Appendix F: Non-Technical Description of Experiment

Recent scientific advances have made it possible to put a new gene into a cell in order to follow its life span. This technology is called "retrovirus mediated gene transfer." A gene is the part of a chromosome (hereditary material) that contains the information needed by cells to make proteins. In this study, a gene is inserted into peripheral blood progenitor cells (PBRC). These are the "mother cells" which produce red blood cells, white blood cells and platelets. It is currently unknown whether these PBRC produce white cells, red cells and platelets in humans only for a short period (weeks to several months), or whether they produce these important cells for the lifetime of a person. The purpose of this study is to investigate this question by marking 25% of the PBRC before transplantation with the marker gene encoding for the protein neomycin phosphotransferase (neo). The neo gene is inserted into the PBRC using the following procedure.

PBRC will be obtained by leukophoresis after the donor has been treated for several days with growth factor to increase the number of PBRC in peripheral blood. 25% of these cells will be used for marker gene insertion. The gene is attached to a virus from mice that can enter the PBRC, taking the gene with it. This virus is modified such that it can enter the blood cells only once, but it cannot spread in the patient's body and cause an infection. PBRC are marked by incubation with the cells producing the virus which contains the marker gene. Virus producing cells are lethally irradiated before PBRC are added to prevent infusion of live virus-producing cells into the patient. After 24 hours of incubation cells are harvested and incubated for 10 days in a specific culture system which supports the survival of PBRC (long-term marrow culture, LTMC). The LTMC contains marker gene containing virus which is added every second day. After four days of LTMC cell samples will be obtained from the cultures and tested for sterility and for absence of infectious "helper" virus. If no abnormalities in the marked cells are identified, they will be given through a large vein to the patient after treatment with high-dose total body irradiation and/or chemotherapy. About three weeks after transplant, when the patient's blood shows sufficient numbers of new white cells, approximately 20 ml of blood will be drawn weekly until discharge home (about 90 days after transplant). Blood cells will be analyzed for the presence of the marker gene. At the times of routine marrow aspirations (days 21, 56 and 84 after transplantation), 5 ml of marrow will be obtained in addition to the routine quantity, and will be analyzed for the presence of the marker gene. The same amount of marrow and blood will be obtained, in addition to routine, at the one year long-term follow up at the transplant center. Blood will also be obtained at yearly intervals for up to ten years by the patient's hometown physician and sent to the transplant center for analysis. These tests will provide information about whether and to what extent the marked PBRC contribute in the long-term to blood cell production.