 groups (184 days versus 511 days, respectively; Fig 2B). One dog that had relapsed and was in clinical remission before PBHCT

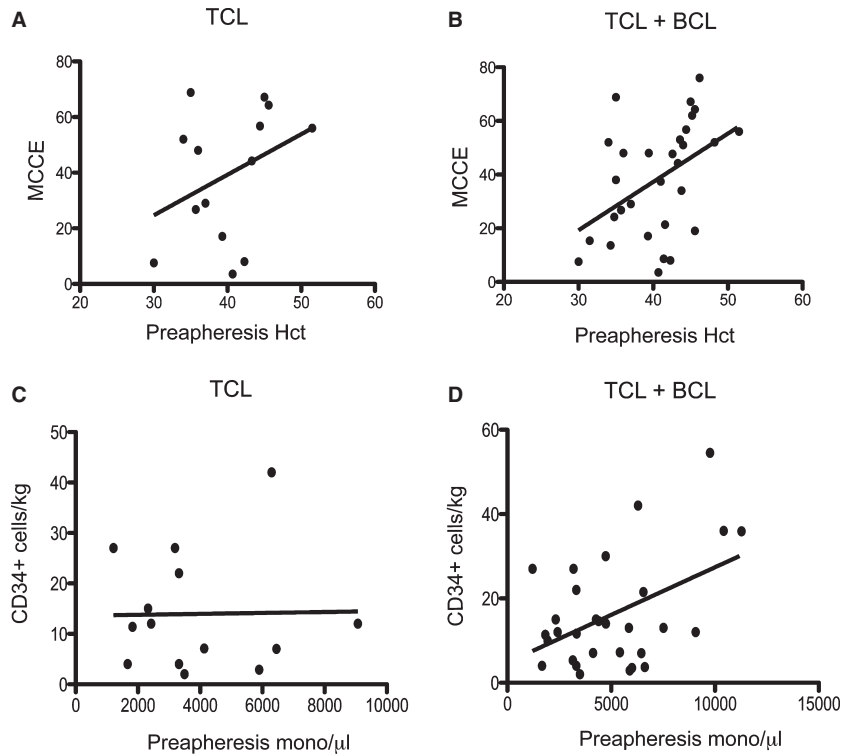


Fig 1. Correlation between preapheresis blood cell parameters and harvest outcomes. (A) Linear regression analysis did not show a correlation in TCL dogs between preapheresis Hct and MCCE ($R^2 = 0.1280$, $P = .2091$) or (C) preapheresis mononuclear cell counts and the number of CD34+ cells/kg harvested ($R^2 = 0.00385$, $P = .9516$). (B) Linear regression analysis did show a correlation between preapheresis Hct and MCCE ($R^2 = 0.2021$, $P = .0127$) and (D) preapheresis mononuclear cell counts and the number of CD34+ cells/kg harvested ($R^2 = 0.2119$, $P = .0105$) when TCL and BCL¹⁰ cases were combined.

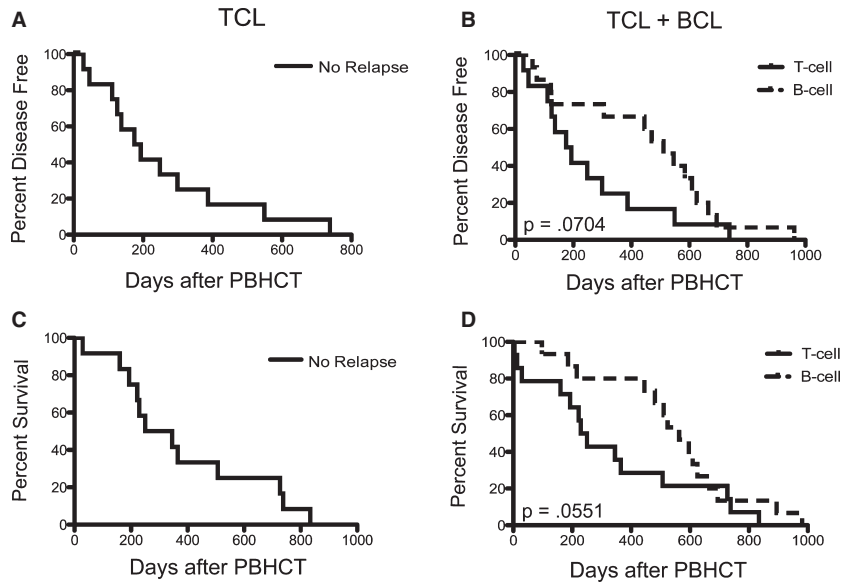


Fig 2. (A) Disease-free interval of TCL and (B) TCL and BCL¹⁰ ($P = .0704$) cases combined. (C) Overall survival of TCL and (D) TCL and BCL¹⁰ ($P = .0551$) cases combined.

remained disease-free for 78 days, whereas 1 dog not in clinical remission on presentation at NCSU died in the hospital.

Overall Survival. The median OS for the 13 dogs that were in CR at the time of PBHCT from the time of PBHCT was 239.5 days (range, 4–738 days;

Fig 2C). When we compared the median OS between the dogs with TCL in this study and the previously reported BCL dogs,¹⁰ there was no statistically significant difference ($P = .0551$) between the 2 groups (240 days versus 531 days, respectively; Fig 2D). The median OS from the time of diagnosis to death of the 13 TCL dogs in CR at the time of PBHCT was 602 days (range, 246–928 days), with 2 (14%) dogs still alive at 741 and 772 days. Stage and substage of disease at diagnosis had no effect on OS ($P = .5862$ and $P = .1303$, respectively).

Discussion

Similar to our previous study using PBHCT to treat dogs with BCL,¹⁰ an adequate number of peripheral blood CD34+ cells could be harvested using a TerumoBCT cell separator for HCT after myeloablative treatment. In addition, the majority of dogs tolerated the toxicities associated with escalating doses of HDC and TBI and achieved complete hematologic reconstitution after transplantation.

In an effort to decrease the potential for minimal residual disease in the blood of the affected dogs, HDC dose intensification was incrementally increased throughout the study. As such, 9 dogs received ≥ 650 mg/m² of cyclophosphamide, with 1 dog receiving 750 mg/m². Although some dogs developed grade-4 neutropenia, only 4 dogs required brief hospitalization (1 received 500 mg/m², 1 received 600 mg/m², 2 received 650 mg/m²), only 1 dog developed cyclophosphamide-induced cystitis, and all dogs were able to proceed with apheresis. These results suggest that cyclophosphamide can be administered to dogs in amounts up to 3.75 times the standard dosage if appropriate protocols are used and supportive care is given if needed.

In a previous report of PBHCT of dogs with BCL,¹⁰ the relapse rate was approximately 65% when 10 Gy of TBI was used to achieve molecular remission. In this study, we continued to escalate the dose of TBI because a higher dose of irradiation should, theoretically, lead to a better clinical outcome^{16–18} and because TCL is a more aggressive disease than BCL. Therefore, we administered >10 Gy TBI to 12 dogs while incorporating more fractionated protocols in an effort to decrease the associated GI toxicities. In addition, many dogs received enteral glutamine, an antioxidant that may protect the GI tract from the adverse effects associated with radiation treatment.^{19,20} Although 2 dogs in this study died in the hospital, only 1 dog, that was not in CR at the time of presentation to NCSU and subsequently received high-dose chemotherapy 1 week before TBI, had evidence of severe GI toxicity at necropsy. Therefore, we conclude that dogs can routinely tolerate >10 Gy TBI when administered in multiday fractionated protocols. In future PBHCT studies, we also will utilize lower dose rates (regardless of overall dose) because it appears that both radiation dose rate and overall dose are the most important factors in acute toxicity of the canine GI tract associated with TBI.¹⁶

Overall, there were only minor differences between the TCL dogs in this study and the BCL dogs in a previous study¹⁰ with respect to apheresis characteristics and apheresis product counts. When we examined a variety of factors affecting apheresis cell yield, preapheresis Hct was not significantly correlated with increased MCCE and preapheresis monocyte count was not significantly correlated with the number of CD34+ cells/kg harvested as we found in the BCL dogs. The lack of correlation between the preapheresis monocyte count and number of CD34+ cells/kg harvested is most likely a reflection of the lower overall number of monocytes before the procedure in the TCL dogs, whereas the reason for the lack of correlation between preapheresis Hct and increased MCCE in these dogs is not clear. However, when combining the data from both studies, these correlations again became significant. Importantly, rhG-CSF administration was well tolerated and the resultant apheresis products contained $>2 \times 10^6$ CD34+ cells/kg (target cell dose) in all TCL dogs.

When apheresis products contain $>1 \times 10^7$ CD34+ cells/kg, we routinely cryopreserve one-half of the product in the event a dog relapses and the owners want to perform a second PBHCT. The cryopreserved product was given to 1 dog that relapsed approximately 93 days after PBHCT after additional chemotherapy (doxorubicin [30 mg/m²] and melphalan [30 mg/m²]) and TBI (6 Gy). Complete hematologic reconstitution was again achieved, and the dog remained in clinical and molecular remission for an additional 225 days. This case underscores the utility of apheresis product cryopreservation and corroborates data from humans showing the feasibility of second hematopoietic cell transplants in patients relapsing after prior PBHCT.^{21,22}

One dog developed a second cancer after PBHCT. Interestingly, this dog developed a B-cell malignancy whereas a dog in a previous report¹⁰ with B-cell lymphoma developed epitheliotropic T-cell lymphoma. Late second malignancies after total body irradiation have been reported in both dogs²³ and humans.²⁴

Two factors that are consistently identified as having negative prognostic importance in dogs with lymphoma are immunophenotype and WHO substage.^{25,26} Therefore, dogs with a T-cell immunophenotype and substage b disease tend to have substantially shorter remission and survival durations. Interestingly, in this study where 40% of the TCL dogs were WHO substage b, the median OS, when defined as time from diagnosis to death, was 602 days, with 2 dogs (1 Stage 3A, 1 Stage 5b) still alive at manuscript preparation. Factors that may have positively influenced the survival of the dogs in this study include the higher doses of HDC and TBI used in the pre-PBHCT conditioning regimen and the unknown number of dogs with low-grade tumors²⁷ because a LN biopsy was obtained in only 3 dogs (all 3 dogs were diagnosed with an aggressive T-cell lymphoma called peripheral T-cell lymphoma). In addition, because 87% of the dogs

maintained a first remission (median, 3.7 months; range, 2–5.8 months) before transplantation, a positive selection bias in this population may have influenced our results. Regardless, it appears that PBHCT achieved a longer OS (602 days) in dogs with TCL than previously reported studies (159 days³ and 235 days²⁸).

In summary, we show that an adequate number of peripheral blood CD34+ cells can be routinely harvested from dogs with TCL and used for autologous PBHCT. Based on this small number of dogs, it appears that, although PBHCT does not lead to a statistically significant increase in DFI in dogs with TCL, it can lead to a longer median OS when compared to historical studies using multiagent chemotherapy regimens. Because 1 dog in this study achieved post-myeloablative hematopoietic reconstitution after a second PBHCT using a cryopreserved autologous apheresis product, we suggest, that, similar to human protocols,²⁹ cryopreserved canine apheresis products can be routinely used for both autologous and allogeneic PBHCT. Based on an apparent increase in the median OS of the dogs in this study (with 2 dogs alive >2 years after diagnosis), PBHCT may be considered as a viable alternative treatment for dogs with high-grade TCL.

Footnotes

- ^a TerumoBCT, Lakewood, CO
^b Baxter Healthcare Corporation, Deerfield, IL
^c Cytosan, Cyclophosphamide for Injection, USP; Baxter Healthcare Corporation, Deerfield, IL
^d Neupogen; Amgen, Thousand Oaks, CA
^e Mesnex; Sagent Pharmaceuticals, Schaumburg, IL
^f L-glutamine; Vitamin Shoppe, North Bergen, NJ
^g DMSO; Amresco, Solon, OH
^h Gambro DF-700 Bag Press; ThermoFisher Scientific, Hudson, NH
ⁱ Varian, Palo Alto, CA
^j GraphPad Software, San Diego, CA
^k Adriamycin; Bedford Laboratories, Bedford, OH
^l Alkeran; GlaxoSmithKline, Brentford, London, UK
^m Mozobil; Genzyme Corporation, Cambridge, MA

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