New World Primate in Columbia: These are the animals selected for this International NTT project.

Date: December 1, 2005

To: NTT International Project Team

From: John W. McDonald III, Director and Vice President

Re: **NTT International Project**

Primary Accomplishments:

- **Lab Transition to KKI/JHH:** Construction of the research laboratory was completed, and equipment procured. Lab is fully operational.

- **Staff Support:** As mentioned previously, Carol Velandia, Columbian, is with Johns Hopkins International and she has joined the team in helping administrate the Spanish side of communications to our Columbian. Dr. Martin Oudega joined the International Center for Spinal Cord Injury from the Miami Project to Cure Paralysis as Associate Professor in Neurology. Martin will be leading the injury and behavioral core at ICSCI and will co-direct the NNT International Project.

- **Post Doc Trip to Columbia**: Drs. Visar Belegu and Andres Hurtado (Columbian, joining us from the Miami Project also) traveled to Bogotá and the Amazon in August to begin the primate collaboration with Dr. Patarroyo as well as the ES cell derivation experiments with Dr. Lucena. Important variables were evaluated during this trip and analysis has presented us with the additional requirements for the project in terms of monetary support (see below). We have imaged the spine and spinal cord of one of the New World monkeys, and have completed the tissue processing for immunoanalysis. The anatomy of the cord and it structures is similar to that of humans. We have purchased important equipment for creating ES cells in Columbia (electroporator, perfusion pump, thin sectioning equipment). In summary, this trip's conclusion was that the primate facility in Columbia was feasible for our project.

- **The Investigator Meeting**: We have completed the investigator meeting in Columbia. The leadership team met in Bogotá and traveled to the Amazon to Leticia. This is the site of the primate facility. The trip was amazingly successful and all facilities are in position to move forward. Building a surgical facility is required and could be completed in less than 2 months. In addition to the Amazon primate facility, we will collaborate with two major scientific institutes in Bogotá.

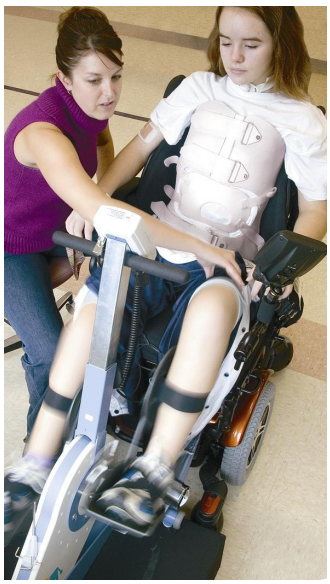
- **International Partnership**: We are creating an international partnership to best accomplish the two goals in this project. Dr. Lucena is centered in Bogotá, Columbia and is an international expert in *in vitro* fertilization and is currently working with ES cells. Dr. Patarroyo has his lab in the Amazon and works primarily with primates. He is internationally recognized for his development of malarial vaccine. We have added governmental leadership in Columbia, Senator

Clopatofsky, who lives personally with spinal cord injury, and is a strong advocate of our project. We will need to add additional South American Scientists to this team. We are evaluating several surgical partners and primate behaviorist partners to complete the team. We continue to have close links with the South Korean's and are utilizing their technology.

- **International Center for Spinal Cord Injury**: The center, opened in June 2005, has a vision and mission that's unique in its focus on effective transition of promising therapies to near-term clinical treatments (<7 year timeframe). To be successful the center has a novel organization of three operating groups, translational basic science, clinical trials, and clinical treatment. These groups work collectively on the mission in the same space under unitary leadership. This timeframe encompasses the "big picture" project limits for the center but individual projects can be shorter in time-frame (ie. upper limit). The three objectives outlined in the attached timetable are designed to run simultaneously in order to minimize the timeframe required for completion. Each project arm is interdependent but capable of being run in parallel.

- **Korean Collaboration**: Dr. S.B. Lee, a South Korean and a SCI injury PM&R doctor, trained at Johns Hopkins University School of Medicine, has joined our group and will have a role of facilitating communication with the S. Korean groups.

Children and adults receive activity based restorative therapy in the Paralysis Restoration Clinic at ICSCI at Kennedy Krieger Institute



Functional Electrical Stimulation (FES): With FES, a computer sends electrical messages to patents arms or legs, signaling their limbs to contract and perform a maneuver – pedaling a bike, for example that would otherwise be impossible.

Partial Weight Supported Gait: Patient with paralysis walks on a treadmill with assistance from a physical therapist.

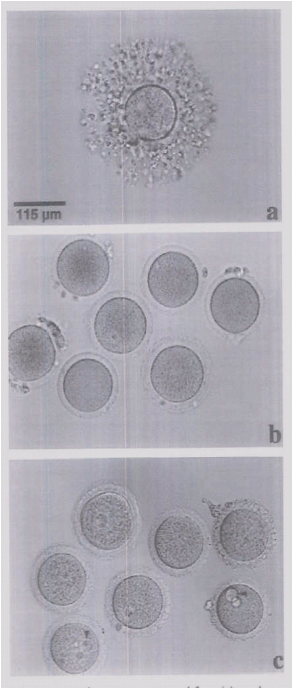
Barriers to successful transplantation and what is needed:

We are ready to move forward, but we need your help.

Critical transplantation work is difficult to get accomplished in the United States, so important steps have been made to assemble an international team for critical pre-clinical work and early human trials. Costs are predictably reduced multi-fold by putting this effort into creating an international team.

There are two primary projects that need to be completed in addition to the barriers the NTT project is already crossing. Thanks all for those of you who have helped to date. Now we need to push forward on additional fronts. The following are brief overviews of the projects requiring funding.

Project 1a: Identifying the ES cell tissue genetic factors critical to prevent rejection of transplanted cells. In today's world overcoming the regeneration issues faced by spinal cord injury will require transplantation to replace the tissue and cells lost. ES cells are tools of discovery. Today, ES cells are the best source of cells as they can create cells normally found in the spinal cord and ES cells can be created with genetic backgrounds acceptable for transplantation in individuals with different genetic backgrounds. Today, it is not practical to generate a new ES cell line for every person with SCI. What is needed are ES cell lines grouped by genetic backgrounds that will allow transplantation into the majority of individuals with spinal cord injury. We have identified that 10 genetic ES cell lines is sufficient to be acceptable to most genetic backgrounds in humans. A proof of principle is required in primates. These large-scale trials are not practical in the United States because of costs of primate studies. However, we have identified an approach to accomplish this in South America. We will isolate 10 different genetic backgrounds of ES cells from monkeys and cross-transplant using advanced methods to quantify cell rejection following transplantation into the injured spinal cord of primates. Similar transplantation of human ES cells will be done in the same New World primates spinal cord for comparison of rejection and growth properties with the primate ES cells.



New World primate oocytes and ES cells: These were isolated in our first trip to Columbian Amazon together with Dr. Lucena and Dr. Patarroyo.

Project 1b: Human ES cell transplantation in primates. This is the proof of principle experiment required to demonstrate recovery of function. Studies in rodents are not sufficient to demonstrate safety and efficacy. We must measure cell survival and rejection with primates.

What is required: We need your support to accomplish these goals. Traditional funding sources have been leveraged maximally. The project requires approximately 1 million dollars. We have recently raised 250K this past month but require another 2 Million dollars. One million is required to initiate construction, hiring of key additional personnel in Columbia and to contract for the primates. Another one million will be required in June 2006 to minimize the timeframe of completion. Delays in funding will result in increasingly greater total delays in the projects completion. Such a large scale international project with an aggressive time-line of less than 1.5 yrs requires automation of procedures, hiring a full time project manager, additional scientist in Bogota, as well as two full time staff scientists here. Please see attached time-line for individual steps and requirements. The footwork has been completed to bring an

international team of scientific leadership together. This is an important opportunity. We must we will. Join us in this mission. A similar project if funded by NIH in the United States would require over 10 million in direct dollars. We are confident the International NTT plan can be accomplished with 5 fold less money and we have a full running primate facility for future studies as well.

Previous NTT Project Summary:

We have assembled an international team with wide expertise to develop effective neural transplantation strategies. The vision of this group is to carry out ground-breaking studies toward human therapies for spinal cord injury and paralysis. One of the goals is to develop and apply an effective transplantation method for embryonic stem cells (ES) for repair of the human spinal cord. Briefly, we will combine new methods for generating ES cells with less invasive approaches for transplantation in the spinal cord. To accomplish this, the group plans to address two big barriers for the development of effective neural transplantation strategies.

Barrier 1: **Overcoming the risks associated with traditional invasive, inpatient surgery for neural transplantation**. ***To overcome this barrier, we proposed to develop a percutaneous method (an injection through the skin into the spinal cord) that can be completed as an outpatient.*** **O**ur goal is to prove that this new application of an old procedure is safe in the eyes of the FDA. To do this we have planned to perform a safety study in primates, which have a similar spine anatomy to that of humans.

Barrier 2: **Overcoming the rejection of transplanted stem cells**. ***To overcome this barrier, we propose to develop methods for somatic nuclear transfer into enucleated ES cells to obtain donor genetic specific ES cells***.

Investment of Kennedy-Krieger Institute and Johns Hopkins International Medicine. KKI has made multi-million dollar investment into the ICSCI and it is this investment that is allowing NTT to operate cost effectively, with no overhead costs and no local staff costs. Therefore, the groups' monies are used maximally. To maximize communication and selection of personnel and services in Columbia, we have developed the first phases of a plan to partner with Johns Hopkins International, a non-for profit entity design to initiate and complete business development at Johns Hopkins International (see attached memo outlining the business project).

Moving forward:

We are continuing to understand factors important for imprinting and nuclear transfer success, both important steps towards development of neural transplantation applications to avoid tissue rejection.

Timeline

Attached is the timeline for this project and monetary requirements for each stage to move forward. Note that projects are run in parallel to shorten the timeline. Each phase must be completed and evaluated for consideration of human application. Important aspects in the timing is achieving one million dollars in fundraising as soon as possible (January 2006), and another one million dollars by June 2006.

Goals for 2006

Future updates will occur with additional funding on quarterly bases with telephone conferences for better communication between written updates. As indicated in the timeline, major goals for 2006 include operationalizing the primate facility (this is a monumental effort), application of percutaneous transplantation, and completion of nearly 1000 primate transplants. Such a rapid timeline requires immediate funding.

GLOSSARY:

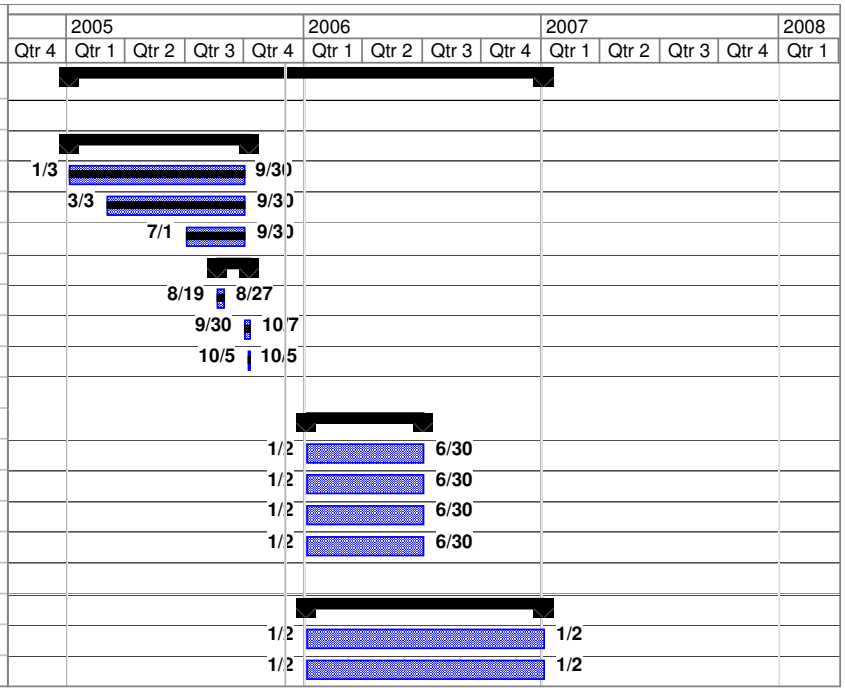
Cytoplasm: the fluid material within a cell body. This fluid contains many complicated molecules and micro-organs.
DNA (or genetic) reprogramming: The molecular alterations that are required to make DNA of somatic cells (such as skin) able to recreate all cell types (become again pluripotent). These are the events that make identical twins not exactly identical.
Enucleated: to remove a cells DNA or genetic material contained inside a structure called a nucleus.
ES cells: Embryonic Stem cell, the earliest stem cells, capable of making every later stem cell and cell in the body.
Genetic make-up: DNA, in this case DNA that is identical to the person receiving the transplant.
Human genome project: The group who sequenced all the genes in the human body.
Immunosuppression: the reduction of the immune systems ability to attach transplanted foreign cells. This is required when transplanting genetically non-identical cells as anything other than what contains your DNA will be recognized as foreign and your body's immune system will attempt to kill them. Typically, immunosuppression is achieved using very strong (and dangerous) medications taken by mouth.
Imprinting: The process where DNA is stripped of its reversible memory banks, enabling the cells to divide, followed by remethylation (imprinting; re-encoding new memories).
In vitro: In culture. In this case it references the process of fertilizing (combining an egg and sperm) a woman's egg in a culture dish.
Neural restoration: repairing the damaged nervous system in this case spinal cord repair
Oocyte: egg; used here in the context of a human egg or oocyte.
Percutaneous: across the skin; used here in the context of transplantation across the skin.
Phase I clinical trial: the first step in human trials. In these studies there is only one group and there are no control groups. These studies evaluate safety of the treatment.
Porcine: Pig; derived from pig.
Recipient: the person that receives the transplant.
Somatic nuclear transfer: The process of transferring a nucleus (containing DNA) from one cell to another.

ABBREVIATIONS:

DNA	Deoxyribonucleic acid
ES cells	Embryonic Stem cells
FDA	Federal Drug Administration
IVF	In Vitro Fertilization
KKI	Kennedy-Krieger Institute
MRI	Magnetic Resonance Imaging
NTT	Nuclear Transfer Transplantation
SCI	Spinal Cord Injury
U.N.	United Nations

Timeline

ID	Task Name	Required Funding	Duration	% Complete	Expense
1	**NTT PROJECT**	**$2,000,000.00**	**26.1 mons**	**29%**	**$410,000.00**
2					
3	**PRIMATE CENTER DEVELOPMENT**	**$0.00**	**10 mons**	**100%**	**$360,000.00**
4	Feasability / Location / Cost Analysis	$0.00	195 days	100%	$250,000.00
5	Collaborative Negotiations / Legal	$0.00	152 days	100%	$50,000.00
6	Equipment	$0.00	66 days	100%	$60,000.00
7	**Travel**	**$0.00**	**1.8 mons**	**100%**	**$50,000.00**
8	Post Doc Visits	$0.00	6 days	100%	$10,000.00
9	Researcher Visits	$0.00	6 days	100%	$15,000.00
10	International NTT meeting	$0.00	1 day	100%	$25,000.00
11					
12	**OPERATIONALIZE PRIMATE CENTER**	**$1,000,000.00**	**6.5 mons**	**0%**	**$0.00**
13	Construction	$300,000.00	130 days	0%	$0.00
14	Secure Primates	$200,000.00	130 days	0%	$0.00
15	Establish Primate ES Cell Lines	$250,000.00	130 days	0%	$0.00
16	Percutaneous Transplant	$250,000.00	130 days	0%	$0.00
17					
18	**ESC GENETIC GROUP TRANSPLANTATION**	**$1,000,000.00**	**13.1 mons**	**0%**	**$0.00**
19	1250 Primates	$500,000.00	262 days	0%	$0.00
20	Facility Overhead	$500,000.00	262 days	0%	$0.00



**Days = Working Days (five days a week - 52 weeks per year)