Gene Therapy (2009) 16, 943

www.nature.com/gt

Gene therapy for sickle cell disease

Translational stability for sickle gene therapy

MC Walters

Gene Therapy (2009) **16,** 943; doi:10.1038/gt.2009.70; published online 25 June 2009

Since the discovery of sickle cell anemia and its genetic origin, the possibility of cure by correcting the altered nucleotide has intrigued a generation of scientists. The establishment of viral transduction into hematopoietic stem cells (HSCs) to generate safe, stable, erythroid-specific replacement gene expression at a level that is sufficient to have a clinical effect has, however, been elusive, and the necessity of fulfilling all of these criteria makes this genetic disorder one among the most complex disorders to treat successfully by gene therapy. Not surprisingly, advances in this endeavor have been incremental, and Persons and colleagues[1] report another very important advancement in replacement gene vector design in a recent *Molecular Therapy* paper.

Pestina *et al*.[1] engineered a novel globin gene therapy vector by capitalizing on earlier observations made by Russell and colleagues[2] that a pyrimidine-rich, stem-loop structure in the 3′ untranslated region (3′ UTR) of the β-globin mRNA (and absent from the γ-globin mRNA) accounts for the hyperstability of the β-globin transcript. Using a fusion gene that linked γ-globin coding sequences to the β-globin 3′ UTR, Pestina *et al*.[1] created a lentiviral vector with potent anti-sickling activity in a mouse model of sickle cell anemia, which was sufficient for significant improvements in the hematological and pathophysiological features of sickle cell disease in this mouse model. Thus, by improving the stability of the anti-sickling γ-globin RNA transcript and promoting the translation of a greater quantity of fetal hemoglobin in red blood cells, the authors seem to have overcome a very important obstacle to successful gene therapy for sickle cell anemia: establishing a high level of sustained γ-globin expression that is sufficient for a clinical effect.

The report also alluded to areas where future efforts should be focused. Although the authors demonstrated significant γ-globin expression even at a very low vector copy number, the integrations did not generate copy number-independent expression and were therefore subject to position effect variegation. This raises the possibility that the expression from lentiviral genomic integrations might be influenced by local chromatin effects and, conversely, that regulatory elements contained in these vectors may influence adjacent chromatin structures. This possibility was illustrated by an observation by Persons and colleagues in this report that a control vector containing the green fluorescent protein in lieu of γ-globin was expressed in non-erythroid cells, consistent with the notion that some lentiviral integration sites in the HSC may be occurring at sites generally permissive for transcription in hematopoietic tissues. Although it is possible that bracketing the gene expression cassette by a transcription insulator such as the chicken hypersensitive site-4 element may help overcome this problem,[3,4] the possibility of disrupting native gene expression by having a transcriptional insulator inserted in or near regulatory elements in an active locus would remain. This study did not focus on vector safety, but it is encouraging to note that none of the mice exhibited malignant transformations during the course of these investigations. Nonetheless, it is of paramount importance that issues related to genotoxicity are resolved before any clinical trials of gene therapy for sickle cell anemia are embarked upon.

The authors correctly assert that their vector design may be particularly applicable to the clinical setting of sickle β° thalassemia, which is the human genotype that most closely resembles the mouse model of sickle cell anemia that is used in these studies. This is presumably in part due to the relative α- to β-globin polypeptide chain imbalance that is corrected by overexpression of the transduced γ-globin and its preferential association with α-globin to form fetal hemoglobin. This would also increase the ratio of β/α-chains so that it approaches 1.0 and thereby inhibits the formation of unstable α-globin tetramers. Therefore, it is tempting to speculate that as we get closer to the clinical application of gene therapy for sickle cell anemia, brought about by advances such as those reported by Pestina *et al*.,[1] it is possible that more careful attention to vector design will be influenced by genotype/phenotype characteristics expressed by the individual sickle cell disease recipients. ■

Dr MC Walters is at the Blood and Marrow Transplant Program, Children's Hospital and Research Center, 747-52nd Street, Oakland, CA 94609, USA.
E-mail: mwalters@mail.cho.org

1 Pestina TI, Hargrove PW, Jay D, Gray JT, Boyd KM, Persons DA. Correction of murine sickle cell disease using γ-globin lentiviral vectors to mediate high-level expression of fetal hemoglobin. *Mol Ther* 2009; **17**: 245–252.
2 Jiang Y, Xu XS, Russell JE. A nucleolin-binding 3′ untranslated region element stabilizes beta-globin mRNA *in vivo*. *Mol Cell Biol* 2006; **26**: 2419–2429.
3 Aker M, Tubb J, Groth AC, Bukovsky AA, Bell AC, Felsenfeld G *et al*. Extended core sequences from the cHS4 insulator are necessary for protecting retroviral vectors from silencing position effects. *Hum Gene Ther* 2007; **18**: 333–343.
4 Arumugam PI, Scholes J, Perelman N, Xia P, Yee JK, Malik P. Improved human beta-globin expression from self-inactivating lentiviral vectors carrying the chicken hypersensitive site-4 (cHS4) insulator element. *Mol Ther* 2007; **15**: 1863–1871.