IN UTERO HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CANINES: THE GESTATIONAL WINDOW OF OPPORTUNITY TO MAXIMIZE ENGRAFTMENT

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Introduction

- **In Utero Hematopoietic Stem Cell Transplantation (IUHSCT)** is a promising therapy that may circumvent many of the morbidities and limitations of postnatal BMT.
Introduction

- Candidate diseases for human application of IUHSCT include
  - Hemoglobinopathies
  - Granulocyte disorders
  - Leukodystrophies
  - Storage disorders
Theoretical Benefits of IUHSCT

- Avoid ablative therapy required for donor cell expansion in postnatal HSCT
- GVHD is less likely to occur
- Avoid the need for a HLA-matched donor
- Low-level engraftment may be curative or allow same-donor postnatal HSCT by induction of host tolerance
- Avoid organ damage if performed early enough
Previous IUHSCT in Humans

- The only clear successes to date have been with immunodeficiencies (X-linked SCID)
- The major problem has been lack of engraftment
In Utero Bone Marrow Transplantation

Pre-Clinical  Clinical
Our overall objective is to increase our understanding of the parameters involved in safe and successful \textit{in utero} BMT in an animal model which will be relevant to the human.
Our long term goal is to establish a therapeutic protocol to cure congenital hemoglobinopathies and metabolic disorders such as congenital leukodystrophies.
Canine Model

- We have developed a model of IUHSCT in fetal canines which, similar to humans, develop immunocompetency prior to birth, in order to investigate the roles of donor cell dosing and gestational age in engraftment and GVHD.
Benefits of Canine Model

- Relatively short gestational length compared to other animal models
- Litter size allows multiple experiments to be performed within the same gestation
- Uterus is tolerant of surgical manipulation and few obstetrical complications
- Canines have many analogous medical conditions for future treatment trials
Benefits of Canine Model

- Availability of:
  - Canine DNA markers for engraftment analyses
  - Canine CD34 antibody
  - Hematopoietic stem cells
  - Canine CD3 and CD5 antibodies
  - T cells
Background

- The development of the canine fetal immune system shares similarities with humans that are relevant to a preclinical model of IUHSCT.
Canine Immune Development

- Unlike rodents, dogs appear to be immunologically mature at birth.
- The canine fetus exhibits bacteriophage recognition at 40 gestational days (term day 63).
- Peripheralization of lymphocytes begins to occur between day 45-50.
Canine Immune Development

- Thymectomy of the 48 day gestational dog produces deficient humoral antibody and cellular hypersensitivity response.
- The earliest detectable antibody production occurs at ~day 56.
Background

- Previous work by others have proposed a gestational age “window of opportunity” which permits successful engraftment and avoidance of GVHD following IUHSCT.
  a). Early enough to avoid graft rejection
  b). Late enough to minimize competition from highly proliferative fetal host cells
The Concept of a Window of Opportunity

a). The “window of opportunity” for IUHSCT should precede the establishment of host immunologic competence.

b). IUHSCT too early might be less successful during the rapid cellular expansion of fetal hematopoietic cells.
Objective

- To investigate the optimal time in gestation for IUHSCT in a canine model.
Study Design

- Eight bred animal pairs were procured for IUHSCT at post-fertilization day 31-50.
Study design: Donor HSC Preparation

- Paternal canine bone marrow harvested
- Mononuclear cells isolated
  - $CD34^+$ selection with Miltenyi magnetic bead system (1H6 clone)
  - or
    - Entire ficoll fraction used
- Flow cytometry for CD34 cell purity (2E9 clone)
- Flow cytometry for CD3/CD5 percentages
Study design: Donor T cell Preparation

- Paternal canine peripheral blood harvested
- Mononuclear cells isolated
- CD3$^+$ selection with Miltenyi magnetic bead system
- Flow cytometry to determine cell purity using CD3 and CD5 antibodies
Study design: *In Utero* HSCT procedure

- Sonography to verify gestational age
- General anesthesia
- Laparotomy to expose the uterus
- Intraperitoneal fetal injection under ultrasound guidance
  - HSCs and T cells (recipients)
  - Ethiodol dye (controls)
- Close abdomen after checking fetal viability
Study design:  
Postnatal Studies for Engraftment

- DNA Engraftment Analyses
  - TaqMan canine SRY for female recipients
  - Unique microsatellite loci using capillary electrophoresis for both sexes
Study design:
Tissues examined

- Blood
- Bone Marrow
- Liver
- Spleen
- Thymus
- Brain
- Kidney
- Heart
- Skin
- Large Intestine
- Tongue
Results

Term survival 77% (36/47); total of 22 recipient animals.

<table>
<thead>
<tr>
<th>Days @Tx</th>
<th>CD34 (10E9) cells/kg</th>
<th>T-cell (10E9) cells/kg</th>
<th>Max Engraftment Blood/BM %</th>
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</thead>
<tbody>
<tr>
<td>31</td>
<td>3.2</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>3.4</td>
<td>1.1</td>
<td>0.53</td>
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<tr>
<td>36</td>
<td>2.5</td>
<td>0.63</td>
<td>FDIU @ IUHSCT</td>
</tr>
<tr>
<td>38</td>
<td>1.1</td>
<td>0.65</td>
<td>3</td>
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<tr>
<td>42</td>
<td>0.35</td>
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<td>0.11</td>
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<td>0.09</td>
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<tr>
<td>50</td>
<td>0.24</td>
<td>0.19</td>
<td>0.51</td>
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</tbody>
</table>
Gestational Day at IUHSCT

% Engraftment hematopoietic tissues
Days gestation at transplant

Engraft-Blood

Engraft-BM
Gestational Day at IUHSCT

% Engraftment of hematopoietic tissues
Conclusions

- Fetal age at time of injection of paternally derived HSCs may play a key role in achieving engraftment in our canine model of IUHSCT.
Conclusions

- A gestational age window at ~42 days may permit maximal engraftment opportunity without GVHD in the fetal canine.
Our work supports the concept that

- At early gestational ages, rapid proliferation of fetal hematopoietic cells may prohibit engraftment.

- At later gestational ages, maturation of the fetal immunologic response may lead to graft rejection.
A window in gestation in which host cellular proliferation is slowing yet spaces for donor cells to occupy are still available may prescribe the optimal time to perform IUHSCT.
Future Directions

Future studies will focus on cell dosaging within the gestational age window of 39-45 days to determine parameters for maximal engraftment in this mammalian model of IUHSCT.
Future Directions

- Incremental adjustments in CD34+ and T cell dosages.
- Long term engraftment studies.
- Investigate parameters that achieve GVHD in the canine model.
Future Directions

- Test the therapeutic potential of engraftment achieved through IUHSCT in canine models of human disorders.
- Studies in animal models such as the canine will provide a framework for advancing IUHSCT towards human application.
In Utero Bone Marrow Transplantation

Pre-Clinical → Clinical
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