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Gene Therapy for β -Thalassemia: Development of Therapeutically Consistent γ -Globin Lentiviral Vectors, In Vivo Selection of Genetically Modified Cells, and Testing in a Large Animal Model

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The hemoglobin disorders of β -thalassemia and sickle cell disease together constitute the most prevalent group of human monogenic diseases. Though curative allogeneic stem cell transplantation therapy and palliative therapies have been developed for these disorders, the majority of patients still suffer significant morbidity and early mortality. The development of therapeutic approaches based on genetic manipulation of autologous stem cells therefore remains an attractive alternative. Successful gene therapy for beta-thalassemia and sickle cell disease will require high-level globin expression in a substantial proportion of the developing erythroblasts of an affected individual. Since increased fetal hemoglobin diminishes the severity of β -thalassemia and sickle cell anemia, a strategy using autologous, stem cell-targeted gene transfer of a γ -globin gene may be therapeutically useful. We have focused our efforts on 1) developing lentiviral vectors containing gamma-globin cassettes capable of therapeutic expression levels and 2) incorporating into the vectors a second expression cassette containing the methylguanine methyltransferase (MGMT) drug resistance gene, which can be used to accomplish in vivo selection of genetically modified hematopoietic stem cells.

We previously found that a γ -globin lentiviral vector utilizing the β -globin promoter and elements from the β -globin locus control region (LCR) totaling 1.7 kb could correct murine β -thalassemia. However, therapeutic consistency was compromised by chromosomal position effects on vector expression and high level γ -globin expression

often required multiple vector copies. We therefore tested whether increasing the size of the individual elements from the LCR in the vector would yield higher and more consistent expression. Using a new vector containing 3.2 kb of LCR sequences, the majority of animals transplanted with genetically modified β -thalassemic stem cells expressed high levels of fetal hemoglobin (17-33%), with an average vector copy number of 1.3. This resulted in a mean 2.6 g/dL increase in hemoglobin concentration and amelioration of other hematologic parameters. Analysis of clonal erythroid cells of secondary spleen colonies from transplanted mice demonstrated that this vector was more resistant to position effects. Mapping the genomic sites of vector insertion in the single copy erythroid clones (n=22) showed that 45% of vector insertions were located in the introns of genes. Globin expression from the smaller vector was often compromised in such sites whereas the larger vector performed more consistently. Although intergenic vector insertions were generally associated with a higher probability of expression, the 3.2 kb LCR vector performed more consistently at these sites as well.

We have recently shown that treatment with temozolomide plus benzylguanine can be used to increase a minor population of transplanted normal hematopoietic stem cells transduced with the MGMT drug resistance gene to therapeutically relevant levels in mice with beta-thalassemic intermedia. This could be a useful strategy to increase the low numbers of corrected cells that may be therapeutically limiting in patients receiving a mild transplant

conditioning regimen. These data will be reviewed. In addition, we constructed a lentiviral vector containing both the above LCR-driven γ -globin expression cassette and an MSCV-driven MGMT cassette. Normal mouse BM cells were transduced at low MOI followed by transplantation into lethally irradiated normal recipient mice. Despite human γ -globin expression in less than 1% of red cells in the cohort at baseline, several mice demonstrated *in vivo* selection of “F” cells to levels of 70-90% after three rounds of treatment with temozolomide and benzylguanine. Optimization of a dual gene vector may ultimately allow successful gene therapy in the absence of aggressive transplant conditioning.

Finally, we have recently developed a simian immunodeficiency virus (SIV)-based lentiviral vector system that successfully circumvents the species-specific block to transduction of rhesus cells by HIV-based lentiviral vectors. This now allows modeling of human stem cell-targeted HIV-based lentiviral vector gene transfer using a rhesus macaque autologous stem cell transplantation system. To date, three animals have been transplanted with autologous CD34⁺ cells transduced with an SIV vector encoding GFP. Greater than six months post-transplantation, each animal demonstrates efficient gene transfer into their repopulating cells, with marking levels ranging between 5-25% in peripheral blood leukocytes. This lentiviral vector system should prove valuable in facilitating the testing of globin lentiviral vectors in the rhesus autologous stem cell transplantation model.