Cytotherapy, 2017; 19: 1096–1112

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STEM CELL TRANSPLANTATON

Preclinical studies for a phase 1 clinical trial of autologous hematopoietic stem cell gene therapy for sickle cell disease

FABRIZIA URBINATI[1], JENNIFER WHERLEY[1], SABINE GEIGER[1], BEATRIZ CAMPO FERNANDEZ[1], MICHAEL L. KAUFMAN[1], AARON COOPER[1], ZULEMA ROMERO[1], FILIPPO MARCHIONI[1], LILITH REEVES[2], ELIZABETH READ[3], BARBARA NOWICKI[4], ELKE GRASSMAN[5], SHIVKUMAR VISWANATHAN[5], XIAOYAN WANG[6], ROGER P. HOLLIS[1] & DONALD B. KOHN[1]

[1]Department of Microbiology, Immunology and Molecular Genetics and the Eli & Edythe Broad Stem Cell Research Center, University of California, Los Angeles, California, USA, [2]Translational Core Laboratory, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA, [3]Consultant, San Francisco, California, USA, [4]UCLA BM/Stem Cell Transplant Laboratory, University of California, Los Angeles, USA, [5]Translational Trials Development and Support Labs, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA, and [6]Department of General Internal Medicine and Health Services Research, University of California, Los Angeles, USA

Abstract

Background aims. Gene therapy by autologous hematopoietic stem cell transplantation (HSCT) represents a new approach to treat sickle cell disease (SCD). Optimization of the manufacture, characterization and testing of the transduced hematopoietic stem cell final cell product (FCP), as well as an in depth *in vivo* toxicology study, are critical for advancing this approach to clinical trials. *Methods.* Data are shown to evaluate and establish the feasibility of isolating, transducing with the Lenti/β^{AS3}-FB vector and cryopreserving $CD34^+$ cells from human bone marrow (BM) at clinical scale. *In vitro* and *in vivo* characterization of the FCP was performed, showing that all the release criteria were successfully met. *In vivo* toxicology studies were conducted to evaluate potential toxicity of the Lenti/β^{AS3}-FB LV in the context of a murine BM transplant. *Results.* Primary and secondary transplantation did not reveal any toxicity from the lentiviral vector. Additionally, vector integration site analysis of murine and human BM cells did not show any clonal skewing caused by insertion of the Lenti/β^{AS3}-FB vector in cells from primary and secondary transplanted mice. *Conclusions.* We present here a complete protocol, thoroughly optimized to manufacture, characterize and establish safety of a FCP for gene therapy of SCD.

Key Words: *gene therapy, hematopoietic stem cells, lentiviral vectors, sickle cell disease*

Introduction

Sickle cell disease (SCD) is a monogenic disease that affects approximately 90 000 to 100 000 Americans. Annually, there are an estimated 275 000 affected births reported worldwide [1], with the highest incidence (>80%) in the sub-Saharan African regions. SCD patients suffer significant neurologic, pulmonary and renal damage, as well as severe chronic pain episodes that adversely affect quality of life. Although current medical therapies for SCD, including supportive measures, can reduce short-term morbidity, the inevitable progressive deterioration in organ function results in a significant decrease in quality of health with early mortality. hematopoietic stem cell transplantation (HSCT) can benefit patients with SCD by providing a source for lifelong production of normal red blood cells (RBCs). However, allogeneic HSCT is limited by the availability of well-matched donors and the immunological complications of graft rejection and graft-versus-host

Study approval: All human samples collected at UCLA have been used following UCLA Institutional Review Board protocol #10–001399. All work with mice performed at UCLA was done under protocols approved by the UCLA Institutional Animal Research Committee.

Correspondence: **Donald B. Kohn**, MD, Department of Microbiology, Immunology and Molecular Genetics and the Eli & Edythe Broad Stem Cell Research Center, University of California, 3163 Terasaki Life Science Building, 610 Charles E. Young Drive East, Los Angeles, CA 90027, USA. E-mail: e-dkohn@mednet.ucla.edu

(Received 17 March 2017; accepted 6 June 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.06.002

disease. Autologous HSCT may provide a better therapeutic alternative to allogeneic HSCT. Stem cell therapy in which the patient's own bone marrow (BM) stem cells have been corrected by transfer of a modified human β-globin gene that inhibits polymerization of the HbS would avoid the immunologic complications and donor limitations of allogeneic HSCT.

We have optimized a lentiviral vector (Lenti/β^{AS3}-FB) carrying a previously described anti-sickling β-globin cassette [2]. Our earlier studies [3] provided preclinical evidence to support translation of this approach to a clinical trial of gene therapy for SCD, showing an average Vector Copy/Cell of 1 in BM $CD34^+$ cells from SCD donors; transduction of approximately 35% of the colony-forming progenitors; and sufficient expression of the anti-sickling β-globin gene (15–20% of total β-globin mRNA and protein) to prevent *ex vivo* sickling of RBCs produced by *in vitro* erythroid differentiation.

Here, we present the step-by-step translational process we followed to move from proof of concept to a phase I clinical trial of gene therapy for SCD to produce a clear road map for future early-phase hematopoietic stem cell (HSC)-based gene transfer trials. Because of risks associated with granulocyte colony-stimulating factor (G-CSF) administration to patients with SCD, BM was used as the source of HSCs instead of G-CSF-mobilized apheresis collections, which are often used in other adult patient populations [4]. Our process development therefore required scaling up the RBC depletion and $CD34^+$ cell isolation from a large volume of BM, followed by *ex vivo* manipulation for transduction and cryopreservation of the final cell product. Additionally, because several previous gene therapy clinical trials have been complicated by genotoxicity, mainly due to the insertion of gammaretroviral vectors into the host genome, we carefully investigated the possible genotoxicity of the Lenti/β^{AS3}-FB lentiviral vector. We performed an *in vivo* toxicology study using murine BM and additional genotoxicity studies in human cells engrafted *in vivo* in immune-deficient mice, as an addition to the In Vitro Insertional Mutagenesis (IVIM) assay reported previously [3]. The relevance of this work can be extended beyond SCD to guide translation of preclinical work to a gene therapy clinical trial.

Methods

BM samples

BM aspirates from SCD patients were obtained from the posterior iliac crest, either with local anesthesia or when under general anesthesia for a clinical indication, following written informed consent in accordance with the institutional review boards. Control healthy donor BM samples were purchased from commercial sources (AllCells or Hemacare) obtaining aspirates from volunteer donors. The ages of the five healthy donors used for the clinical scale BM processing were 20, 24, 27, 28 and 37 years old.

Large-scale BM volume reduction by centrifugation

The high RBC content in BM can interfere with immunomagnetic $CD34^+$ selection of $CD34^+$ cells. Therefore, a clinical-scale protocol for RBC depletion/volume reduction of the BM by Hetastarch sedimentation and centrifugation of the blood bag was developed, based on the literature [5] and procedures previously used at the UCLA Clinical Bone Marrow and Stem Cell Lab. After adjusting the hematocrit to 25% with Plasmalyte A, 6% Hetastarch (Hospira) was added to the sample (one-fifth of the total volume), and it was transferred into a 300-mL transfer pack (Fenwal). Centrifugation was performed at 40*g* for 5 min to allow RBCs to sediment to the bottom of the bags. After RBC removal, a second centrifugation was performed at 400*g* for 10 min, and supernatant containing the buffy coat was expressed from the top with a plasma extractor.

BM volume reduction and RBC depletion using the CliniMACS prodigy

As an alternative method to deplete RBC content, 6% Hetastarch was added (one-fifth of the total volume) to the initial fresh BM product, and the sample was centrifuged using the automated CliniMACS Prodigy (Miltenyi Biotec), with TS710 tubing set, using a protocol developed by Miltenyi specifically for this application. Automated cell labeling with anti-CD34 monoclonal antibody was also performed on the Prodigy system. Subsequently, $CD34^+$ cells were isolated by immunomagnetic selection on the CliniMACS system (Miltenyi Biotec). After isolation, cells were prestimulated and transduced using the conditions described below.

Small-scale BM $CD34^+$ isolation

BM aspirate samples (5–15 mL) were separated by Ficoll-Hypaque gradient to isolate mononuclear cells and then further processed to isolate $CD34^+$ cells, using CD34 MicroBead Kit (Miltenyi Biotec); the isolated cells were cryopreserved in liquid nitrogen with 10% dimethyl sulfoxide.

Lentiviral vector production and characterization

The Lenti/β^{AS3}-FB vector has been described previously [3]. The LV vector carries a human β-globin cassette including portion of the globin LCRs (HS2-HS3-HS4), portion of the endogenous β-globin promoter, and the β-globin gene carrying three amino