Gene Therapy Approaches to Hemoglobinopathies

Giuliana Ferrari, PhD[a,b], Marina Cavazzana, MD[c,d], Fulvio Mavilio, PhD[e,*]

KEYWORDS

• Thalassemia • Sickle cell disease • Gene transfer • Retroviral vectors
• Lentiviral vectors • Globin gene regulation • Stem cell transplantation
• Hematopoiesis

KEY POINTS

- Gene therapy for hemoglobinopathies requires the transfer to hematopoietic stem cells of large globin gene expression cassettes driven by complex regulatory elements.
- Preclinical and early clinical studies proved safety and efficacy of stem cell–based gene therapy while showing hurdles and limitations of the existing technology, particularly for sickle cell disease.
- Stem cell procurement, cell dose, transduction efficiency, gene expression level, conditioning regimen, and patient's age at the time of intervention are key factors affecting the therapeutic range and clinical efficacy of gene therapy.
- The bone marrow microenvironment is a crucial yet poorly understood factor for both stem cell mobilization and engraftment.

INTRODUCTION

Hemoglobinopathies are a family of inherited blood disorders characterized by the defective synthesis of 1 of the 2 polypeptide chains of hemoglobin (α- or β-thalassemia [β-thal]) or by the synthesis of an abnormal hemoglobin variant, such as the hemoglobin S (HbS) mutation (β^{A-E6V}) that causes sickle cell diseases (SCD). Hemoglobinopathies are found all over the world, although the carriers' relative resistance to malaria established a high frequency of thalassemia in the Mediterranean areas, India, and the Far East, and of SCD in sub-Saharan Africa.[1] Migration

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[a] San Raffaele-Telethon Institute for Gene Therapy (SR-TIGET), Istituto Scientifico Ospedale San Raffaele, Via Olgettina 58, Milan 20132, Italy; [b] Vita-Salute San Raffaele University, Milan, Italy; [c] Biotherapy Department, Necker Children's Hospital, Imagine Institute, 149 rue de Sèvres, Paris 75015, France; [d] Paris Descartes University, INSERM UMR 1163, Paris, France; [e] Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 287, 41125 Modena, Italy
* Corresponding author.
E-mail address: fulvio.mavilio@unimore.it

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phenomena caused their spreading to other regions of the world, notably the United States and Europe. Overall, hemoglobinopathies are the most frequent monogenic diseases worldwide, with approximately 5% of the world population carrying a hemoglobin disorder trait.[2]

The only curative treatment of thalassemias and SCD is allogeneic transplantation of hematopoietic stem cells (HSCs) from HLA-matched sibling donors, which is associated with greater than 90% disease-free survival but is available to only less than 20% of patients.[3] Transplants with matched unrelated or mismatched donors carry a progressively higher risk of morbidity and mortality, unacceptable given the current high standards of care for both diseases. Gene therapy, or the autologous transplantation of genetically corrected HSCs, would be a potential alternative available to all patients and carries low transplant–related risks.

Over the past 2 decades, gene therapy has been successfully applied to primary immunodeficiencies, such as adenosine deaminase–deficient severe combined immunodeficiency (ADA-SCID), X-linked SCID (SCID-X1), Wiskott-Aldrich syndrome, and X-linked chronic granulomatous disease (X-CGD) (reviewed in Booth and colleagues, 2016[4]). The first clinical trials were based on vectors derived from the Moloney murine leukemia retrovirus (MLV), carrying a therapeutic gene under the control of the promoter/enhancer elements contained in the MLV long terminal repeats (LTRs). Although gene therapy has been beneficial for most patients, all trials except those for ADA-SCID were characterized by the occurrence of leukemia or myelodysplasia in most patients.[4] The molecular bases of these events are still ill defined, although it is likely that insertional deregulation of proto-oncogenes led to clonal expansion and eventually malignant progression.[5,6] The recognition of the MLV LTR as a major component of proto-oncogene activation led to the design of self-inactivating (SIN) vectors devoid of LTR regulatory sequences and incorporating cellular, short-range promoters to drive the expression of the therapeutic gene. This vector design proved safe and efficacious in a clinical trial of gene therapy for SCID-X1.[7]

To reduce the risk of insertional oncogenesis, SIN lentiviral vectors (LVs) derived from the human immunodeficiency virus–replaced MLV-derived vectors in all clinical trials involving genetically modified HSCs. Although MLV preferentially targets transcriptional regulatory elements,[5,8,9] LVs integrate throughout transcribed genes[5,6] in proximity of the nuclear membrane,[10] an inherently safer integration pattern as indicated by in vitro as well as in vivo studies (reviewed in Naldini, 2015[11]). LVs are currently used in early- or advanced-phase clinical trials of gene therapy for Wiskott-Aldrich syndrome, ADA-SCID, X-CGD, and nonimmune disorders such as adrenoleukodystrophy or metachromatic leukodystrophy. These trials are providing strong evidence of clinical efficacy in the absence of treatment-related adverse events or clonal abnormalities in the genetically modified HSC repertoire.[11]

Gene therapy for hemoglobinopathies presents additional challenges. Globin genes are subjected to a sophisticated regulation relying on the molecular interaction of globin promoters with a locus-control region (LCR), which regulates high-level, lineage-restricted gene expression as well as the choice of embryonic, fetal, or adult genes in different phases of development.[12,13] The combination of a full LCR and an adult β-globin gene, which requires its introns and 3′ untranslated region for proper function, is too large to be accommodated by an LV. Size reduction has been achieved, although globin-expressing LVs remain large and complex and have a lower transduction efficiency compared with simpler vectors. LVs have been developed and successfully tested in preclinical models of hemoglobinopathies, whereas recent clinical trials are showing remarkable safety and promising clinical efficacy.