

**Allogeneic Hematopoietic Stem Cell Transplantation for Treatment of Canine Lymphoma**  
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## **INTRODUCTION**

Allogeneic hematopoietic stem cell transplantation is a common treatment option for humans with hematopoietic malignancies. Much of the basic principles and techniques of transplantation was completed in canines as a preclinical large animal model, however, transplantation as a treatment option has not been routinely available to dogs with spontaneous lymphoma.<sup>1,2</sup> In this presentation, we discuss the recent advances that have made these transplantation techniques available to client owned dogs, and we outline the steps needed to successfully complete an allogeneic stem cell transplant.

## **BACKGROUND**

In the late 1970's it was demonstrated that 25% of canine lymphoma cases could be cured with a combination of chemotherapy, total body irradiation, and autologous marrow recovery.<sup>3-6</sup> Later, similar results were obtained with the use of peripheral blood mobilized stem cells as the recovery cells rather than marrow.<sup>7</sup> Additional studies found that allogeneic stem cells from dog leukocyte antigen (DLA) matched donors could also cure lymphoma.<sup>8,9</sup> All of these transplant procedures were hampered by significant procedure related toxicities (marrow failure, overwhelming infections, and radiation toxicity), and the allogeneic procedures were additionally plagued by potentially fatal graft versus host disease (GVHD).<sup>10</sup> Years of intense investigation of the molecular immunogenetics of the major histocompatibility complex of dogs lead to the development of techniques to rapidly identify DLA-matched family members for use in allogeneic transplantation procedures.<sup>11-21</sup> Subsequent advancements identified treatment regimens for controlling GVHD, and better post-transplant medical support (with broad spectrum antibiotics and transfusion support with irradiated blood products) lead to less treatment related morbidity and mortality. Collection of peripheral blood mobilized stem cells using a dual lumen central venous catheter and an automated apheresis machine further reduced morbidity to the donor by eliminating the need for marrow harvesting.<sup>22</sup> In the last three years, these techniques have been expanded further to be available outside of the laboratory setting to include client owned dogs.<sup>23</sup>

## **MATERIALS AND METHODS**

### **Patient Staging and Induction of Remission**

Standard staging should be performed to characterize the type of lymphoma before chemotherapy is initiated. Additionally, flow cytometry, immunohistochemistry, and/or gene rearrangements, should be performed to identify a molecular marker of disease for use in identifying complete remission and relapse. Standard chemotherapy protocols can be used to achieve a complete remission, and a transplant procedure is expected to be more successful if performed while the patient is in the first remission. A rest period of 3 weeks after the last dose of chemotherapy (including prednisone) is advised before the transplant. During the induction chemotherapy an effort to identify a donor is made.

### **Identification of a Donor-Recipient Pair**

Allogeneic transplant procedures in client owned dogs are limited by the donor pool being confined to siblings of the patient with lymphoma. Additionally, a DNA sample from at least one of the parents (and in some cases both) is needed to help identify DLA matched donor-recipient pairs. Because it can sometimes be difficult to find siblings, it is important to initiate a search for a potential donor as soon as possible. We have found that breeders often keep good records on the location of littermates, and we have left the process of finding the potential donors up to the patient's owner. A good family tree is diagrammed, and a blood sample (5cc whole blood in EDTA) from the patient, the sire and dam, and as many siblings from the same or subsequent litters of the same breeding pair, are collected and sent chilled overnight to the lab where the matching techniques are performed. As blood samples are delivered to the lab, the DNA is extracted and frozen for future analysis. Once all samples are delivered to the lab the matching process is completed (25% of siblings are expected to be DLA-identical), and a donor is selected who is as big as or bigger than the patient if possible. Also, willingness of the potential donor's owner to participate is critical.

### **Donor Preparation**

Once a donor has been identified and the patient is in confirmed remission, a time-line for the transplant day can be prepared. The transplant day is defined as day zero, and important preparations are made in reference to this day. Beginning day -30 the donor is screened for tick borne diseases, heart worms, and a CBC, chemistry, and a urinalysis are performed. A pre transplant chimerism whole blood sample (10cc whole blood in EDTA) is collected and sent to the lab for DNA extraction and cryopreservation. On day -14 a blood prime (~200cc whole blood in ACD solution) is collected from the donor for use in priming the apheresis machine on the apheresis day. This blood is kept under refrigeration until the apheresis is performed. A blood prime is not needed for dogs over 30kg. Starting on day -6 the donor is given 5ug/kg Neupogen SQ BID for five days with the apheresis scheduled for day -1. Beginning day -4 a daily CBC and peripheral blood CD34+ count are done two hours after the morning dose of Neupogen in order to document adequate mobilization of progenitor stem cells from the marrow into the peripheral blood. CD34+ counts will range from 0.5 to 2.5% of the total nucleated cell count, and total WBC counts will range between 30,000 to 65,000 cells/uL.

On the morning of day -1 (the apheresis day), the Neupogen dose is increased to 10ug/kg SQ 2 hours before the apheresis procedure is started. The donor is placed under general anesthesia and a dual lumen central venous catheter is placed in a jugular vein (Arrow Dual Lumen Catheter—12f, 15cm). A CBC is run on the donor to help set the collection parameters on the apheresis machine. The donor's body temperature is monitored and regulated during the apheresis procedure, and serial blood calcium measurements are made every 20 min. A 10% calcium gluconate solution is administered via IV pump at a rate of 10ml/h during the apheresis to avoid hypocalcemia from anticoagulant induced calcium depletion, and adjustments to the infusion rate are made as indicated. The apheresis

machine (COBE Spectra from Gambro BCT) is set to run a standard mononuclear cell cycle using a closed collection set. Half way through the apheresis procedure a sample of the harvest is evaluated with a CBC and a CD34+ cell count. The target CD34+ dose is  $4 \times 10^6$  cells/kg body weight of the recipient. An adjustment in the apheresis time frame can then be made based upon the harvest quantity, total white blood cell count, and % CD34+ cells. The apheresis procedure may take between two to four hours, and once complete, the central venous catheter is removed.

After completion of the apheresis, confirmation that the CD34+ target dose has been reached is made with an additional CBC and CD34+ cell count on the total apheresis harvest. The harvest is kept under refrigeration until infusion into the recipient immediately after total body irradiation.

### **Recipient Preparation**

On day -8 (just before the mobilization of the donor is started) the recipient is evaluated for confirmation of remission using molecular markers established prior to chemotherapy. A pre transplant chimerism whole blood sample (10cc whole blood in EDTA) is collected and sent to the lab for DNA extraction and cryopreservation. In addition, a urinalysis with culture and sensitivity are performed and a dental prophylaxis is completed if needed. On day -5 oral antibiotics are initiated including neomycin sulfate (6mg/kg PO q8hrs), polymyxin B (25,000 U/kg/d PO q 8hrs), and enrofloxacin (10mg/kg SC q24hrs). These medications are continued from day -5 until the after the neutrophils recover to above 1,000cells/ul after the HCT. Lactobacillus, a probiotic (80mg PO bid), is also started on day -5 and continued until day +40. On day -1 cyclosporine (5mg/kg PO bid) is started and continued to day +35 or longer if needed to control GVHD. Cyclosporine assays are performed 2-3 times per week as needed to establish therapeutic serum concentrations from 400 to 600ng/ml. Blood samples for the cyclosporine assays are collect 12 hours after the last dose.

On day 0 the patient is prepared for total body irradiation (TBI). TBI is delivered with a linear accelerator at a total dose of 8Gy in two fractions of 4Gy with a three hour rest period between fractions. Radiation is delivered at a rate of 7cGy/min, and the patient is rotated 180 degrees after 2Gy during each fraction. Immediately following the TBI the harvested progenitor cells are administered to the recipient through a peripheral intravenous catheter.

### **Immediate Post Transplant Care**

Beginning the night of day 0 and continuing through day 4, the patient is not allowed to take in anything PO. **All steroid use should be strictly avoided (including topical use) as this has been shown to interfere with engraftment and cause graft failure.** Fluid support is intravenous or subcutaneous (standard maintenance dose) with lactated ringer's solution or saline. Daily inspections of the skin for lesions associated with GVHD are made and noted. These lesions include a red, slightly raised, expanding pruritic dermatitis of the inside of the pinnae, the dorsal and lateral surfaces of the muzzle, the skin around the eyes, and the ventral abdominal midline. If any of these lesions are found, a chemistry profile to evaluate liver enzymes should be completed, as the primary organs affected by GVHD are the skin, liver, and gastrointestinal tract. Increases in the cyclosporine dose can be made to try to bring GVHD under control. Vomiting is controlled with antiemetics as needed, and diarrhea is controlled with Imodium as needed. Antibiotics, probiotics, and cyclosporine are continued as described above, and the body temperature is monitored twice daily. During the first 5-6 days the patient is kept in a clean, semi isolated environment. Starting on day 4 once or twice daily CBCs and serum chemistry profiles are performed. Oral food and water are allowed beginning on day 5. When the neutrophil count goes below 1000cells/uL the patient is kept in an isolation room and not allowed to leave until the count recovers to above 1000cells/uL. The neutrophil nadir (counts below 100cells/uL) occurs around day 7 and can continue for 24-48 hours. The platelet nadir (counts 10,000platelets/uL or below) occurs around day 10 and can last for 48-72 hours. During this time transfusion support may be needed using cross matched fresh whole blood or platelets. All blood products should be irradiated before use (18-25Gy) in order to prevent allo-competition and potential graft failure. Cyclosporine assays are run every 2-3 days as described above in order to keep the serum cyclosporine level between 400-600ng/ml. Adjustments in the cyclosporine dose are made according to the assay results. Once the neutrophils have recovered to above 1000cells/uL (usually by day 10) the patient is allowed outside of the isolation room. Platelet recovery to a safe level (usually by day 14) allows the patient to go home and be monitored on an out-patient basis. Cyclosporine is discontinued on day 35 if there are no signs of GVHD.

### **Long Term Post Transplant Care**

On days 30, 60, 90, 120, and 150 whole blood samples (3cc in EDTA) are submitted to the lab for chimerism assays. With myeloablative TBI, autologous marrow recovery is not expected, however, if a state of mixed chimerism exists (the presence of both donor and host origin blood cells), then a donor lymphocyte infusion (DLI) may be performed to boost the patient into 100% donor chimerism. Identification of residual neoplastic cells is another indication for a DLI. A DLI consists of donor lymphocytes collected from the peripheral blood of the donor with a target of delivering  $2 \times 10^5$  CD3 cells/kg body weight of the recipient. GVHD may develop 3-6 weeks after a DLI, which may then require medical management. A DLI can also be used as an adjunct therapy for relapse of disease. In this case the DLI is given after chemotherapy induced remission. Additional long term follow-up procedures include bone marrow and lymph node aspirates to monitor for residual disease using molecular markers identified before the HCT. Chronic low grade GVHD is associated with longer survival in humans after HCT. For this reason, low grade GVHD is desirable as it infers the presence of the beneficial effect of graft versus tumor (GVT). Indeed, GVT is likely the most important benefit of an allogeneic HCT.

### **CONCLUSIONS**

To date, we have completed three allogeneic HCT on dogs with lymphoma. Two of these dogs had T cell lymphoma grade 5b, and one had B cell lymphoma grade 3a. All three dogs survived the transplant procedure with mild complications; however the B cell dog relapsed six months after the HCT and later succumbed to advanced lymphoma. She survived 20 months from the time of

initial diagnosis until death. Both of the T cell dogs continue to thrive, one now 3 years and 4 months since the original diagnosis, and the second 18 months since the original diagnosis. Neither of these two dogs needed a DLI, nor have they been on any transplant related medications.

All of the steps needed to perform a successful HCT are well described and repeatable. Additionally, many motivated pet owners are aware of the idea of a bone marrow stem cell transplant, and even though the costs and risks associated with this procedure are substantial, informed pet owners are capable of making the decision to pursue a transplant in the hope of achieving a long term treatment for lymphoma.

## REFERENCES

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## KEYWORDS

Bone Marrow, Autologous, Apheresis, Dog Leukocyte Antigen, Graft-versus-host disease, Graft-versus-tumor, Chimerism, Donor Lymphocyte Infusion.