

Scientific Abstract

Investigators at Indiana University have extensive experience in cord blood transplantation. Indiana University is 1 of 6 transplant centers in the United States funded by the National Heart, Lung and Blood Institute (F.O. Smith, Principal Investigator) to study the transplantation of cord blood. This study proposed in this application is a Phase I/II study to evaluate the toxicities associated with the infusion of gene marked, *ex vivo* expanded cord blood cells. This study will also determine, by the presence or absence of the xenogeneic gene sequence for the neomycin resistance gene in bone marrow and peripheral blood, whether *ex vivo* expanded cord blood cells contribute to short and long term hematopoiesis.

Patients with certain genetic and malignant diseases may benefit from the transplantation of hematopoietic stem cells following myeloablative chemotherapy and radiation. Stem cells have traditionally been obtained from the bone marrow, but more recently, investigators have studied the potential use of stem cells obtained from the placenta and umbilical cord of newborn infants as an alternate source of stem cells suitable for transplantation. Preliminary studies at Indiana University and elsewhere have suggested that while cord blood is capable of reconstituting hematopoiesis in a portion of patients, non-engraftment may be problematic. In addition, the rate of myeloid and platelet engraftment appears to be slower than that expected from stem cells obtained from bone marrow. An additional concern is that a unit of cord blood may not contain enough stem cells to reliably engraft larger recipients. In attempts to address these issues, investigators at Indiana University and the University of Colorado are currently conducting Phase I studies utilizing similar technologies for CD34+ cell selection and *ex vivo* expansion of cord blood. If these studies demonstrate that it is safe to infuse CD34+ selected, *ex vivo* expanded cord blood cells into children and adults undergoing cord blood transplantation, this proposed study will address the safety of infusing retroviral-mediated, gene marked *ex vivo* expanded cord blood cells as a means to determine if *ex vivo* expanded cells contribute to hematopoiesis.

Eligible patients will receive a preparative regimen of total body irradiation, melphalan and antithymocyte globulin (ATG) or busulfan, melphalan and ATG as indicated in the protocol based upon their primary disease. For patients whose cord blood unit is divided into two aliquots (either 50%/50% or 60%/40%), at the time of cryopreservation, an aliquot (50% or 60%) of the unit will be infused, unmanipulated, on Day 0. The other aliquot (50% or 40%) will be selected, transduced and expanded, then infused on day +10. For all cord blood units frozen in a single bag, 60% will be infused, unmanipulated, on Day 0. The remaining 40% will be selected, transduced and expanded, then infused on day +10. The CD34+ cells will be transduced with a retrovirus construct carrying the mutated, non-expressing neomycin resistance gene (G1PLII). The transduction will be performed utilizing fibronectin fragments and recombinant cytokines (SCF, G-CSF and PEG-rHuMGDF).

The retroviral construct G1PLII was chosen because it is based upon the commercially available G1Na backbone. The neomycin-resistance gene and β -gal gene have been mutated so that they are non-expressing, potentially decreasing the probability of immunologic responses against the xenogeneic gene segments.

Preliminary data utilizing fibronectin and the combination of SCF, G-CSF and PEG-rHuMGDF suggest greater gene transfer efficiency than previous methods (see attached cover letter). The genetically modified cells will be expanded in American Fluoroseal bags in defined media and recombinant growth factors (SCF, G-CSF and PEG-rHuMGDF) for a total gene transduction and *ex vivo* expansion period of 10 days. On Day 10 post-transplant, these genetically modified cells will be infused into the transplant recipient. Patients will be assessed for acute infusion related toxicities as well as for the presence of replication competent retrovirus (RCR) by the S+L- assay and the 4070A PCR. Transfer of the retroviral construct will be determined in peripheral blood and bone marrow at various time points post-transplant. This study will enroll 10 patients. The

study will be stopped for unacceptable toxicity defined as grade IV acute infusion-related toxicity, high rate of non-engraftment, a high rate of acute grade IV graft-versus-host disease (GVHD) and the presence of RCR.

Since the G1PLII vector has not yet been produced for our use, we have conducted *in vitro* and animal studies using other retroviral vectors. Therefore, the G1PLII vector has not yet been used to transduce *ex vivo* expanded cord blood cells. Our preliminary studies of gene transfer into *ex vivo* expanded cord blood cells have used the MFG-EGFP retrovirus. However, due to concerns about the immunogenicity of retroviral constructs that contain the neomycin resistance gene, we propose to use the G1PLII vector, currently being produced by the NGVL, to mark *ex vivo* expanded cord blood cells.