Children's Hospital of Philadelphia

Annual Progress Report: 2007 Nonformula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Nonformula Grant Overview

The Children's Hospital of Philadelphia received $2,110,362 in nonformula funds for the grant award period June 1, 2008 through May 31, 2012. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Prenatal Stem Cell Therapy for Sickle Cell Disease - We are proposing a new and novel approach to cure Sickle Cell Disease. In utero hematopoietic stem cell transplantation is the transplantation of blood forming stem cells into the fetus. Because of unique aspects of fetal biology, these cells can engraft and produce a fraction of the blood cells in the recipient. While this fraction is too small to treat Sickle Cell Disease, it results in permanent immunologic tolerance for the specific donor. This tolerance allows a non-toxic bone marrow transplant from the same donor to be performed after birth, resulting in high enough levels of donor cells to cure Sickle Cell Disease. If the aims of this proposal are achieved, it will lead to clinical trials that may change the way Sickle Cell Disease is currently treated, and will provide a non-toxic therapy that will prevent children from ever experiencing the manifestations of this disease.

Anticipated Duration of Project

6/1/2008 - 5/31/2012

Project Overview

In utero hematopoietic stem cell transplantation (IUHCT) is a method of achieving allogeneic mixed hematopoietic chimerism with associated donor specific tolerance without myeloablation or immunosuppression. Individuals made tolerant by IUHCT can undergo a minimal conditioning postnatal hematopoietic stem cell transplant (HSCT) from the same donor without toxicity and improvement of donor cell chimerism to levels that are potentially therapeutic for Sickle Cell Disease (SCD). The broad objective of this proposal is to develop the strategy of IUHCT combined with minimal conditioning postnatal HSCT to cure patients that are prenatally diagnosed with Sickle Cell Disease. The Specific Aims of this proposal are:

Specific Aim #1. To perform pre-clinical studies in the canine model designed to optimize the safety of the IUHCT followed by postnatal minimal conditioning HSCT strategy. Further studies will be performed in the established pre-clinical canine model to confirm the optimal T-cell dose

for facilitation of engraftment with minimal risk of GVHD. These studies are a priority and will be completed prior to initiation of the Phase I clinical trial.

Specific Aim #2. To experimentally test novel strategies to enhance engraftment and donor specific tolerance after IUHCT. We have developed a protocol in the canine model that can achieve levels of donor cell chimerism that would be anticipated to be curative for SCD patients. However, additional strategies designed to improve the consistency of tolerance induction that are translatable to clinical application will be evaluated.

Specific Aim #3. To establish a model of community based participatory research inclusive of the SCD and African American community and to lay the groundwork for a future clinical trial of IUHCT for treatment of SCD. This aim will be coordinated through the Sickle Cell Center at the Children's Hospital of Philadelphia, Cheyney University, and the Philadelphia/Delaware Valley Chapter of the Sickle Cell Disease Association of America. It will include: 1) a social awareness program designed for education of African American couples and others at risk for an affected child with SCD for assessment of their views and opinions regarding a trial of this therapy and 2) research training of undergraduate students and mentoring of junior faculty from Cheyney University through direct involvement in the scientific and social components of this proposal.

Principal Investigator

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Other Participating Researchers

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Adedoyin Adeyiga, PhD - employed by Cheyney University
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Expected Research Outcomes and Benefits

Sickle Cell Disease (SCD) is a major cause of morbidity, mortality, shortened life-span, and health care costs among African Americans. It is the most common genetic disorder among African Americans with 1 in 8 African Americans carrying a mutation and over 50,000 homozygous, affected individuals in the US alone. On a worldwide basis, hemoglobinopathies, i.e., SCD and Thalassemia are among the most common of all genetic disorders causing untold human suffering and cost to society. At present there is no curative therapy for SCD that can be widely applied. While hematopoietic stem cell transplantation (HSCT) is curative, the morbidity and mortality of standard HSCT, except in the few individuals with a sibling matched donor, is

prohibitive. The goal of this proposal, if successful, would be the development of a therapy that would result in the complete correction of SCD in prenatally diagnosed patients. Because of treatment early in life, affected children and adults would never experience the manifestations of the disease and would be expected to have a normal life span without the sequelae of sickling red cells and the associated complications related to microvascular occlusion. Moreover, the treatment would be anticipated to be non-toxic with few short or long-term complications allowing the beneficiaries of the treatment to live normal lives. The approach could be applied to most patients born with SCD in this country and in many areas of the world if population screening for carrier status were instituted and carrier couples underwent early prenatal diagnosis on all pregnancies. Currently, population screening for SCD is incomplete, and early prenatal diagnosis has not been embraced by the African American community due to lack of a therapeutic endpoint. We would predict if this therapy is proven successful, that African American attitudes toward these procedures would change, with institution of widespread prenatal diagnosis for couples at risk. Finally, if successful for SCD, the same approach would be predicted to be applicable to many other disorders, including Thalassemia and many immunodeficiency diseases.

Summary of Research Completed

Specific Aim #1. To perform pre-clinical studies in the canine model designed to optimize the safety of the IUHCT followed by postnatal minimal conditioning HSCT strategy.
Over the past year The researchers have been focused on optimization of the intravascular injection (intracardiac) strategy to achieve consistent and reproducible macrochimerism in recipient dogs after IUHCT. The research team's early experience with this approach was presented in the last Progress Report and was promising with 3 animals demonstrating much higher levels of engraftment than had previously observed with the intraperitoneal approach. Interestingly, 2 of these animals were injected relatively late in gestation (43 days). This led the researchers to the conclusion that the window of opportunity for prenatal engraftment might extend later than originally suggested by the team's work examining the ontogeny of immune development in the canine fetus. In addition, the researchers learned early in this experience that early gestational injections (33 – 38 days) were technically challenging with inconsistent success, and higher mortality of the pups. The researchers have over the past year, systematically investigated later gestational injections with more consistent delivery of higher doses of cells. Because the pups at later gestational time points are significantly larger, the team modified the cell preparation from a CD34 enrichment protocol, to a CD3 depletion protocol. This significantly improves the yield of the cell preparations with no loss of CD34+ cells. The researchers' experience over the past year is detailed in Table 1. Overall, the entire litters of seven dams have undergone IUHCT by intracardiac injection totaling 33 pups injected. One litter (4 pups) was lost on the second post operative day to chorioamnionitis. Of the remaining 29 pups, 20 were born alive (69%). One pup that was macrochimeric after birth (6%) died as a complication of the busulfan boosting regimen. The other 19 animals have survived for long term analysis. Of the 19, 6 pups (2 litters) were transplanted prior to 40 days and only one of six was chimeric by VNTR analysis (sensitivity > 2.5%). The remaining 13 were injected at 40 days or later and 12/13 demonstrated macrochimerism after birth with levels of chimerism ranging from 2.8% to 23.6%. If the 2/2 macrochimeric pups from a litter injected at 43 days last year (see

last years progress report) is included, 14/15 pups undergoing IUHCT at 40 days gestation or later by intracardiac injection are macrochimeric with an average level of chimerism of 8.43%. This is in marked contrast to the previous experience with intraperitoneal injections where the average level of chimerism was under 1% (detected in CLAD dogs by the CD18 antigen and pcr for male sry) and the frequency of detectable chimerism was less than 50%. The ability to reproducibly achieve macrochimerism in nearly every recipient at sufficient levels to induce associated tolerance represents an accomplishment of a major milestone toward clinical application of this approach and was the primary goal of this proposal. A second goal was to demonstrate stability of engraftment after IUHCT and/or boosting. The researchers have continued to follow a cohort of animals that have received IUHCT with or without boosting and now have data extending for as long as 3 years in some animals (Figure 1). The research team is pleased to report that the animals have remained healthy, and have maintained their levels of chimerism (with increases over time in some cases). In no instance have any of the chimeric pups lost chimerism after it was established. It should be noted that all of the animals in the long-term group received intraperitoneal transplants and had initial chimerism levels of < 2% prior to postnatal boosting. The more recent cohort of intracardiac transplants are still relatively young and the researchers will continue to follow them to assess long-term engraftment. This is also a major finding with respect to clinical application. In addition, we have also been tracking the relationship of chimerism to the ability to "boost" engraftment. As the researchers would expect from our experience in the murine system, all of the animals with documented macrochimerism after IUHCT alone, that the team has attempted to boost postnatally by a minimal conditioning BMT, have been "boostable" with either maintenance or significant increases in their levels of chimerism. The increase in engraftment after boosting has been highly variable in the canine model and there has been some unexpected toxicity from the single dose Busulfan regimen with demise of one animal from hepatic toxicity. This may be due to variable pharmacokinetics of Busulfan in dogs. The researchers are in the midst of changing our protocol to Melphalan conditioning, which is known to provide much more consistent blood levels with standard dosing, and is equally effective as a conditioning agent. The research team does not plan to boost the current cohort of dogs to assess long-term maintenance of chimerism, but will generate a cohort to test the new conditioning regimen. Finally, the researchers have been assessing the threshold for GVHD in the canine system. Interestingly, the researchers have not seen any GVHD in the surviving animals despite delivery of as high as 5×10^9 CD3+ cells/kg fetal wt. This suggests that there may be regulatory activity generated after IUHCT that suppresses GVHD in the recipients. This is another important observation, supporting the safety of IUHCT for clinical application.

Specific Aim #2. To experimentally test novel strategies to enhance engraftment and donor specific tolerance after IUHCT.

Dr. Edwin Horwitz's group has completed work toward the generation of a transplantable population of canine mesenchymal stromal cells (MSC) to facilitate the performance of Experiment #2a. The researchers have elected not to perform further injections of these cells until a cohort of chimeric animals have been produced as a baseline for the facilitation studies. As discussed above, the researchers now have this cohort and can begin to test whether cotransplantation of MSC can improve levels of hematopoietic engraftment after IUHCT.

Experiment 2b Design of an in situ gelation system for sustained cell delivery
Dr. Levy and Chorny have not pursued this strategy for maintaining injected cells at the site of delivery further in the past year. In last year's Progress Report the research team described the problems encountered with this technology in the murine model and the need to test the approach in dogs once our intravascular injection model is optimized.

Experiment 2c To selectively enhance the competitive capacity of the donor cell population
Over the past year Dr. Brazelton has continued his work in the optimized in vitro culture system. In this system, enriched HSC are cultured in hypoxic conditions with factors that may increase donor cell competitive capacity by a number of established pathways (Wnt, HoxB4, SCF, SDF-1a, VEGF-A, Ang-1, PGE2, diprotin A, SB21) involving stem cell proliferation or homing interactions. Once the dose responses for these factors have been determined by a number of criteria including assessment of target gene response the optimal dose can be assessed by incubation of donor cells and in vivo transplantation. Over the past year, in vivo studies of pre-incubation of donor HSC with each of these factors at the predetermined optimal dosage have been investigated in the murine model. Each factor has been assessed for impact on homing, short term engraftment, and long term (> 4 months) engraftment. The results of these studies over the past year have been somewhat disappointing. Of the factors investigated, only culture in hypoxia alone and Wnt5a have demonstrated improved short term engraftment over baseline. The researchers are currently awaiting data on whether this benefit to short term (2 month) engraftment will be sustained long term (4 month).

Specific Aim #3. To establish a model of community based participatory research inclusive of the SCD and African American community and to lay the groundwork for a future clinical trial of IUHCT for treatment of SCD.

The second year of the The Applied Biological Methods course was completed and the third year has been initiated as designed in our original proposal in collaboration with Cheyney University. In the past year (third year of the course) 6 Cheyney undergraduates were identified and completed an 8 week long, 4 hr/week, hands on didactic training course at the Children's Hospital of Philadelphia (CHOP) on basic biological research methods. These courses are held in Dr. Flake's laboratory and were instructed by Dr. Brazelton and other laboratory personnel. Of the 7 participants in the second year, 3 wished to participate in the Summer internship program and 3 students were selected and completed the internship this year. Of this year's students in the didactic course, 2 students were recommended by Dr. Adeyiga for the summer internship and accepted offered positions. One of these students did not show up to perform the internship and the other completed the internship successfully. This year there were no applicants for the Faculty Summer Research Sabbatical at CHOP.

Sub Aim #3a. Social awareness program designed for education of African American couples and others at risk for an effected child with SCD for assessment of their views and opinions regarding a trial of this therapy. This cooperative effort headed by Dr. Ohene Frempong between the CHOP Sickle Cell Center, the Delaware Valley Chapter of the Sickle Cell Disease Association, and Cheyney University has been initiated. Questionnaires have been designed and and pilot surveys have been performed related to African American attitudes toward prenatal treatment of SCD. The PI is awaiting feedback on these efforts from Dr. Ohene-Frempong and

his associates.

Table 1. Summary of Canine IUHCT Experiments 6/30/10 – 6/30/11

Mom	# cells/kg per pup	CD34 cells/kg	CD3 cells/kg	Enrichment	GA	#Injected	# Live Births	Puppy	Gender	Dye	Outcome	Chimerism post IUHCT	Chimerism post boost
Symphony	1.18 x 10^11	5.06x10^9	1.03x10^9	CD3 depletion	38	(6/6)	3	Mozart	Female	n/a	boosted	0	N
							3	Brahms	Female	n/a	boosted	0	LOST
							3	Beethoven	Male	n/a	alive, on protocol	0	n/a
Snuffleupha	1.15 x 10^11	2.86x10^9	1.10x10^9	CD3 depletion	33.7	(7/7)	3	Bert	Female	n/a	alive, on protocol	0	n/a
							3	Ernie	Female	n/a	alive, on protocol	0	n/a
							3	Oscar	Male	n/a	boosted	2.39	Y
Hollywood	2.98 x 10^11	5.82x10^9	2.72x10^9	CD3 depletion	35.5	(1/1)	1	Neo	Female	n/a	boosted	5.2	Y
Voodoo	3.99 x 10^10	1.07x10^9	4.07x10^8	CD3 depletion	40.6	(5/5)	4	Maverick	Male	n/a	died shortly after	5.93	n/a
							4	Ice	Female	n/a	alive, on protocol	10	n/a
							4	Goose	Female	n/a	alive, on protocol	6.16	n/a
							4	Jester	Female	n/a	boosted	6.35	Y
Chanel	8.22x10^9	3.5x10^8	6.4x10^7	CD3 depletion	45	(4/4)	0				All aborted POD 3 2/2 chorioamnionitis		
Minnow	5.23x10^10	1.73x10^9	3.71x10^8	CD3 depletion	40	(4/4)	3	Gilligan	Male	n/a	alive, on protocol	23.6	n/a
							3	Skipper	Male	n/a	alive, on protocol	16.9	n/a
							3	Ginger	Female	n/a	alive, on protocol	2.84	n/a
Vinnie	4.6x10^10	9.05x10^8	3.95x10^8	CD3 depletion	40.5	(6/6)	6	Picasso	Male	n/a	alive, on protocol	5.29	n/a
							6	Michaelangel	Male	n/a	alive, on protocol	3.53	n/a
							6	Georgia	Female	n/a	alive, on protocol	15	n/a
							6	Frida	Female	n/a	alive, on protocol	3.75	n/a
							6	Monet	Female	n/a	alive, on protocol	6.5	n/a
							6	Matisse	Female	n/a	alive, on protocol	0	n/a
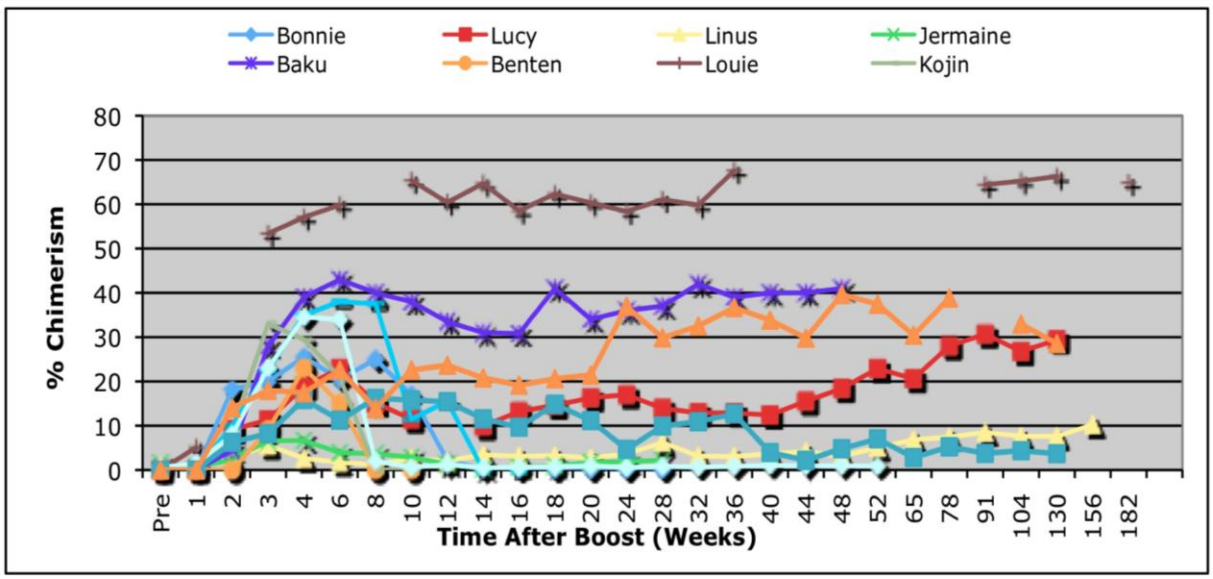
Figure 1. Long-term maintenance of chimerism after IUHCT