SCIENTIFIC ABSTRACT

Bone marrow contains stem/progenitor cells capable of engrafting in bone and differentiating to osteoblasts. In preclinical models, whole unmanipulated bone marrow transplanted into nonablated recipients results in donor osteoblast engraftment. In our clinical trial of conventional bone marrow transplantation (BMT) for children with severe osteogenesis imperfecta (OI), a genetic disorder of bone in which short stature and severe osteopenia are principle manifestations, we demonstrated 1.5-2.0% engraftment of donor osteoblasts within 100 days after transplant. These donor osteoblasts appear to be functional and therapeutic. Within the first 6 months post BMT, a biopsy revealed an improvement in the microscopic structure of the bone, and childrens' growth velocity accelerated to approximate the median growth velocity of unaffected age- and sex-matched children. Moreover, with 36 months of follow-up, their total body bone mineral content increases at a rate similar to that of unaffected children. However, the growth velocity slows, but remains greater than controls.

In our second clinical trial for children with severe OI, we showed that allogeneic gene-marked marrow stromal cells (MSCs) engraft in the bones after infusion without chemotherapy conditioning. Subsequent to engraftment, the growth velocity of these children accelerated similar to the results after the initial bone marrow transplant. These cells were selected because, in 1999, when the trial opened, these were the best-characterized marrow cells with osteoprogenitor potential. Our data suggests that these cells have biologic and therapeutic capacity and augmenting the bone marrow graft may enhance the benefits of BMT. However, we now recognize that other marrow cells also seem to have such differentiation potential and may be a superior source of osteoprogenitor activity. In principle, identifying the marrow cell population with the greatest osteoprogenitor potential would allow investigators to more specifically focus future efforts in the development of cell therapy for all genetic diseases of bone.

This protocol is designed to test the hypotheses that BMT with MSC graft augmentation is safe and feasible for children with OI. We will also study the clinical outcome to gain insight into the benefits of such a strategy. The secondary, but equally important, aims of this protocol are to investigate the biology of marrow osteoprogenitor cells using a double gene marking strategy to track, the fate of two marrow cell populations and investigate the osteoprogenitor potential.

MARKING STRATEGY

Our marking strategy has two goals. First, it will allow us to assess whether CD34+ cells give rise to cells that engraft in the bone of recipients after chemotherapy conditioning, and whether isolated marrow stromal cells will similarly engraft. Second, it will allow us to assess the fraction of donor osteoblasts and the duration of osteoprogenitor activity derived from each marrow cell source. Finally we may be able to investigate the plasticity of marrow stem/progenitor cells by clone specific PCR analysis. We will mark the marrow cells with two distinguishable retroviral vectors. Envelopes were selected for the two vectors to maximize the transduction efficiency of the target cells. Each encodes nonexpressed genes to avoid a biased outcome due to potential bioactivity of an expressed protein. One vector, the G1Nm, is identical to the G1Na vector, containing a neomycin phosphotransferase gene (neo^R), except that the initiator codon has been

mutated to a stop codon. The G1Na vector has been used in previous human studies at St. Jude (RAC #9303-039; IND 5009). The other vector, GCsamPLI, is nearly identical to the G1PLII, containing a nonexpressing neo^R cassette and a lacZ cassette, which has been previously used in clinical trials at the NIH (IND 6568) and at St. Jude (RAC #9904-310; IND 8257). This vector differs from the G1PLII only in the length of the polylinker and the MFG viral backbone (instead of the G1). The MFG backbone had been used in Dr. Alain Fischer's (Hôpital Necker, Paris, France) study of gene therapy for children with severe combined immunodeficiency syndrome (SCID), and has been approved by both the FDA (IND 8828) and the RAC (0001-370) for a pilot study of gene therapy for patients with Fanconi's anemia (PI: James Croop, MD, PhD, Indiana University School of Medicine).

In our study, CD34+cells and MSCs will be isolated from donor marrow and transduced with the retroviral vectors. Marrow without experimental manipulation will be infused with the engineered samples after chemotherapy conditioning. This strategy will allow us to assess the safety and feasibility of MSC graft augmentation and to independently follow the fate of co-infused CD34+ cells and MSCs. We hypothesize that the MSCs will engraft in bone, but also that cells derived from the CD34 fraction will be identified as donor osteoblasts. From the animal data prepared for publication and presented in the background of the protocol (Section 2.0), we further hypothesize that the CD34+ cells will possess greater osteoprogenitor potential.

TREATMENT PLAN

This study is designed as a pilot study of BMT with graft augmentation for children with severe OI. The patients will be stratified according to donor: matched sibling and matched unrelated. About 4-6 weeks prior to the BMT, marrow will be harvested from the donor. About 25 ml will be used to isolate donor MSCs and then the cells will be transduced and modestly expanded *ex vivo.* The remaining marrow will be divided into two equal volumes. One will be cryopreserved either without further manipulation (matched sibling donor) or after T-cell depletion (matched unrelated donor). This component of the graft will serve to provide the same therapy, and presumably benefits, as in our previous trial. The remaining volume of marrow will be used to isolate CD34+ cells, which will then be transduced and cryopreserved. After the patients receive a conditioning regimen of fludarabine, pharmacokinetically targeted busulfan and ATG, the cryopreserved bulk bone marrow, gene marked CD34+ cells, and freshly prepared gene marked MSCs will be infused into the patients. The patients will be followed closely for toxicity of BMT with graft engineering. To assess the fate of the gene marked marrow cells, blood and bone marrow will be assayed by PCR for the presence of proviral sequences at various time points after BMT. At 2-3 months and again at 12 months post BMT, we will obtain a biopsy of bone, skin, (muscle, fat, and cartilage if within the surgical field) and an aspirate of bone marrow to assess for proviral sequences as an indication of donor engraftment. Additionally, we will attempt to determine proviral integration sites of the CFC derived from the marrow post BMT by inverse PCR, which will allow us to investigate the fate and potential plasticity of individual stem/progenitor cells.