Bone Marrow Transplantation for the Treatment of Canine Hematologic Malignancies

Steven Suter VMD PhD DACVIM (Oncology)
Director: Canine Bone Marrow Transplant Unit
North Carolina State University
Canine Lymphoma

Treatment:

L-asparaginase
CHOP
COPLA
AMC
UW
MOPP
DMAC
COAP

Hit the wall???

YES!
Canine Lymphoma

Bottom line: Canine lymphoma, while very treatable, remains an essentially incurable disease using current chemotherapy doses and dosing regimes.

Canine B-cell LSA: 12-16 months

Canine T-cell LSA (High-grade): 6-9 months
Canine Lymphoma

So what else is there??

- Autologous peripheral blood hematopoietic cell transplantation (autoPBHCT)
  - (auto=Self) cells are obtained from the patient

- Allogeneic peripheral blood hematopoietic cell transplantation (alloPBHCT)
  - (definition- “transported to its present position from elsewhere”) cells are obtained from a donor

This is crazy talk, right!?!?
Humans need approximately 12 trillion new peripheral blood cells (8 lineages) produced on a daily basis.

The source of these cells must be inexhaustible throughout life.

Hematopoietic stem cells—a self-renewing cell that can give rise to the multi-lineage clonal expansion of all peripheral blood cell lineages.
Human/Primate HPC

- BM CD34+ cells (1-5%) shown to reconstitute hematopoiesis in humans and primates undergoing autologous marrow reinfusion after myeloablative therapy

- CD34+ cells—phenotypically and functionally heterogeneous, so other antigens such as CD38, c-kit, HLA-DR have also been used
Canine CD34

-1996-Canine CD34 was cloned and mouse anti-canine CD34 monoclonals became available\(^1\)

-Comprised ~1-3% of total BM cells

-25 to 50-fold enriched for colony-forming units-granulocyte-macrophage when compared to unfractionated BM cells\(^2\)

-Autologous CD34+ BMT after marrow ablative TBI(920 cGy) resulted in prompt hematopoietic recovery in 3 dogs\(^2\)

Currently, the “cells of choice” for BMT remain CD34+ BM cells: they are reasonably plentiful, easy to isolate, have a long history of being able to reconstitute the immune system, and can be easily cultured \textit{ex vivo}.

Other sources of CD34+ cells include:

- Umbilical cord blood-highly enriched for CD34+ cells. Cord blood banking is routinely offered to all parents
- Peripheral blood
BMT 101

• BMT was revolutionized by 4 significant discoveries:
  
  • CD34+ progenitor cells are able to reconstitute all blood cell lineages after lethal marrow ablation
  • Peripheral blood contains a small number of CD34+ and this number can be dramatically increased using hematopoietic cytokines
  • Cloning and large-scale production of recombinant hematopoietic cytokines
  • Development of sophisticated continuous centrifugation cell separation machines
Stem cell transplantation was pioneered using bone marrow-derived stem cells by a team at the Fred Hutchinson Cancer Research Center starting in the early 1970s led by Drs. Thomas Donnall, Joseph Murray and Ranier Storb.

- Proved that whole bone marrow and/or CD34+ cells infused intravenously could repopulate the bone marrow and produce new blood cells.
- Work was recognized in 1990 with a Nobel Prize in Physiology and Medicine.
- 95% of all human BMT protocols were first perfected in dogs.

Not true!

Dr. Ranier Storb
Peripheral blood stem cells: CD34+

- PBSCs are readily collected by continuous-flow apheresis from patients and healthy donors after the administration of s.c. recombinant colony-stimulating factors with only minimal morbidity and discomfort.

- Autologous PBSC BMT in humans is routinely used for tx of chronic myelogenous leukemia (CML), acute leukemia, myelodysplasia, multiple myeloma, and non-Hodgkin’s LSA.

- Autologous transplantation for relapsed intermediate and high-grade NHL has clear survival advantages over chemotherapy-cure rates of 45%-60%.

- The use of PBSCs in leukemia, myeloma, non-Hodgkin’s lymphoma, and myelodysplasia has resulted in shorter times to neutrophil and platelet engraftment at the expense of increased rates of chronic graft-versus-host disease.
Apheresis (Greek: "to take away")

Uses:
- Platelet depletion/collection
- Plasma exchange
- RBC exchange/depletion
- WBC depletion/collection
- Granulocyte depletion/collection
BMT Justification

Dogs have been undergoing BMT in an experimental setting to treat lymphoma since the early 1970s:

And, CD34+ PBSC can be harvested from dogs and used to treat and/or cure dogs with lymphoma:

  25% long-term disease free survivors


Canine BMT

Leukaphoresis

Baxter-Fenwal apheresis machines kindly donated by the Mayo Clinic, Rochester, MN*

(2) TerumoBCT machines purchased for $18,000/each

(1) TerumoBCT Optia ($70,000)

*With many thanks to Dr. Nick Bandarenko MD, Head, Duke University Apheresis Unit and Dr. Jeffrey Winters MD, Head, Mayo Clinic Apheresis Unit
Canine BMT

Leukaphoresis

Spectra tubing kit

Buffy coat formation and cell collection
Canine BMT

Leukaphoresis

Initial leukaphoresis studies using a Baxter-Fenwal cell separator

- 2 unprimed (no G-CSF) normal mongrel dogs:
  - Both dogs tolerated procedure well
  - Propofol for jugular catheter placement, followed by dexmedetomidine CRI
  - Able to document CD34+ cell recovery
Calculations:
1. 56,340 WBC/ul x 50 mls = \(2.817 \times 10^9\) total cells harvested
2. 0.35% CD34+ cells x \(2.817 \times 10^9\) = \(9.859 \times 10^6\) total CD34+ cells
3. \(9.859 \times 10^6\) CD34+ cells/34 kg = \(~2.9 \times 10^5\) CD34+ cells/kg

Conclusion: Target dose of \(2 \times 10^6\) CD34+ cells/kg was not reached
Canine BMT

Leukaphoresis

- 6 Neupogen-primed normal mongrel dogs:
  - Able to monitor effects of Neupogen @ 5 ug/kg SQ BID x 6 days
  - Continued protocol refinement
  - CD34+ enumeration and transplant calculations
  - Harvest product storage
Canine BMT

Neupogen Priming Outcome

Total WBC

Days of Neupogen

DD= d6 AM double dose of Neupogen
PL= d6 3 PM post-leukopheresis

Ikester
Isabelle
Strawberry
Shortcake
Marcello
Marco
Canine BMT

Neupogen Priming Outcome

Neutrophils

DD = d6 7 AM double dose of Neupogen
PL = d6 3 PM post-leukaphoresis
Canine BMT

Neupogen Priming Outcome

Monocytes

Days of Neupogen

Monocytes/ul

0 500 1000 1500 2000 2500 3000 3500 4000 4500 5000

1 2 3 4 5 6 7

Pre DD PL

DD= d6 7AM double dose of Neupogen
PL= d6 3 PM post-lukaphoresis
Canine BMT

Neupogen Priming Outcome

Strawberry

*No adverse side-effects noted

**Pre-leukaphoresis CBC:**
- WBC 42.95 x 10^3/ul
- Hct 50
- Seg neuts 34.790 x 10^3/ul
- Lymphocytes 2.577 x 10^3/ul
- Monocytes 2.143 x 10^3/ul

*Leukaphoresis CBC:*
- WBC 199.30 x 10^3/ul
- Hct 27
- Seg Neuts 1.993 x 10^3/ul
- Lymphocytes 93.671 x 10^3/ul
- Monocytes 91.678 x 10^3/ul
Canine BMT

Neupogen Priming Outcome

Strawberry

Calculations:
1. $1.199.30 \text{ WBC/ul} \times 50 \text{ mls} = 9.965 \times 10^9$ total cells harvested
2. $5\% \text{ CD34+ cells} \times 9.965 \times 10^9 = 3.487 \times 10^8$ total CD34+ cells
3. $487 \times 10^8 \text{ CD34+ cells/kg} = \sim 1.94 \times 10^7 \text{ CD34+ cells/kg}$

Conclusion: Target dose of $2 \times 10^6 \text{ CD34+ cells/kg}^{1-3}$ was easily reached

Leukaphoresis product flow cytometric analysis:
Upper left quadrant are CD34+ cells

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Neupogen Priming Outcome
Shortcake*

<table>
<thead>
<tr>
<th>Pre-leukaphoresis CBC:</th>
<th>Leukaphoresis CBC:</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC 31.91 x 10^3/ul</td>
<td>WBC 150.74 x 10^3/ul</td>
</tr>
<tr>
<td>Hct 45</td>
<td>Hct 24</td>
</tr>
<tr>
<td>Seg neuts 27.762 x 10^3/ul</td>
<td>Seg Neuts 1.507 x 10^3/ul</td>
</tr>
<tr>
<td>Lymphocytes 1.276 x 10^3/ul</td>
<td>Lymphocytes 60.29 x 10^3/ul</td>
</tr>
<tr>
<td>Monocytes 1.276 x 10^3/ul</td>
<td>Monocytes 75.37 x 10^3/ul</td>
</tr>
</tbody>
</table>

*No adverse side-effects noted
Canine BMT

Neupogen Priming Outcome

Shortcake*

Leukaphoresis product flow cytometric analysis:
Upper left quadrant are CD34+ cells

Calculations:
1. 150.74 WBC/ul x 50 mls = 7.537 x 10^9 total cells harvested
2. 3.62% CD34+ cells x 7.537 x 10^9 = 2.728 x 10^8 total CD34+ cells
3. 2.728 x 10^8 CD34+ cells/21 kg = ~1.30 x 10^7 CD34+ cells/kg

Conclusion: Target dose of 2 x 10^6 CD34+ cells/kg was easily reached
Neupogen Priming Conclusions

- Normal dogs responded appropriately to “G-priming” with no adverse side effects.
- The Baxter-Fenwal apheresis machines were able to harvest canine mononuclear cells.
- “G-priming” led to a significant increase in the number of CD34+ cells harvested from peripheral blood.
- The number of CD34+ cells harvested was above the target dose of $2 \times 10^6$ cells/kg, making BMT a possibility.
Canine BMT

NC State Canine Bone Marrow Transplant Unit

Collection of Peripheral Blood CD34+ Progenitor Cells From Healthy Dogs and Dogs Diagnosed with Lymphoproliferative Diseases Using a Baxter-Fenwal CS-3000 Plus Blood Cell Separator


<table>
<thead>
<tr>
<th></th>
<th>WBCs (cells/μl)</th>
<th>Pits (cells/μl)</th>
<th>*Grans (%)</th>
<th>Hct (%)</th>
<th>*Mono (cells/μl)</th>
<th>CD34+%</th>
<th>CD34+ cells/kg</th>
<th>MCCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmobilized normal (n = 2)</td>
<td>45,879</td>
<td>5,187</td>
<td>1</td>
<td>21</td>
<td>41,345</td>
<td>0.40</td>
<td>3 x 10^5</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>56,340</td>
<td>8,520</td>
<td>3</td>
<td>32</td>
<td>46,879</td>
<td>0.35</td>
<td>5.3 x 10^3</td>
<td>19</td>
</tr>
<tr>
<td>Mobilized normal (n = 6)</td>
<td>99,020-297,440</td>
<td>3,726-8,520</td>
<td>1-5</td>
<td>24.5-38</td>
<td>92,089-282,568</td>
<td>1-4.3</td>
<td>4 x 10^5-2.5 x 10^7</td>
<td>27.5-77</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>214,858 ± 78,741</td>
<td>5,763 ± 1,624</td>
<td>2.33 ± 1.5</td>
<td>30.4 ± 6</td>
<td>195,384 ± 72,516</td>
<td>2.7 ± 1.4</td>
<td>1.1 x 10^7 ± 8.2 x 10^6</td>
<td>44 ± 17.8</td>
</tr>
<tr>
<td>Mobilized LPD (n = 11)</td>
<td>18,855-280,160</td>
<td>2,899-11,942</td>
<td>0-7</td>
<td>19-51.3</td>
<td>18,690-252,144</td>
<td>1.67-4.83</td>
<td>1.87 x 10^5-13.7 x 10^6</td>
<td>4-63.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>140,748 ± 89,396</td>
<td>5806.8 ± 3346.4</td>
<td>5.36 ± 7</td>
<td>35 ± 10</td>
<td>135,869 ± 68,535.2</td>
<td>2.4 ± 1.08</td>
<td>5.4 x 10^5 ± 3.25 x 10^6</td>
<td>25 ± 19.3</td>
</tr>
<tr>
<td>Average difference (95% CI, P-value) for mobilized normal versus mobilized LPD</td>
<td>(-25126.42 to 153274.90, P = .1406)</td>
<td>(-2632.85 to 2546.54, P = .0720)</td>
<td>(-7.92 to 1.86, P = .1955)</td>
<td>(-14.237 to 5.055, P = .3265)</td>
<td>(-22155.98 to 141187.31, P = .1337)</td>
<td>(-1.2392 to 1.7877, P = .6870)</td>
<td>(-3374773.98 to 14594167.92, P = .6682)</td>
<td>(-39.3826 to 1.6742, P = .0682)</td>
</tr>
</tbody>
</table>

*One dog underwent apheresis twice

![Graph](image1)

![Graph](image2)
NC State Canine Bone Marrow Transplant Unit

- Opened in October, 2008 after construction of a small, 2 run semi-isolation room in our old hospital, BMT SOP VTH board approval, fiscal responsibility approval (ie, how much money will we make), nursing coordinator approval (ie, we are chronically short-staffed and have no money to hire more technicians), legal dept. approval (ie, no client can ever sue us, ever!!), client consent form generation and approval, and ~4,000 other meetings over a period of 1.5 years.

- The BMT service was fully integrated into the NCSU VTH with Medical Oncology residents, other house officers, and 4th year student participation. The BMT Unit also supported a full time BMT nurse and trained 2 veterinarians to perform apheresis and canine BMT via a one-year BMT Fellowship.

http://www.cvm.ncsu.edu/vth/clinical_services/onco/BoneMarrowTransplant.html
Canine BMT

NC State Canine BMT Experience

- Performed 73 autologous and 5 allogeneic transplants of dogs with lymphoid malignancies:
  - High-dose cytoxan (750 mg/m² IV) to “clear” cancer from the peripheral blood 2 weeks before BMT at rDVM
  - G-CSF (Neupogen, 5 ug/kg SQ BID) starting ~14 days later to drive CD34+ progenitor cells from the bone marrow-given for 5 days-Arrive at NCSU on Thursday
  - Elspar @ 400 IU/kg SQ on Saturday
  - Day 5 (Monday) of Neupogen (>30,000 WBC/ul): double dose at 7AM followed by leukapheresis starting within 2 hrs
Canine BMT

NC State Canine BMT Experience

- Leukapheresis: TerumoBCT Spectra
- Goal is to apherese at least 2 -3 total blood volumes
  - 25 kg dog=~2,250 mls blood (90mls/kg BW)=6,750 mls through machine
  - Procedure takes ~ 3-5 hrs (1,500 mls/hr), depending on BW
  - 12g 12 F double-lumen Arrow jugular hemodialysis catheter
  - Propofol sedation for jugular catheter placement and 2 lateral saphenous catheters for Ca gluc infusion and ionized Ca monitoring (q ½ hr.)
  - Dexmedetomidine CRI for mild sedation throughout procedure
  - Target: 2 x 10⁶/kg CD34+ cells

*Average specific gravity of cell type shown
Leukapheresis
Canine BMT

NC State Canine BMT Experience

- Harvested cells refrigerated and/or frozen
- Next day:
  - Total body radiation: 10-12 Gy @7cGy/min
  - Lethal to >95% of dogs without BMT
- Infusion of harvested cells immediately after TBI (BMT)
- Frozen portion of harvest stored at -80° indefinitely in case of relapse
Canine BMT

Varian Novalis Linear Accelerator
NC State Canine BMT Experience

– Post-transplant Care:
  • Essentially supportive:
    Antibiotics, anti-emetics, anti-diarrheals, fluids
  • TBI complications (all temporary):
    Diarrhea at day 3-5
    Neutrophil nadir (0/ul) at day 5-7 (when <1,000/ul dogs go into isolation ward)
    < 1,000/ul dogs go into an isolation suite)
    Platelet nadir (0/ul) at day 10-12 (when <10,000/ul dogs go into ICU)
  • Daily monitoring (CBC, serum chems) to assess engraftment-
    when neutrophils >1,000/ul for 3 days and platelets ~20k/ul
    time to go home-usually 2 weeks post-BMT
Canine BMT

Terry Center

Isolation Rooms
Canine BMT

NC State Canine BMT Experience

Total White Blood cells

Days of Neupogen

WBC/ul

Tina
Cody
Molly
Scooby
Dusty
Madison

DD= d6 7AM double dose of Neupogen
PL= d6 3 PM post-leukaphoresis
Canine BMT

NC State Canine BMT Experience

Neutrophils

DD= d6 7AM double dose of Neupogen
PL= d6 3 PM post-leukaphoresis
Canine BMT

NC State Canine BMT Experience

Monocytes

DD = d6 7AM double dose of Neupogen
PL = d6 3 PM post-leukaphoresis
Canine BMT

NC State Canine BMT Experience

– Tina Ottworth

- 11/16: WBC 24,970, Monos 2,340
- 11/17: WBC 39,970, Monos 1,376
- 11/18: WBC 39,270, Monos 1,571

OK for leukophoresis

Mike, Kate, and Tina Ottworth
Canine BMT

NC State Canine BMT Experience

Leukaphoresis procedure

Dexmedetomidine infusion

10% Ca Gluc infusion

i-Stat

Harvested mononuclear cells
NC State Canine BMT Experience

- Harvest Calculations:
  - 11/18: WBC 21.23 x 10^3/ul x 50 ml = 1.062 x 10^9
  - 1.59% CD34+ x 1.062 x 10^9 = 1.6 x 10^7 total CD34+ cells
  - 1.6 x 10^7/19.6 kg = 8.9 x 10^5/kg
  - 11/19: WBC 72,420, Monos 5,069
  - WBC 16.43 x 10^3/ul x 50 ml = 8.215 x 10^8
  - 1.34% CD34+ x 8.215 x 10^8 = 1.1 x 10^7 total CD34+ cells
  - 1.1 x 10^7/19.6 kg = 5.8 x 10^5/kg
  - Combined harvests: ~1.5 x 10^6 CD34+ cells/kg
Canine BMT

NC State Canine BMT Experience

Total body irradiation

2-5Gy fractions @7cGy/min with a 3 hr interfraction interval
Canine BMT

NC State Canine BMT Experience

Harvest infusion

Cells brought to room temperature and infused via the jugular catheter over ~30 m
Canine BMT

NC State Canine BMT Experience

Happy Thanksgiving
My friend
No help from the turkey
Pondering the big escape!

Thanksgiving Day

Ready to go home
NC State Canine BMT Experience

—Cody McGrath: 4YO MC Golden

- Dx stage IVa B-cell LSA 7/24/08
- In CR with induction therapy and maintained 1\textsuperscript{st} CR until BMT
- BMT on 2/20/09
Canine BMT

NC State Canine BMT Experience

Leukapheresis
Canine BMT

NC State Canine BMT Experience

—Harvest calculations:

• $94.53 \times 10^3/\text{ul} \times 50 \text{ ml} = 4.726 \times 10^9$ total cells
• 3.0% CD34+ cells $= 1.418 \times 10^8$ CD34+ cells
• $1.418 \times 10^8$ CD34+ cells/36kg $= 3.9 \times 10^6$ CD34+ cells/kg
• Target dose of $2 \times 10^6$ CD34+ cells/kg reached

Harvest CBC:
Neuts 2,836/ul
Lymphs 58,509/ul
Monos 33,086/ul
Hct 27

3.0% CD34+
Canine BMT

TBI/BMT
Canine BMT

Heading home

3/10/09-Going home

Cody 11/14/10
Canine BMT

Hematologic Changes After Total Body Irradiation and Autologous Transplantation of Hematopoietic Peripheral Blood Progenitor Cells in Dogs With Lymphoma

C. Escobar, C. Grindem, J. A. Neel, and S. E. Suter


Figure 1. Box and whisker plots of red blood cell (a), neutrophil (b), lymphocyte (c), and platelet (d) dyscrasias for 18 days after lethal total body irradiation, followed by peripheral blood CD3+ progenitor cell transplantation.
Canine BMT

Canine BMT-B cell LSA (24 dogs)

- >2 x 10^6 CD34+ cells/kg harvested in all dogs- 23/24 dogs tolerated apheresis
- 87.5% (21/24) engrafted appropriately
- 12.5% (3/21) experienced TRM (treatment related mortality)
  - 1 dog died of anuric renal failure 9 days after BMT and was euthanized (only had 1 kidney)
  - 1 dog vomited during apheresis, developed tachycarida and a fever, and went into cardiac arrest 9 days after BMT
  - 1 dog died at rDVM with apparent incomplete engraftment

Canine BMT-B cell LSA (24 dogs)

No relapse: Median DFI 456d post BMT
Relapse: Median DFI 52d post BMT

No relapse: Median OS 531d post BMT
Relapse: Median OS 231d post BMT

Relapse clearly affected median DFI (statistically significant) and median OS (approached significance).
33% of transplanted dogs remain cured of their disease (alive >2yrs post-BMT).

Canine BMT-T cell LSA (15 dogs)

- >2 x 10^6 CD34+ cells/kg harvested in all dogs—all dogs tolerated apheresis
- 87% (13/15) engrafted appropriately
- 13% (2/15) died in the hospital
  - 1 dog developed sepsis and died 13d post-BMT
  - 1 dog, who was not in remission upon arrival at NCSU, received doxorubicin (40mg/m2) and melphalan (30mg/m2) followed by neupogen and Mozibil one week later. After apheresis and BMT, the dog died of severe GI toxicity 4d post-BMT

Canine BMT

Canine BMT-T cell LSA (15 dogs)

- Median DFI 184d
- Median OS 239.5d

184d vs 456d
239.5d vs 531d

15% (2/15) of transplanted dogs remain cured of their disease (alive >2yrs post-BMT).

Canine BMT

Canine BMT-LGL leukemia

– Zeke: 3YO 10kg MC Cavalier king charles
  • Dx with a rare acute LGL leukemia of splenic origin by rDVM
  • Underwent induction CHOP chemotherapy while an appropriate donor was located
  • DLA-matching performed at FHCRC

MHCII VNTR analysis

Canine BMT

Canine BMT-LGL leukemia

DLA-88 & DRB1 sequence identities

Canine BMT

Canine BMT-LGL leukemia

Zeke remains disease free now >5yrs post-BMT
NC State Canine BMT Experience

The BIG question:

- Can we cure dogs with lymphoma with autologous PBSCT and, if so, what will the cure rate be??
- Human cure rates are ~40%-55%
- Very old veterinary literature describes an ~30% cure rate
- Anything better than the standard chemotherapy protocols in use now (~5% cure rate?) would be an improvement

Molly Goodwin
The other BIG question:

- How much does this cost?
  - $13,000-$18,000 if Neupogen is supplied by client
  - $17,000-$22,000 if NC State supplies Neupogen
  - Includes leukaphoresis, TBI, BMT, all drugs, 3 week hospital stay
  - Does not include blood products (if needed), intensive ICU care (if needed), additional diagnostics
  - Duke: ~$60,000-$75,000 for BMT procedure alone
NC State Canine BMT Experience

- The tough question for clients:
  - Should I spend upwards of $8,000-$12,000 over the period of a year and a half on a protocol that is essentially palliative with an extremely low cure rate or should I spend ~$17,000-$22,000 on a protocol that may have an ~ 35-50% cure rate (B-cell LSA)?

Sadie Phares

http://www.cvm.ncsu.edu/vth/clinical_services/onco/BoneMarrowTransplant.html
Canine BMT

Acknowledgements

– Dean Arden, NCSU
– Dr. Ed Sullivan, Bellingham Veterinary & Critical Care, Seattle WA
– Dr. Ranier Storb, Fred Hutchinson Cancer Research Center, Seattle WA
– Dr. Nick Bandarenko, Duke University, NC
– Dr. Jeffrey Winters, Mayo Clinic, Rochester, MN
– NCSU CVM Faculty and staff
– BMT dog owners—Truly my heroes!
Canine BMT

QUESTIONS?

Dr. Suter
Break