Oncology Department
VCA West Los Angeles Animal Hospital
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VCAWLAspecialty.com

Tucker (r) and donor sibling Rusty (l)

CASE STUDY

Resistant B-Cell Lymphoma in a 5-year-old Vizsla

John Chretin, DVM, DACVIM (Oncology)

"Tucker," a 5-year-old MC Vizsla, was referred for rescue chemotherapy and evaluation for hematopoietic stem cell transplantation (HSCT)...

1. Referral

Tucker is a 5-year-old, castrated male Vizsla that presented to his referring veterinarian on June 11, 2012 for acute lymphadenopathy. He was otherwise reported to be acting normally. Full blood, urinalysis, thoracic and abdominal radiographs were performed along with fine needle aspiration of a peripheral lymph node. Results revealed lymphoblastic lymphoma with evidence of splenic and both peripheral and abdominal lymph node involvement. Tucker was placed onto an alternating cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) based chemotherapy protocol and quickly achieved full clinical remission. On November, 16 2012, the day of his final scheduled chemotherapy, Tucker remained in complete remission and was reported to be doing excellent at home. Three weeks later he returned to his referring veterinarian for

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recurrence of his lymphadenopathy. Repeat fine needle aspiration of an enlarged peripheral lymph node was submitted. Results revealed Tucker to be out of remission. He was administered oral lomustine chemotherapy for re-induction but failed to respond. Tucker was referred to VCA West Los Angeles Animal Hospital for rescue chemotherapy and evaluation for hematopoietic stem cell transplantation (HSCT).

2. Examination

On presentation Tucker's only exam change was generalized moderate lymphadenopathy. Owing to his quick relapse after completion of his CHOP based protocol and resistance to lomustine, a grave long term prognosis was given. Allogeneic HSCT was recommended. To proceed, a Dog Leukocyte Antigen (DLA) matched donor was required. A single litter mate of Tucker's -- "Rusty" -- was available for testing. Lymphoma phenotyping, current complete blood count, serum chemistry, urinalysis and peripheral blood, lymphocyte clonality testing was performed. He was confirmed to have a B-cell lymphoma and clonality testing was positive for circulating malignant lymphocytes.

3. Treatment

MOPP (mustargen, vincristine, procarbazine and prednisone) rescue chemotherapy was initiated. Tucker returned one week later for his ongoing care. He had experienced a partial response (>50% reduction in size of peripheral lymph nodes). His owner elected to proceed with allogeneic HSCT. A whole blood sample from Tucker and his littermate were submitted for DLA typing. Tucker received his scheduled MOPP chemotherapy and was discharged. Three weeks later he presented with stable disease. A second course of MOPP chemotherapy was administered. One week following this visit, Tucker's lymphoma was again progressive. L-asparaginase was administered. DLA typing had determined that his littermate was a suitable hematopoietic stem cell donor. Tucker underwent complete pre-transplantation staging on February 17, 2013 (echocardiogram, abdominal ultrasound, bone marrow aspiration, complete blood count, serum chemistry, urinalysis,

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urine culture). Concurrently, heath screening of the donor was performed and included physical examination, complete blood count, serum chemistry, urinalysis and tick borne PCR panel testing on peripheral blood.

Tucker and Rusty at the hospital the day the donor lymphocyte infusion (DHL) was performed..

All findings for the donor were within normal limits. No evidence of blood borne pathogens were detected. Tucker's pre-transplantation staging revealed that a significant tumor burden was present. Splenectomy along with lymphadenectomy of his largest peripheral lymph node (right submandibular) was performed on February 19, 2013. Three days later Tucker began gastrointestinal sterilization with oral enrofloxacin, neomycin and polymyxin B. Immunosuppression with oral cyclosporine was instituted on February 23rd. The donor was mobilized with granulocyte colony-stimulating factor subcutaneously every 12 hours for 5 days.

Tucker on the day of the DHL.

On the 6th day of mobilization, the donor underwent mononuclear cell apheresis for stem cell collection. A stem cell harvest volume of 363ml was obtained with a hematopoietic stem cell (CD34+) yield of 1.2%. Tucker was admitted for transplantation on February 26th and underwent conditioning with total body irradiation (4Gy x 2 with a minimum of 3 hour rest between fractions). Immediately following his second radiation fraction the donor stem cells were infused

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Tucker on day 11+, his first day out of isolation.

intravenously ($6.3x10^6$ CD34+ cells/kg). Tucker remained on intravenous fluid therapy for the first 24 hours following transplantation. On day 3+ Tucker was placed into isolation. On day 9 and 10+ he was administered prophylactic transfusions of fresh, irradiated platelets. On day 11+ his leukocyte and platelet counts were $1.8x10^3$/ul and $49x10^3$/ul respectively. Due to white cell engraftment, Tucker was removed from isolation. All antibiotics were discontinued and he remained on cyclosporine. Day 13+ Tucker's leukocyte and platelets counts were $3x10^6$/ul and $73x10^3$ respectively. Tucker was clinically well with a persistently finicky appetite that began on Day 2+. He was discharged with stable lymphadenopathy.

4. Follow-up

On day 15+ Tucker presented for recheck. Chimera testing had revealed him to be 99.4% donor. His appetite remained poor, but he was otherwise reported to be doing well at home. Lymph node assessment revealed mild progression of his lymphadenopathy. Due to early lymphoma progression, his cyclosporine was discontinued, and he was scheduled to return 10 in days for recheck.

Tucker being fed by a veterinary technician in the Oncology Isolation Ward, 8 days post-transplant. Although stable, initially he had a finicky appetite which later improved.

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On day 26+ Tucker's lymph nodes were again slightly progressive (14% increase from discontinuation of cyclosporine, 38% post day 13+). Clinically he was doing well and his appetite was improved. Recheck chimera testing revealed him to be a full chimera (100% of circulating hematopoietic cells donor in origin). Owing to a lack of improvement /mild progression of his lymphoma, a Donor Leukocyte Infusion (DLI) was recommended. On day 33+, a non-mobilized, mononuclear apheresis was performed on the donor and an estimated $1x10^8$ CD3+ cells/kg was given intravenously to Tucker.

Day 48+ Tucker was reported to be doing excellent at home with no abnormal changes. His peripheral lymphadenopathy was 20% improved. Day 107+, Tucker was clinically well, but his lymph nodes were again mildly progressive. Whole blood was collected from the donor for ex-vivo expansion of donor CD8+ cells (adoptive immunotherapy).

Tucker, post-transplant, with his brother Rusty.

On June 26, 2013 (119+) $1x10^7$ CD8+ cells were given without incident. Three weeks later a second infusion of $4.2x10^8$ CD8+ cells was administered. At time of the second infusion all peripheral lymph nodes were improved. Six months after beginning DLI's all peripheral lymph nodes were within normal limits with exception to Tucker's right submandibular lymph node. Peripheral blood clonality testing remained positive for the presence of malignant lymphocytes. Due to a lack of complete clinical and molecular remission, on October 13, 2013 (210+) a second course of two DLI's was scheduled. The first ($2x10^8$ CD8+ cells) was followed one week later with excision of Tucker's right submandibular lymph node. Clonality testing on his peripheral blood at time of surgery was negative for malignant cells, but remained positive in his right suprascapular lymph node that palpated clinically normal. On October, 17 a final DLI ($3x10^8$ CD8+ cells) was administered.

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On November 21, 2013 (268+) Tucker remained in clinical remission. Clonality testing of his right suprascapular lymph node and peripheral blood were both negative. Tucker's most recent recheck was performed on June 24, 2016. He remains in clinical and molecular remission and as a full chimera.

5. Discussion

Standard chemotherapy for dogs with lymphoma results in an approximate 3% cure for the B-cell type and lower rate for those with the T-cell form. In the absence of cure, most patients will ultimately succumb to chemotherapy resistance. Initially dogs may receive 1-2 courses of standard CHOP-based chemotherapy. Once resistant they will continuously and sequentially be administered rescue chemotherapy protocols until all options have been exhausted.

An estimated 300 dogs diagnosed with lymphoma have undergone HSCT over the past 50 years, many of which were performed as a part of the body of research for the development of the procedure. Large studies are presently lacking and only several clinical veterinary transplant centers are presently in existence. Given these factors, a true cure rate is not known. However, based on available information in human and veterinary literature, HSCT is a proven advanced therapy that offers a higher potential of cure for lymphoma compared to chemotherapy alone. Current estimates for dogs are approximately 30-40% with the B-cell lymphoma undergoing autologous and 60% of those undergoing allogeneic transplantation will experience durable long term control/cure.

The two main types of HSCTs performed in dogs are autologous and allogeneic. Both utilize total body irradiation (TBI). For those undergoing autologous transplantation TBI is the sole method for eradication of disease. Whereas TBI for those undergoing allogenic transplant, is a means of conditioning or weakening the recipient's innate immune system for engraftment of the allogenic hematopoietic stem cells. The ensuing result is a graft versus tumor (GVT) effect that is mediated by the alloreactive donor T and B cells.

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Allogeneic transplantation requires a DLA matched donor, is associated with a higher financial cost, and carries a higher risk for toxicity. Therefore, autologous transplantation in dogs is more commonly performed.

Minimal residual disease influences both clinical outcome and risk of complications with HSCT. Both clinical and molecular remission (clonality testing unable to detect malignant cells) is recommended for either form of transplantation. With autologous HSCT, it is a requirement as the hematopoietic stem cells are collected from peripheral blood during the mononuclear apheresis. For these reasons, Tucker was not considered a candidate for autologous transplantation.

Tucker had a significant tumor burden identified just prior to his scheduled transplantation. To mitigate the potential for tumor lysis like syndrome following TBI and to potentially improve outcome, splenectomy along with removal of his largest peripheral lymph node was performed. Although he was considered to be at a high risk for complications, the only significant toxicity encountered during the transplant period was a prolonged lack of interest in food. This change immediately began to improve after cyclosporine was discontinued.

Cyclosporine is an integral component of allogeneic transplantation in dogs as it allows for the establishment of graft-host tolerance thereby mitigating the risk for potentially fatal graft versus host disease (GVHD). While being administered, GVT is also abrogated. Standard of care treatment for early relapse or progression of malignancy, during the immediate post-transplant period, is discontinuation of immunosuppression. The response is low but it may lead to reestablishment of cancer control by inducing GVT. When not effective, DLI's, a form of cellular adoptive immunotherapy, are commonly pursued. The aim is to harness the powerful benefit of the donor's immune system to eliminate residual host tumor and establish recipient immunization against the malignancy via memory T-cells. Tucker initially received an apheresed DLI and temporarily responded. Due to the need for subsequent infusions, ex vivo expansion of CD8+ T-cells was pursued. His initial

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two infusions were escalated. The attempt was to induce GVT without leading to the development of significant GVHD. Clinically significant GVHD did not occur. In an effort to maximize his response the last remaining enlarged lymph node was surgically removed. Two additional DLI's were administered surrounding the surgery. Tucker is presently day 1264+ HSCT. He has been in clinical and molecular remission for 1020 and 1055 days respectively. ■

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Dr. John Chretin, head of oncology at VCA West Los Angeles Animal Hospital, obtained his DVM degree in 1994 from Colorado State University. He completed a small animal internship at North Carolina State University and then went on to receive board certification in medical oncology after a three year residency at Tufts University. He has been a member of our staff since 2002. Dr. Chretin's 4 core principles are educating owners, providing progressive cancer care, advancing veterinary oncology and improving the quality of life for all animals. Dr. Chretin stresses client education foremost and has the unique first-hand experience of treating two of his own dogs for cancer. Dr. Chretin is an active participant in clinical studies and has co-authored numerous publications. In July 2010 he established our bone marrow transplant program for dogs with lymphoma.