

Approaches to the Gene Therapy of the β -hemoglobinopathies

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The β -hemoglobinopathies are the most prevalent genetic disorders worldwide. They are caused by mutations that result in massive decrease in β -globin gene expression (β -thalassemia) or production of an abnormal β -globin protein that polymerizes within red blood cells (sickle cell anemia [SCA]). Despite currently available treatments, the life expectancy of patients with the severe forms of these diseases remains shortened by several decades unless allogeneic bone marrow transplantation (BMT) is performed with an HLA geno-identical sibling donor before irreversible organ damage occurs. Unfortunately, most patients do not have a suitable donor and those who do face an increased risk of morbidity and mortality from graft versus host disease (GVHD). Because many different mutations and deletions are responsible for the various cases of β -thalassemias, gene therapy by gene addition remains the preferred approach rather than gene repair/homologous recombination or inhibition of spurious splicing events. Devising an effective gene therapy for these disorders by transfer of a therapeutic gene into autologous hematopoietic stem cells (HSCs) has proven especially challenging, as oncoretroviral vectors containing β -globin gene and locus control region (LCR) derivatives have a high propensity for low titers and multiple provirus rearrangements. Earlier studies led us to conclude that spurious viral RNA splicing and inadequate nucleo-cytoplasmic export were the main determinants of globin vector instability. As a remedy, we thus proposed the use of RNA exports elements that include HIV-1 Rev/RRE. In agreement with this model, major advances were recently achieved by means of Rev-dependent lentiviral vectors, making the initiation of clinical trials now warranted. In the first Phase I/II clinical trial of lentivirus-mediated gene therapy of the β -hemoglobinopathies, the overall study objective is to test the safety tolerance, and therapeutic efficacy (biological and clinical) of transplantation with autologous bone marrow-derived CD34+ cells that have been transduced ex-vivo with a lentiviral vector expressing, in a tissue specific manner, a variant of the wild-type adult human β^A -globin, β^{A-T87Q} , which has been shown to have anti-sickling properties. In addition, the in-vivo protein level expressed by this variant can be quantified by mass spectrometry and distinguished from that of wild-type HbA derived from transfused red blood cells. This property is important for demonstrating a biological effect in β -thalassemia patients by detection of engraftment with cells expressing the transferred gene, since patients may be transfused with normal HbA-containing blood during the trial. Extensive preclinical studies in mouse models have demonstrated reliable long-term correction of most pathological features of both β -thalassemia and SCA with this lentiviral vector. Complementary studies in human cells showed effective transduction of primitive human HSCs and high expression levels of the therapeutic protein in their red blood cell progeny. The therapeutic β -globin gene is not intrinsically oncogenic and the vector has been extensively modified to further decrease the risk of leukemogenesis by insertional mutagenesis (e.g., self-inactivating vector deletions, chromatin

insulators bordering the provirus). The vector has not presented an oncogenic risk in animals followed for up to 2 years after treatment, including primary and secondary transplants with transduced marrow cells in pre-leukemia mouse models. On the basis of these combined preclinical results, the first human clinical trial is likely to be initiated in Europe before the end of 2005 for both SCA and β -thalassemia major. The population to be enrolled in this study are patients with severe, life-threatening disease, who would be eligible for allogeneic BMT but do not have an HLA geno-identical sibling donor. I will review the various steps involved in the development of this approach to the stage of human clinical trial (vector design, preclinical efficacy and safety studies, manufacturing and controls, clinical trial design). Complementary and alternative approaches involving stem cell selection/amplification and RNAi will be discussed.